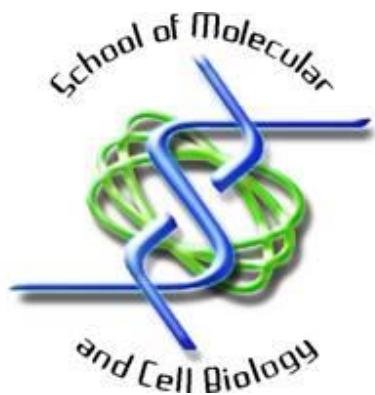


**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

**SCHOOL OF MOLECULAR AND CELL BIOLOGY**



**MOLECULAR AND CELL BIOLOGY IIB: CONCEPTS (MCBG2032)**

**COURSE INFORMATION**

**AND**

**PRACTICAL SCHEDULE**

**2018**

## **MOLECULAR AND CELL BIOLOGY IIB: CONCEPTS COURSE (MCBG2032) – 2018**

Welcome to the Molecular and Cell Biology IIB: Concepts Course. This course is designed to cover the concepts required for the major third year courses offered in the School of Molecular and Cell Biology. One of the interactive ways in which it is taught is to promote critical engagement using writing (CEW). Therefore, you will do informal, exploratory writing during lectures, on the Sakai discussion forum and during practicals. In addition, you will be required to complete a number of formal writing assignments during the year. You will also be using clickers.

### **Course Information:**

**Course Co-ordinator** Dr Meyer (vanessa.meyer@wits.ac.za; GH512)

**Textbooks:** **Book Bundle ISBN 9781473757776**

**Prescribed:** Garrett, RH Grisham, CM. *Biochemistry* 6<sup>th</sup> edition, Cengage.  
Supplementary Material for Molecular and Cell Biology: Scientific Practice and Concepts (Custom book)

**Recommended:** Jemiolo, DK Theg, SM. *Student Solutions Manual, Study Guide and Problems Book*. Cengage.

**Lecturers:**

|         |  |
|---------|--|
| Block 1 | Dr Nikitina (natalya.nikitina@wits.ac.za; GH701) |
|         | Dr Meyer (vanessa.meyer@wits.ac.za; GH512)       |
| Block 2 | Prof Brenner (liz.brenner@wits.ac.za; GH510)     |
|         | Dr Gentle (nikki.gentle@wits.ac.za; GH108)       |
| Block 3 | Dr Fanucchi (sylvia.fanucchi@wits.ac.za; GH416)  |
| Block 4 | Prof Veale (rob.veale@wits.ac.za; GH001D)        |

**Assessment:** There will be at least one major test per block. Continuous and formative assessment will take place during tutorials, lectures and practical sessions, in the form of tests, writing, inquiry, other assignments and practical reports.

**Major Block Tests:**

|        |             |            |
|--------|-------------|------------|
| Test 1 | 27 February | (30 marks) |
| Test 2 | 6 April     | (50 marks) |
| Test 3 | 26 April    | (30 marks) |
| Test 4 | 24 August   | (50 marks) |
| Test 5 | 12 October  | (60 marks) |

To apply for a deferred test, you will need to provide an original medical certificate **within 3 working days** of the original test date to Mrs Louise Giustinoni (GH001).

**Examinations:** Summative assessment for each semester will include a 3 hour formal exam. To apply for a deferred examination, you will need to complete the **Faculty's** official forms **within 3 working days** of the original examination date (see notice board for details). Information pertaining to the deferred examinations will be communicated through Sakai.

**Mark Allocation:** Final examination 50%; Class mark 50% (the class mark will be compiled from: major block tests – 50%; assignments and tutorials – 25%; practical reports and practical tests – 25%).

**Practicals:** Practical work is an important part of the course. This is because all knowledge and theory in science has originated from practical observation and experimentation and, as budding scientists, it is essential that you learn to design and perform experiments successfully. You may be given a short practical test before or after the afternoon contact sessions to make sure that you have prepared for or have understood the practical session and that you understand and can apply the theory that has been covered during the week. Your practical mark will be a composite mark comprised mainly of results from these tests and practical reports. Unless otherwise stated,

practical reports are to be signed by your TA at the end of the practical session before you leave the laboratory. In some instances you may be required to complete sections at home for submission the next week.

**You will get the most out of practical sessions if you prepare well in advance. Do not go into a practical session assuming that everything will be provided without any input on your part. Before you enter the laboratory make sure that you understand the purpose of the practical and the particular skills involved. In some cases you will required to design your own experiments. This must obviously be done before you enter the laboratory.**

**All practical work must be carried out with safety in mind, to minimize the risk of harm to you and to others - SAFETY IS EVERYONE'S RESPONSIBILITY! Make sure that you familiarize yourself with the general laboratory safety procedures before you enter the laboratory (on Sakai).**

**Plagiarism:** The following transcript is based on material in *How to Write Better Essays*, by Bryan Greetham (2001).

Plagiarism is presenting someone else's ideas or arguments as your own, or taking credit for someone else's work. Plagiarism is considered as a form of academic dishonesty; it is illegal and unethical. Don't commit plagiarism intentionally (e.g. if you are pressed for time). Never cut and paste information from electronic sources. The following 'six-point code' could help you decide when and how to cite. It might be useful to write out the points and keep them with you as a quick reference. Basically, you need to provide a reference for:

1. Distinctive ideas (this means you have to cite even when you are rewriting in your own words)
2. Distinctive structure or organizing strategy
3. Information or data from a particular source
4. Verbatim phrase or passage
5. Anything that's not common knowledge
6. Finally, if in doubt, cite!

All formal scientific reports and writing assignments must be submitted to Turnitin®. If your report or assignment displays a **similarity index above 15%, you will be given a zero** for the report or assignment in question.

**Satisfactory Performance:**

You must attend all tutorials and practicals, hand in all practical reports and write all tests: absence is condoned only by a medical certificate or some other satisfactory reason. Medical certificates must be handed to the Mrs Giustinoni (GH001) within 3 working days. Records will be kept of attendances during laboratory periods and spot checks made to ensure that you are present during the tutorials. No satisfactory performance certificate will be issued unless you have given satisfactory performance.

**NB: Students with a course semester mark of less than 40% will not be allowed to present themselves for examination and will be required to deregister from the course.**

## MOLECULAR AND CELL BIOLOGY IIB: CONCEPTS ANTICIPATED LECTURING SCHEDULE 2018 (B diagonal)

**BLOCK 1:** Monday 5 February – Friday 23 March

### Week 1: Mon 5 – Fri 9 Feb (Dr Nikitina)

|         |             |   |
|---------|-------------|---|
| Tue 6   | 8h00-9h45   | Mendelian genetics - review of principles and concepts      |
| Wed 7   | 10h15-12h00 | Tutorial: solving genetic problems                          |
| Thurs 8 | 12h30-13h15 | Tutorial on <i>D. melanogaster</i> dihybrid cross           |
| Fri 9   | 14h00-15h00 | <b><i>Scientific Report writing workshop (Dr Meyer)</i></b> |
|         | 15h00-17:00 | <b><i>D. melanogaster dihybrid cross set up</i></b>         |

### Week 2: Mon 12 – Fri 16 Feb (Dr Nikitina)

|          |             |   |
|----------|-------------|---|
| Tue 13   | 8h00-9h45   | Chromosome structure and changes, Imprinting and X-inactivation |
| Wed 14   | 10h15-12h00 | Sex determination and dosage compensation mechanisms            |
|          |             | Tutorial: chromosomes   |
| Thurs 15 | 12h30-13h15 | Tutorial: chromosomes   |
| Fri 16   | 14h00-15h30 | <b><i>Basic calculations tutorial (Dr Meyer)</i></b>            |
|          | 15h30-17:00 | <b><i>Dihybrid cross 2</i></b>                                  |

### Week 3: Mon 19 – Fri 23 Feb (Dr Nikitina)

|          |             |   |
|----------|-------------|---|
| Tue 20   | 8h00-9h45   | Basic structures & processes in vertebrate embryology               |
| Wed 21   | 10h15-12h00 | Vertebrate embryology: eye development                              |
| Thurs 22 | 12h30-13h15 | Tutorial on vertebrate embryology                                   |
| Fri 23   | 14h00-17h00 | <b><i>3D models of vertebrate development; Dihybrid cross 3</i></b> |

### Week 4: Mon 26 Feb – Fri 2 Mar (Dr Meyer)

|         |             |   |
|---------|-------------|---|
| Tue 27  | 8h00-9h45   | Block Test 1 (Dr Nikitina)                              |
| Wed 28  | 10h15-12h00 | DNA replication - prokaryotes vs eukaryotes             |
| Thurs 1 | 12h30-13h15 | Tutorial: DNA replication                               |
| Fri 2   | 14h00-17h00 | <b><i>Chick embryology slides; Dihybrid cross 4</i></b> |

### Week 5: Mon 5 – Fri 9 Mar (Dr Meyer)

|         |             |   |
|---------|-------------|---|
| Tue 6   | 8h00-9h45   | Genes structure in prokaryotes and eukaryotes; gene mapping                     |
| Wed 7   | 10h15-12:00 | Principles of transcription; Basic regulation                                   |
| Thurs 8 | 12h30-13h45 | Tutorial on Gene expression   |
| Fri 9   | 14:00-17:00 | <b><i>DNA replication (Dr Meyer); Dihybrid cross analysis (Dr Nikitina)</i></b> |

### Week 6: Mon 12 - Fri 16 Mar (Dr Meyer)

|          |             |  |
|----------|-------------|--|
| Tue 13   | 8h00-9h45   | Molecular Biology techniques: nucleic acid extraction & quantification |
| Wed 14   | 10h15-12h00 | Molecular Biology techniques: DNA manipulation                         |
| Thurs 15 | 12h30-13h15 | Tutorial: techniques   |
| Fri 16   | 14h00-17h00 | <b><i>DNA extraction</i></b>   |

### Week 7: Mon 19 – Fri 23 Mar (Dr Meyer)

|          |  |  |
|----------|--|--|
| Tue 20   | 8h00-9h45                                  | Molecular Biology techniques: PCR, sequencing and related applications |
| Wed 21   | <b>Public holiday – University closed.</b> |  |
| Thurs 22 | 12h00-13h15                                | Tutorial: techniques   |

**Study break:** Saturday 24 March - Sunday 01 April.

1. **Assignment:** Write a 3-5 page practical report on the five week *Drosophila melanogaster* dihybrid cross. Refer to the marking guidelines to guide your writing.  
**Due date:** Tuesday 3 April The report must be placed in the box marked: "Concepts" by 12h00 and submitted electronically on Sakai by 18h00.
  2. **Reading for Inquiry project:** To what extent does primary structure define the final structure of a protein? Choose a protein to study in depth to help you answer this question. During the study-break, do some reading about proteins to help you select the protein that your group could use to answer the question. Write a short motivation (1 page) arguing why your protein should be the one of choice.  
**Due date:** A copy of your motivation should be handed in to your TA at the start of the practical on Friday 13 April.
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**BLOCK 2:**      **Monday 3 April – Fri 19 May**

**Week 8: Mon 2 – Fri 6 Apr (Dr Meyer)**

|         |             |  |
|---------|-------------|--|
| Tue 3   | 8h00-9h45   | Genetic manipulation techniques                      |
| Wed 4   | 10h15-12h00 | Translation; from translation to functional proteins |
| Thurs 5 | 12h30-13h15 | Tutorial: Translation                                |
| Fri 6   | 14h00-17h00 | Block test 2 (Dr Meyer)                              |

**Week 9: Mon 9 – Fri 13 Apr (Prof Brenner)**

|          |             |  |
|----------|-------------|--|
| Tue 10   | 8h00-9h45   | Overview of proteins and amino acid structures                   |
| Wed 11   | 10h15-12h00 | Acid base properties of amino acids                              |
| Thurs 12 | 12h30-13h15 | Practical tutorial and Inquiry Project overview and requirements |
| Fri 13   | 14h00-17h00 | <b><i>Chromatography of a mixture of amino acids</i></b>         |

**Week 10: Mon 16 – Fri 20 Apr (Prof Brenner)**

|          |             |   |
|----------|-------------|---|
| Tue 18   | 08h00-9h45  | Acid base properties of amino acids   |
| Wed 19   | 10h15-12h00 | Peptides & peptide bonds, overview of protein structure & structural integrity  |
| Thurs 20 | 12h30-13h15 | Tutorial on practical; standard curves  |
| Fri 21   | 14h00-17h00 | <b><i>Properties of proteins and determination of protein concentration</i></b> |

**Week 11: Mon 23 – Fri 27 Apr (Prof Brenner)**

|          |             |   |
|----------|-------------|---|
| Tue 24   | 8h00-9h45   | Primary and higher order protein structure & evolutionary trees |
| Wed 25   | 10h15-12h00 | Protein structure and function relationships                    |
| Thurs 26 | 12h30-13h15 | Block Test 3 (Prof Brenner)                                     |
| Fri 27   |             | <b><i>Public holiday – University closed.</i></b>               |

**Week 12: Mon 30 Apr – Fri 4 May (Prof. Brenner & Dr Gentle)**

|         |             |   |
|---------|-------------|---|
| Mon 30  |             | Inquiry project submission by email               |
| Tue 1   |             | <b><i>Public holiday – University closed.</i></b> |
| Wed 2   | 10h15-12h00 | Inquiry project presentation and discussion       |
| Thurs 3 | 12h30-13h15 | Introduction to Bioinformatics (Dr Gentle)        |
| Fri 4   | 14h00-17h00 | <b><i>Exploring biological databases</i></b>      |

**Week 13:** **Mon 7 – Fri 11 May (Dr Gentle)**

|          |             |  |
|----------|-------------|--|
| Tue 8    | 08h00-9h45  | Biological databases                             |
| Wed 9    | 10h15-12h00 | Principles of sequence alignment                 |
| Thurs 10 | 12h30-13h15 | Basic Local Alignment Search Tool (BLAST)        |
| Fri 11   | 14h00-17h00 | <b>Basic Local Alignment Search Tool (BLAST)</b> |

**Week 14:** **Mon 14 – Fri 17 May (Dr Gentle)**

|          |             |                               |
|----------|-------------|-------------------------------|
| Tue 15   | 8h00-9h45   | Multiple sequence alignment   |
| Wed 16   | 10h15-12h00 | Protein motifs and signatures |
| Thurs 17 | 12h30-13h15 | Revision                      |

**Examination Session: Monday 28 May - Friday 22 June**

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**Winter vacation: Saturday 23 June – Sunday 15 July**

**Preparation for Block 3 writing assignment:** - The MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay) is a combination of two types of diet; namely, Mediterranean diet and DASH diet. This diet was developed by Martha Clare Morris, a nutritional epidemiologist at Rush University Medical Centre, Chicago, USA. Recent studies in the USA have found evidence of significantly slower mental decline in people who ate at least two servings of vegetables per day and with at least six servings of leafy green vegetables per week. Animal studies have also shown that eating a variety of berries may result in improved memory performance. Additionally, population studies suggest eating a single fish meal per week may be related to the prevention of Alzheimer's disease. Apart from the mental health benefit, the diet also claims to assist in weight loss.

1. Write an **argumentative essay** in which you critically evaluate Martha Clare Morris' diet in terms of the effect on Alzheimer's disease and weight loss by making reference to metabolic pathways in intermediary metabolism. Your argument may be for or against her diet plan. You must supply reasons for your argument and evidence that is based on literature to support the reasons you choose.
2. Design a **digital poster** based on the argument of your essay.

**Due date:** Hand in your essay to your TA at the start of the practical on **Friday 20 July**. The digital poster should be submitted electronically on **Sakai by 12h00 on 20 July**.

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**BLOCK 3:** **Monday 16 July - Friday 31 August**

**WEEK 15:** **Mon 16 – Fri 20 Jul (Dr Fanucchi)**

|          |             |  |
|----------|-------------|--|
| Tue 17   | 8h00-9h45   | Characteristics and specificity of enzymes; coenzymes                                    |
| Wed 18   | 10h15-12h00 | Michaelis-Menten kinetics  |
| Thurs 19 | 12h30-13h15 | Tutorial: enzymes & practical  |
| Fri 20   | 14h00-17h00 | <b>Michaelis constant of the hydrolysis of salicin by <math>\beta</math>-glucosidase</b> |

**Week 16:** **Mon 23– Fri 27 Jul (Dr Fanucchi)**

|        |             |  |
|--------|-------------|--|
| Tue 24 | 8h00-9h45   | Enzyme inhibition  |
| Wed 25 | 10h15-12h00 | Kinetics of bimolecular reactions; Overarching principles of enzyme regulation |

|          |             |  |
|----------|-------------|--|
| Thurs 26 | 12h30-13h15 | Basic concepts of metabolism & the laws of thermodynamics                  |
| Fri 27   | 14h00-17h00 | <b><i>The importance of pH in the functioning of digestive enzymes</i></b> |

**Week 17:** **Mon 30 Jul – Fri 3 Aug (Dr Fanucchi).**

|         |             |  |
|---------|-------------|--|
| Tue 31  | 8h00-9h45   | Overview of metabolism and nutrition; Metabolic maps                 |
| Wed 1   | 10h15-12h00 | Glycolysis   |
| Thurs 2 | 12h30-13h15 | Tutorial: glycolysis & practical                                     |
| Fri 3   | 14h00-17h00 | <b><i>The calorimetric determination of enthalpy and entropy</i></b> |

**Week 18:** **Mon 6 – Fri 10 Aug (Dr Fanucchi)**

|         |             |   |
|---------|-------------|---|
| Tue 7   | 8h00-9h45   | TCA cycle                                       |
| Wed 8   | 10h15-12h00 | TCA cycle; Anaerobic respiration - fermentation |
| Thurs 9 |             | <b>Public holiday – University closed.</b>      |
| Fri 10  | 14h00-17h00 | <b><i>Alcoholic fermentation</i></b>            |

**Week 19:** **Mon 13 – Fri 17 Aug (Dr Fanucchi).**

|          |             |  |
|----------|-------------|--|
| Tue 14   | 8h00-9h45   | Electron transport chain   |
| Wed 15   | 10h15-12h00 | ATP synthesis  |
| Thurs 16 | 12h30-13h15 | Tutorial   |
| Fri 17   | 14h00-17h00 | <b><i>The use of redox to determine thermodynamic parameters</i></b> |

**Week 20:** **Mon 20– Fri 24 Aug (Dr Fanucchi)**

|          |             |   |
|----------|-------------|---|
| Tue 21   | 8h00-9h45   | Glycogen metabolism - example of regulation |
| Wed 22   | 10h15-12h00 | Gluconeogenesis                             |
| Thurs 23 | 12h30-13h15 | Urea cycle                                  |
| Fri 24   | 14h00-17h00 | <b>Tutorial</b>                             |

**Week 21:** **Mon 27 – Fri 31 Aug (Dr Fanucchi)**

|          |             |                            |
|----------|-------------|----------------------------|
| Tue 28   | 8h00-9h45   | $\beta$ -oxidation         |
| Wed 29   | 10h15-12h00 | Lipid biosynthesis         |
| Thurs 30 | 12h30-13h15 | Tutorial                   |
| Fri 31   | 14h00-17h00 | Block Test 4 (Dr Fanucchi) |

**STUDY BREAK: Saturday 1 September - Sunday 9 September.**

*Preparation for Block 4 writing assignment:*

*The development of specialised coherent tissues required i) multicellularity and ii) the presence of a range of extracellular matrix molecules. From an evolutionary perspective, present an argument for whether the relationship between these two elements was sequential or simultaneous. Your argument must be supported by the appropriate evidence and should be presented in not more than 2 typed pages.*

**Due date: Friday 28 September.** A hard copy of your argument must be handed in, in person, to your lecturer.

|                        |   |  |
|------------------------|---|--|
| <b><u>BLOCK 4:</u></b> | <b>Monday 10 September – Tuesday 23 October</b> |  |
| <b><u>Week 22:</u></b> | <b>Mon 10 – Fri 14 Sept (Prof Veale)</b>        |  |
| Tue 11                 | 8h00-9h45                                       | Reducing atmospheres & evolution of first organisms; RNA world & ribozymes       |
| Wed 12                 | 10h15-12h00                                     | DNA world - information & stability; Membrane encapsulation - amphipathism       |
| Thurs 13               | 12h30-13h15                                     | Eukaryotes - information separated from expression                               |
| Fri 14                 | 14h00-17h00                                     | <b><i>The effect of detergents on protein determination in cell extracts</i></b> |
| <b><u>Week 23:</u></b> | <b>Mon 17 – Fri 21 Sept (Prof Veale)</b>        |  |
| Tue 18                 | 8h00-9h45                                       | Membranes, lipid anchoring motifs, homeostasis & membrane transport              |
| Wed 19                 | 10h15-12h00                                     | Membrane transport   |
| Thurs 20               | 12h30-13h15                                     | Membrane transport   |
| Fri 21                 | 14h00-17h00                                     | <b><i>Adhesion of eukaryotic cells</i></b>                                       |
| <b><u>Week 24:</u></b> | <b>Mon 24– Fri 28 Sep (Prof Veale)</b>          |  |
| Tue 25                 | 8h00-9h45                                       | Sorting & distribution of protein; targeting ER, ER signals                      |
| Wed 26                 | 10h15-12h00                                     | ECM: components, proteins, polysaccharides & GAGS                                |
| Thurs 27               | 12h30-13h15                                     | Tutorial: practical  |
| Fri 28                 | 14h00-17h00                                     | <b><i>Effect of ECM on cell adhesion</i></b>                                     |
| <b><u>Week 25:</u></b> | <b>Mon 1 – Fri 5 Oct (Prof Veale)</b>           |  |
| Tue 2                  | 8h00-9h45                                       | ECM: Cadherins, integrins, focal adhesions & hemi-desmosomes                     |
| Wed 3                  | 10h15-12h00                                     | Cytoskeleton microfilaments & intermediate filaments                             |
| Thurs 4                | 12h30-13h15                                     | Microtubules   |
| Fri 5                  | 14h00-17h00                                     | <b><i>Cell proliferation assay MIT</i></b>                                       |
| <b><u>Week 26:</u></b> | <b>Mon 8 – Fri 12 Oct (Prof Veale)</b>          |  |
| Tue 9                  | 8h00-9h45                                       | Dynein and microtubule motors; cytoskeleton abnormalities & disease              |
| Wed 10                 | 10h15-12h00                                     | Cell locomotion, Cell signalling - receptor response pathways                    |
| Thurs 11               | 12h30-13h15                                     | Tutorial: synthesis of information   |
| Fri 12                 | 14h00-17h00                                     | Block Test 5   |
| <b><u>Week 27:</u></b> | <b>Mon 15 – Fri 19 Oct (Prof Veale)</b>         |  |
| Tue 16                 | 8h00-9h45                                       | Pathways triggered by integral protein kinases                                   |
| Wed 17                 | 10h15-12h00                                     | Pathways triggered by separate protein kinases; G proteins; INsP3/DAG etc.       |
| Thurs 18               | 12h30-13h15                                     | Cell communication   |
| Fri 19                 | 14h00-17h00                                     | No practical scheduled   |
| <b><u>Week 28:</u></b> | <b>Mon 22 – Tues 23 Oct (Prof Veale)</b>        |  |
| Tue 23                 | 8h00-9h45                                       | <b><i>Test Feedback (Prof. Veale)</i></b>  |

**Examination session: Friday 31 October - Tuesday 27 November**

**Vacation commences: Wednesday 28 November**

**LIST OF PRACTICALS – MOLECULAR AND CELL BIOLOGY IIB: CONCEPTS (MCBG2032) 2017**

| <b>PRAC</b> | <b>TITLE OF PRACTICAL</b>   | <b>WEEK</b> | <b>DATE</b> | <b>PAGE</b> |
|-------------|---|-------------|-------------|-------------|
| 1           | The Scientific Report; <i>D. melanogaster</i> dihybrid cross Part 1     | 1           | 9 Feb       | 10          |
| 2           | Basic calculations; Dihybrid cross Part 2                               | 2           | 16 Feb      | 17          |
| 3           | 3D models of vertebrate development; Dihybrid cross Part 3              | 3           | 23 Feb      | 23          |
| 4           | Chick embryology slides; Dihybrid cross Part 4                          | 4           | 2 Mar       | 26          |
| 5           | DNA replication; Dihybrid cross Part 5                                  | 5           | 9 Mar       | 30          |
| 6           | DNA extraction  | 6           | 16 Mar      | 39          |
| 7           | Visualisation of nucleic acids; agarose gel electrophoresis             | 7           | 23 Mar      | 43          |
| 8           | Chromatography of a mixture of amino acids                              | 9           | 13 Apr      | 48          |
| 9           | Properties of proteins and determination of protein concentration       | 10          | 21 Apr      | 60          |
| 10          | Exploring biological databases  | 12          | 4 May       | 73          |
| 11          | Basic Local Alignment Search Tool (BLAST)                               | 13          | 11 May      | 76          |
| 12          | Michaelis constant of the hydrolysis of salicin by $\beta$ -glucosidase | 15          | 20 July     | 80          |
| 13          | The importance of pH in the functioning of digestive enzymes            | 16          | 27 Jul      | 89          |
| 14          | The calorimetric determination of enthalpy and entropy                  | 17          | 3 Aug       | 97          |
| 15          | Alcoholic fermentation  | 18          | 10 Aug      | 102         |
| 16          | The use of redox to determine thermodynamic parameters                  | 19          | 17 Aug      | 110         |
| 17          | The effect of detergents on protein determination in cell extracts      | 22          | 14 Sep      | 114         |
| 18          | Adhesion of eukaryotic cells  | 23          | 21 Sep      | 120         |
| 19          | Effect of ECM on cell adhesion  | 24          | 28 Sep      | 124         |
| 20          | Cell proliferation assay MIT  | 25          | 5 Oct       | 128         |

## **PRACTICAL 1: THE SCIENTIFIC REPORT & DROSOPHILA MELANOGLASTER DIHYBRID CROSS PART 1**

### **THE SCIENTIFIC REPORT**

#### **HOW TO WRITE A REPORT**

As a scientist, the main way in which we communicate our work is through scientific reports which are published as articles in scientific journals. During this session, you will learn how to write a report, what the key components are and what is needed for each section.

#### **STRUCTURAL GUIDELINES FOR THE PREPARATION AND FORMAT OF A PRACTICAL REPORT**

The following was adapted from:

1. Dr Barbara S Polla, former head, Laboratoire de Physiologie Respiratoire, Paris University V, Paris, France.
2. Prof Chris Bornman, former editor of the international journal, *Physiologia Plantarum*.
3. Davis M (1997) Scientific papers and presentations. Academic Press, New York.

#### **General**

- Write your text in Standard English (American or British). Refer to the Oxford English dictionary when you are struggling ([www.oup.com/elt/oald/](http://www.oup.com/elt/oald/)).
- Always use clear, simple and straight forward English
- Do not use *Italics* for words like *in vivo*, *et al.*, *per se*.
- Use *Italics* for genus and species. E.g. *Drosophila melanogaster*
- Use decimal points (not commas)
- Use a space for thousands (10 000 and above)

The scientific report has a very set order:

1. Title
2. Abstract
3. Introduction
4. Materials and Methods\*
5. Results
6. Discussion
7. Conclusion\*\*
8. References

\*Some journals will place the materials and methods after the conclusion.

\*\*Some journals allow the final paragraph of the discussion to be the concluding paragraph.

Despite the specific order in which the scientific report is presented, the order in which you need to work on the document is quite different:

1. References
2. Methods
3. Results
4. Introduction/Discussion and conclusion
5. Discussion and conclusion/ Introduction
6. Abstract
7. Title

## Title [7]

Should be concise and informative.

1. Start with a keyword.
2. The title should tell you something about the result or conclusion.
3. Avoid abbreviations and formulae where possible.

**Abstract [6]: A paragraph of 5 – 10 lines long and can be centered and/or in *italics*. Your abstract is essentially a short summary of your entire experiment.**

1. Must contain the following elements in the stipulated sequence:
  - a. **Background** – provide a short description to contextualise the work (1-2 sentence/s).
  - b. Formulate a **hypothesis or a question** (1 sentence).

A hypothesis is a statement on the assumption you are testing. Sometimes the study/experiment leans itself more towards answering a **question** and not testing a **hypothesis**. You can use a question or a hypothesis to motivate the study.

- c. State the **Objective(s)** (1 sentence).
- d. Briefly describe the major **methods** used (1/2 sentences).
- e. Summarise the main **Result(s)** (1/2 sentences).
- f. In the **Conclusion**, you need to answer the question or accept/reject the hypothesis and comment on the new knowledge gained during the study (1/2 sentences).
2. It helps to use the words in **bold** above as headings in your abstract.
3. The abstract should never contain things that is not elsewhere in your report.
4. No need to reference (you reference in the report introduction and discussion, and sometimes in the methods).
5. Avoid uncommon abbreviations and acronyms.

## Introduction [4/5]: A paragraph of about 1 page long.

This is the place to introduce your experiment/work/ study. You need to state the objectives of the work and provide enough background to convince the reader why this work is so important. You should refer to the literature while:

1. Introducing the concept/topic and specifically the problem you are addressing.
2. Defining / explaining all specialized terms, concepts and theories necessary to follow the rest of your report.
3. Orientating the reader to the relevant literature. Remember to refer to references which you list at the end of your report.
4. Stating the problem, question or hypothesis that is being investigated/ tested. Say **WHY** was this work was done and **WHAT** it was that you hoped to find.

## Materials and Methods [2]

**Nomenclature and units.** Follow internationally accepted rules and conventions: use the international system of units (SI).

1. This methods should explain what was done in the lab, what reagents were used, what equipment was used and all conditions employed. You should provide sufficient detail to allow the work to be reproduced.
2. This section should NOT be bulleted. It should be written in paragraph format, in third person and past tense. Remember: One method = One paragraph. So if you can't make a sub-heading, then don't break the paragraph.

3. Manufacturers of reagents should be included, i.e. the company, city and state (in case of the USA) or country. If in the USA, USA is never mentioned and the state is treated as a country.
4. Write the "Materials and methods" in the same order as "Results" for which they have been used.

## Results [3]

1. Results should be clear and concise.
2. Results are presented in tables or graphs/figures with a paragraph describing what is shown in them.
3. Each figure should have a **figure legend** below the figure. Each table should have a **heading** above the table. Figure legends and table headings should be descriptive and numbered in the order in which they appear in the report.
4. The descriptive paragraphs between your figures and tables should only say what is shown and **NOT WHAT IT MEANS** – what is meant is meant for the discussion.

## Discussion [4/5]

A 'Discussion' is basically the interpretation of your results, and showing its relationships with other research in the scientific literature (books or papers in journals in the library). The Discussion should be well organized and should discuss all questions asked or hypotheses tested, in a logical and straightforward manner.

**This is the most important part of your report and should be at least 1 page long.** This section explains WHY you got the results you got. In this section you should answer the question WHY? Why did you do each step of the experiment (e.g. why did you use the reagents you used)? What results (according to literature, cited) were you supposed to get? Why were you supposed to get them? How did your results compare? Why were your results different? What errors could have been made and why?

## Conclusion

1. This is the "So what" paragraph.
2. Did you achieve the aim of your experiment? What can you conclude?
3. Answer your study objectives/hypothesis/question.
4. Say what the impact is of your finding on the pool of scientific information available in the literature in this field. Indicate how your results have contributed to a better understanding of the problem.

## References [1]

1. **Citations in the text.** Please ensure that every reference cited in the text is present in the reference list (and vice versa).
2. The preference for referencing is: use the surname and year of publication in the text, e.g. xxx (Veale and Weiss, 2005) and list references in alphabetical order under 'References' at the end of your report. For one author use only his/her surname (Nikitina, 2006) for two, use for example (Nikitina and Veale, 2006), for more than 2, use for example (Nikitina et al., 2006).
3. The format of references (Author surnames and initials/names with/without full stops, order of year, title, journal volume, page number etc.) differs, depending on the journal or your lecturer's preference. The following is a general guideline:

### For a book:

Authors (just surname and no full stops after initials e.g. van Heerden H (date) *Title*, Edition, Publisher, Place of publication, page numbers.

Strunk Jr., W., & White, E. B. (1979). *The elements of style* (3rd ed.). New York: Macmillan (Chapter 4).

**Reference to a chapter in an edited book:**

Mettam, G. R., & Adams, L. B. (1994). How to prepare an electronic version of your article. In B. S. Jones, & R. Z. Smith (Eds.), *Introduction to the electronic age* (pp. 281-304). New York: E-Publishing Inc.

**For a journal article:**

Authors, e.g. James J, Snyman M (date) *Title of article*. Abbreviated Journal name (as listed on PubMed), volume number: first page number – last page number.

Kim, Ch.-H. (2004). Increased expression of *N*-acetylglucosaminyltransferase-V in human hepatoma cells by retinoic acid and 1 $\alpha$ ,25-dihydroxyvitamin D3. *The International Journal of Biochemistry and Cell Biology*, 36, 2307-2319.

**For the worldwide web:**

Authors, (date updated) *Title of homepage*, URL / web address, date accessed. If all this information is not available you may not use this information.

**Citing and listing of Web references.** As a minimum, the full URL should be given. Any further information, if known (Author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

## DROSOPHILA MELANOGASTER DIHYBRID CROSS

This dihybrid cross will run from weeks 1-5, concurrently with other practicals as shown in the table of practicals.

The aim of the dihybrid cross is to introduce the basic concepts of **transmission genetics**. This branch of genetics deals with the way genes are transmitted between generations. Contributions to this field have been obtained from breeding experiments and the analysis of their results. In this practical you will learn how this is done. First, some background.

### THE DIHYBRID CROSS

A **dihybrid cross** is a cross involving two traits. The purpose of this cross is:

- To demonstrate **recessive alleles**. For example, both dumpy and sepia are recessive alleles and the heterozygotes for the mutations are indistinguishable from the homozygous wild type form.
- To demonstrate **independent assortment**. Independent assortment is the segregation of a pair of alleles independently of the segregation of another pair of alleles. This can be seen in the F<sub>2</sub> generation because the F<sub>1</sub> individuals are all double heterozygotes.

The first step to setting up a breeding experiment is to choose an organism that meets the following criteria:

- Has a relatively short generation time
- Breeds well
- Can survive laboratory conditions
- Has observable phenotypic genetic mutations

There are a number of organisms that fit these criteria, namely: the bacterium (*Escherichia coli*), the fruit fly (*Drosophila melanogaster*), yeast (*Saccharomyces cerevisiae*), the mouse (*Mus musculus*), the round worm (*Caenorhabditis elegans*), and the mustard plant (*Arabidopsis thaliana*).

### THE FLY AND TERMINOLOGY

The fruit fly, *Drosophila melanogaster*, is ideally suited for the demonstration of basic genetic principles. It has a small genome size and a haploid chromosome number of four. Fruit flies have a generation time of 10 days, and produce hundreds of offspring at a time. There are a large number of visible genetic markers that have been found over the many years that geneticists have been studying fruit flies.

To begin to understand transmission genetics, one needs to know the basic terminology and symbols used. In genetics, a characteristic (or trait), is often controlled by a single gene. A mutation in that gene produces a new allele, or alternative version of that gene. Alleles differ slightly from each other in DNA sequence, and have the same basic function of the gene. The mutations we will be dealing with are alleles that have lost all activity of the gene and result in a big phenotypic difference from the flies that do not have the mutation, also known as wild type flies. Most of the mutations are expressed recessively, meaning that the fly must have two copies of the mutation to display the mutant phenotype.

In the naming of genes, the mutant phenotype is used. For example, a fly with vestigial wings (very small wings) is denoted *vg*. To indicate the wild type of that gene, we use the same symbol with a plus sign (+) to distinguish it from the mutant. Therefore, *vg* is the symbol for the mutant allele and *vg<sup>+</sup>* or *vg+* is the wild type alternative. Often the wild type is abbreviated further and indicated with only the +.

Diploid genotypes are written with a slash separating the symbols for alleles: *vg/vg* or *vg+/vg+* or *vg+/vg*. As a rule, only alleles of interest are written in the genotype, and if any gene is not written, it is assumed

to be homozygous for the wild type. For crosses involving more than one gene, a semi-colon (;) is used to separate the genes. So a double heterozygote fly for vestigial (*vg*) and ebony (*e*) would be written as *vg<sup>+</sup>/vg; e<sup>+</sup>/e*, the wild type always being written first in a heterozygote. In the case of a cross, the genotype of the female parent is written first, followed by a X and then the genotype of the male parent.

## MAKING CROSSES

The first requirement for a cross is a batch of virgin females. Females may mate with different males and can store and utilise sperm cells from one mating for a large part of their reproductive lives. Therefore, only unmated or virgin females are used for making controlled crosses.

To collect virgins, bottles are cleared of all adult flies and newly hatched flies are collected over the next 7 -8 hours. Females less than 8 hours old normally do not mate and are almost always virgin. Bottles are cleared of all adult flies (for example at 8 am) and not longer than 8 hours later (i.e. before 4 pm) the newly hatched flies are collected: females from this batch can be presumed to be virgins. Alternatively, virgins can be collected by sexing larvae or pupae and then allowing the females to hatch in a separate batch. Males, of course, need not be virgin and can be any age.

To make a cross, three to five virgins from one strain are mated in a culture bottle with a corresponding number of males from the other strain (actually, one male can inseminate many females). These flies are labelled the P<sub>1</sub> (first parental) generation and details of their phenotypes are noted in the lab book and on the bottle. The bottles are incubated to provide the optimal temperature for mating of flies and development of the embryos. When larvae appear (after 4-5 days), the parents are discarded so as not to confuse them with the offspring. The offspring are called the F<sub>1</sub> (first filial) generation. Roughly 200-300 offspring can be bred within about 2 weeks of making the cross. Thereafter the F<sub>1</sub> flies are crossed, making them the P<sub>2</sub> generation now. In this case, it is unnecessary to isolate virgins from the F<sub>1</sub>. This would only be necessary for a back cross. Again the flies must be discarded before the F<sub>2</sub> start hatching.

You will be carrying out one of the following crosses with flies that display the following mutant phenotypes:

- |  |   |   |
|--|---|---|
| 1. Cinnabar brown female<br><i>cn/cn; bw/bw</i>  | X | wild type male<br><i>cn<sup>+</sup>/cn<sup>+</sup>; bw<sup>+</sup>/bw<sup>+</sup></i> |
| 2. Wild type female<br><i>dp<sup>+</sup>/dp<sup>+</sup>; se<sup>+</sup>/se<sup>+</sup></i> | X | dumpy sepia male<br><i>dp/dp; se/se</i>   |

It must be noted that the same results in the F<sub>1</sub> and F<sub>2</sub> generations are achieved in both crosses:

- A. Cinnabar brown X wild type

The cinnabar and brown mutations both affect eye colour, where cinnabar flies have red eyes that are distinctly different from the brick-red colour of wild type flies, and blown flies have brown eyes. The gametes from the P<sub>1</sub> will be *cn*; *bw* and *cn<sup>+</sup>*; *bw<sup>+</sup>*, resulting in all F<sub>1</sub> individuals having the genotype *cn<sup>+</sup>/cn; bw<sup>+</sup>/bw*. In flies that are homozygous for both mutations (*cn/cn; bw/bw*) the mutation genes interact to give white eyes.

- B. Dumpy sepia X wild type

The dumpy mutation produces flies with short wings, shorter or equal length of the body, and the scarlet mutation produces brown eyes that darken to black with age. The gametes from the

$P_1$  will be  $dp; se$  and  $dp^+; se^+$ , resulting in  $F_1$  flies that are  $dp^+/dp; se^+/se$ . Flies that are homozygous for both mutations ( $dp/dp; se/se$ ) will have short wings and black eyes.

### PRACTICAL COMPONENT

1. You will be provided with 2 bottles. The small bottle contains male flies; the large culture bottle contains female virgin flies with fresh culture medium to support the growth of the flies and their offspring. The culture medium is made up of agar, mealie meal, syrup, molasses and water. Once set, the surface is seeded with live yeast.
2. Record the number on the bottle containing the female flies. This is important as you will be carrying out a particular cross (from the list above) and you will need to find your bottle in subsequent weeks. Transfer all the necessary information onto the label that you will stick onto your culture bottle.

Label your cross bottle as follows:

|                                 |          |
|---------------------------------|----------|
| Prac Day                        | Surname  |
|                                 | Seat No. |
| Female genotype X male genotype |          |
| Cross No.                       | Date     |

3. Transfer the male flies into the culture bottle containing the female flies. This is done by lightly tapping the bottle with the female flies, so that they fly away from the neck of the bottle, and then quickly inserting the male flies. This should be carried out quickly and gently so that the flies are not damaged or escape.
4. Return your bottle (with label) to the baskets for incubation.
5. You will need to be able to identify the features of the mutations of your cross. Use the bottles on the side benches for inspection, not the flies from your experiment. Etherize the flies and place them on a white tile under the dissecting microscope. Learn how to identify the different phenotypes (wild type, both single mutants, double mutants) and be able to determine the sex of male and female flies. Record the relevant information in your practical report and answer any questions.

**Every week you will be asked to record your observations and your explanations for these. This exercise will assist you in completing the final report and will require you to link theory to your observations. It will also provide an opportunity to note any questions you have about the practical or theoretical aspects of the dihybrid cross. The better these recordings, the easier the practical report will be.**

| What I observed | What I thought |
|-----------------|----------------|
|                 |                |

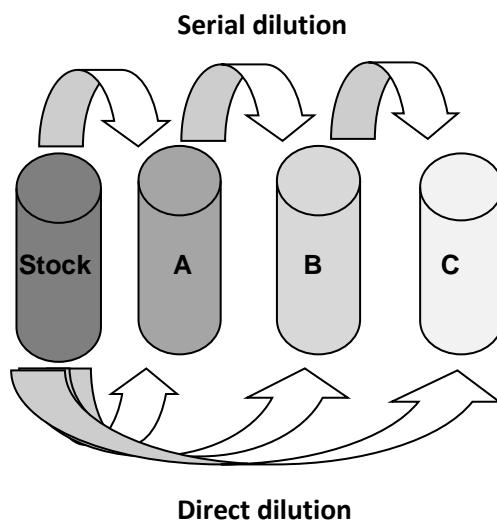
## PRACTICAL 2: BASIC CALCULATIONS & THE DIHYBRID CROSS PART 2

### BASIC CALCULATIONS

Regardless of your majors in life science, mastering basic biochemical calculations is vital for your success in the lab. In this tutorial, you will practice basic calculations related to dilutions and making solutions.

#### Dilutions

Dilution is the action of making a solution more dilute (less concentrated). When diluting a solution in the lab, this can be done as a **direct dilution** or a **serial dilution**:



Dilutions can be expressed as:

|                |          |                         |
|----------------|----------|-------------------------|
| <b>10X</b>     | <b>:</b> | <b>1part + 9 parts</b>  |
| <b>10 fold</b> | <b>:</b> | <b>1part + 9 parts</b>  |
| <b>10Times</b> | <b>:</b> | <b>1part + 9 parts</b>  |
| <b>1:10</b>    | <b>:</b> | <b>1part + 10 parts</b> |

Example: You need 10 ml of 10X diluted buffer A.

10X is 1 part + 9 parts

So, take 1 part buffer A and 9 parts dH<sub>2</sub>O

It is ∴ true to say that buffer A was 10X more concentrated than what you needed.

∴ take 1 ml of buffer A and add 9 ml of dH<sub>2</sub>O to make 10 ml of 10X diluted buffer A

$$C_1V_1=C_2V_2$$

This equation is useful when:

- You want to determine how much of the concentrated solution you need to take to make a set volume of diluted solution (solve for  $V_1$ ).
- You have already diluted a solution by adding water to the solution and you now want to know what the concentration of the diluted solution is (solve for  $C_2$ ).

Example: You have an HCl stock solution with a concentration of 12.2 M. How would you make up 100 ml of 0.1 M HCl?

$$C_1V_1=C_2V_2$$

$$(12.2 \text{ M})(V_1) = (0.1 \text{ M})(100 \text{ ml})$$

$$V_1 = 10/12.2$$

$$V_1 = 0.82 \text{ ml}$$

∴ Take 820 µl of HCl stock solution and add it to 99.18 ml of dH<sub>2</sub>O.

Concentration and volume can be in any unit, as long as it is the **same unit on both sides of the = !!**

## Concentration

Molar concentration (C) refers to the strength of a solution and is the amount of dissolved substance in a given volume (V) of solvent. It is usually expressed as the number of moles (n) per litre.

$$C = n/V$$

Where n = mass (m) / molecular weight (Mr)

If  $C = n/V$  and  $n = m/Mr$ ,

then

$$\boxed{m = C \times V \times Mr}$$

For this equation to work, you can only use **SI units**: grams (g) for mass, molar (M) for concentration, litre (L) for volume and gram/mol (g/mol) for molecular weight.

You can use this equation to:

- Calculate the concentration of a solution you made by dissolving a set amount of salt in dH<sub>2</sub>O (solve for C).
- Calculate how much salt you need to weigh out to make a solution of set concentration (solve for m).

Example: How will you make 200 ml of a 0.3 M NaCl solution (Mr = 58.44 g/mol)?

$$m = C \times V \times Mr$$

$$m = (0.3 \text{ M})(0.2 \text{ L})(58.44 \text{ g/mol})$$

$$m = 3.51 \text{ g}$$

Weigh out 3.51 g of NaCl, dissolve in some dH<sub>2</sub>O and top up to 200 ml with dH<sub>2</sub>O.

But the concentration of a solution can also be expressed as a %.

$$\% = \text{_____ grams / ml in a total volume of 100 ml}$$

Example: How will you make 40 ml of a 7% (w/v) NaCl solution?

% = x grams in 100ml  
∴ 7% is 7 g in 100 ml

w/v stands for weight per volume

v/v stands for volume to volume

That means you need 0.07 g of NaCl for every 1 ml of solution  
Multiply that by the 40 ml that you need and you get 2.8 g in 40 ml.

So, weigh out 2.8 g of NaCl, dissolve in some dH<sub>2</sub>O and top up to 40 ml with dH<sub>2</sub>O.

Example: How will you make 200 ml of a 95% (v/v) ethanol solution?

% = x grams in 100ml  
∴ 95% is 95 ml in 100 ml

That means you need 0.95 ml of ethanol for every 1 ml of solution  
Multiply that by the 200 ml that you need and you get 190 ml in 200 ml.

So, measure out 190 ml of ethanol and add it to 10 ml dH<sub>2</sub>O.

### Practice

1. Describe how you would make a direct dilution of Z to obtain the following:
  - a) 10X
  - b) 100X
  - c) 1000X dilution of Z
2. Describe how you would make a serial dilution of Z to obtain the following:
  - a) 10X
  - b) 100X
  - c) 1000X dilution of Z
3. How is 50 ml of 20 mM NaOH prepared? (Mr = 40)
4. 0.4 mole HCl is dissolved and diluted to 50cm<sup>3</sup>. Calculate the HCl concentration
5. If 25 g NaCl is dissolved into a final volume of 500ml what is the % (w/v) of the NaCl solution?
6. Express 2.5 M NaCl (Mr = 58.44) as a %
7. What is the molar concentration of 10% NaCl solution?

1. Using the values given, calculate the molar masses of the following:

$$C = 12,01$$

$$H = 1,0$$

$$K = 39,1$$

$$S = 32,1$$

$$N = 14,0$$

$$O = 16$$

$$F=19$$

- |    |             |     |
|----|-------------|-----|
| a) | $C_4H_{10}$ | (2) |
| b) | $NH_4OH$    | (2) |
| c) | $K_2SO_4$   | (2) |
| d) | $NH_3$      | (2) |
| e) | $N_2F_2$    | (2) |
| f) | $N_2O_5$    | (2) |

2. Convert the following (8)

- |    |                                 |
|----|---------------------------------|
| a) | 25 nmole = ___?___ $\mu$ moles  |
| b) | 22.2 moles = ___?___ $\mu$ mole |
| c) | 30 $\mu$ mole = ___?___ moles   |
| d) | 10 L = ___?___ $\mu$ l          |
| e) | 0.05 ml = ___?___ $\mu$ l       |
| f) | 250 $\mu$ l = ___?___ ml        |
| g) | 0.02 M = ___?___ mM             |
| h) | 350 ml = ___?___ L              |

If 1 mM =  $1 \times 10^{-3}$  M =  $1 \times 10^{-3}$  mol/L =  $1 \times 10^{-6}$  mol/ml (3)

- |    |   |
|----|---|
| i) | 10 $\mu$ M = ___?___ M = ___?___ mol/L = ___?___ mol/ml |
| j) | 0.25 nM = ___?___ M = ___?___ mol/L = ___?___ mol/ml    |
| k) | 0.95 pM = ___?___ M = ___?___ mol/L = ___?___ mol/ml    |

3. Describe how you would make a dilution of concentrated methanol to obtain 75 ml with the following dilutions: (8)

- |    |                          |
|----|--------------------------|
| a) | 10 X                     |
| b) | 1:10                     |
| c) | 1 in 10 dilution         |
| d) | 10 x or 10-fold dilution |
| e) | 100 X                    |
| f) | 1000 X                   |
| g) | 20 X                     |
| h) | 30 X                     |

4. Calculate how many grams of solid NaOH ( $Mr = 40$  g/mol) are required to prepare 500 ml of a 15% solution? Describe how you would prepare this solution. (3)

5. Describe how you would prepare 20 ml of a 50 mM solution of NaOH. Show all the steps in the calculation. (4)
6. Show and explain how you would prepare 250 ml of 30 mM H<sub>2</sub>SO<sub>4</sub> from a 2 M stock solution? (3)
7. 0.25 mole Hydrochloric acid is dissolved and diluted to 5 dm<sup>3</sup>. Calculate the HCl concentration. (2)
8. Using Table 1 calculate the concentration for tube 3 and 6 if the stock concentration was 18 mg/ml. (4)
9. What is the molar concentration of 35% NaCl solution? (3)

## **DIHYBRID CROSS PART 2**

The adult flies in your culture bottle are the males and females you put there last week. By now they should have mated and laid eggs, and the offspring will still be in the larval stage. The offspring are your F<sub>1</sub> generation. You must now discard the parental generation so they do not interfere with the analysis of the F<sub>1</sub> generation next week.

### **PRACTICAL COMPONENT**

1. Moisten the lid of your etherising jar with ether.
2. Shake all flies from your culture bottle into your etherising jar. Make certain that you discard ALL adult flies.
3. Record the date diagonally across the label on the culture bottle.
4. Replace the lid and return the culture bottle to the basket for incubation.

| What I observed | What I thought |
|-----------------|----------------|
|                 |                |

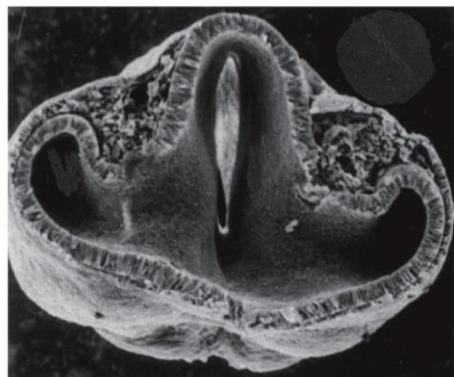
## **PRACTICAL 3: 3D MODELS OF VERTABRATE DEVELOPMENT & DIHYBRID CROSS PART 3**

### **3D MODELS OF VERTABRATE DEVELOPMENT**

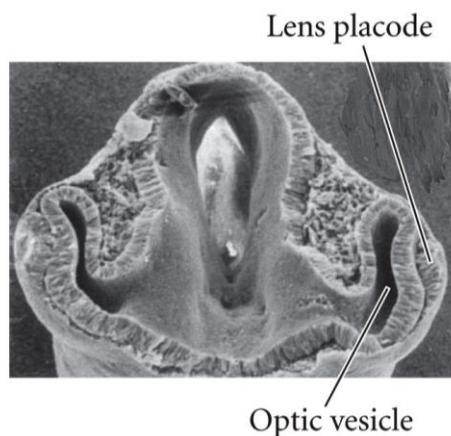
You will be provided with three pieces of different colour plasticine. Use them to build a series of 3-D models to illustrate the process of eye development in vertebrates. Use blue plasticine for non-neural ectoderm derived tissues, yellow for neural ectoderm derived tissues, and red for neural crest derived tissues. Use the cross-sections below as a guide.

After you complete the models, show them to your TAs for critical evaluation and comments. A mark will be assigned for your group's model.

(A) 4-mm embryo



(B) 4.5-mm embryo

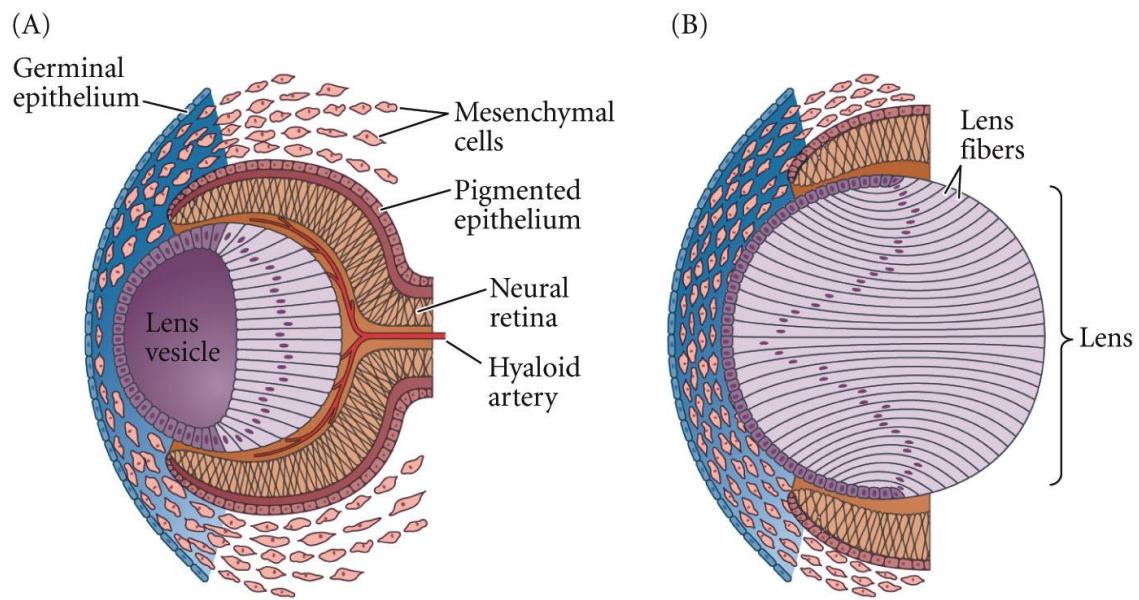


(C) 5-mm embryo



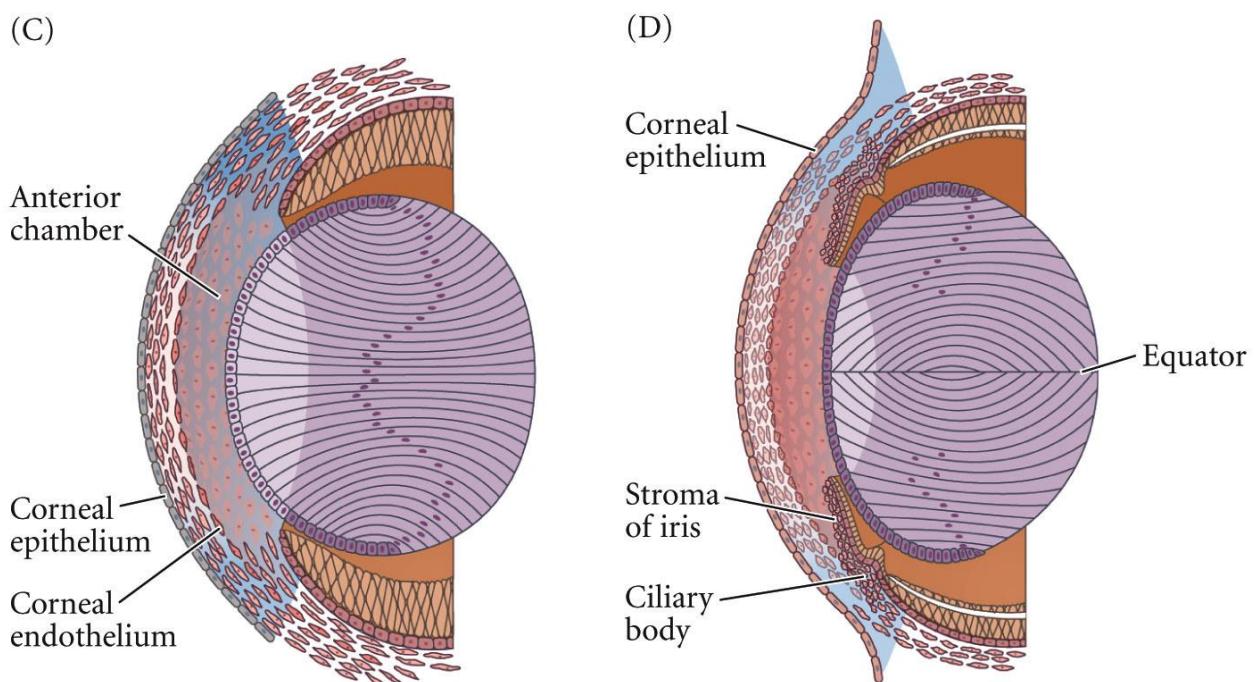
(D) 7-mm embryo





**DEVELOPMENTAL BIOLOGY, 9e, Figure 9.36 (Part 1)**

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**DEVELOPMENTAL BIOLOGY, 9e, Figure 9.36 (Part 2)**

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### **DIHYBRID CROSS PART 3**

By now the larvae of the P<sub>1</sub> should have hatched. In this practical, you are going to select four males and four females from your F<sub>1</sub> generation. These flies should be heterozygous as the P<sub>1</sub> had different homozygous genotypes, and will form the P<sub>2</sub> generation of your cross.

#### **PRACTICAL COMPONENT**

1. **Lightly** etherise the F<sub>1</sub> flies in your bottle.
2. Pick out four males and four females and place them in the vial. When you are sure all the flies are still alive, transfer them to the fresh culture bottle provided. Be careful not to drown your flies in the medium.
3. Label the fresh culture bottle correctly with your details (as in the first week of this practical) and return the bottle to the basket for incubation.
4. Examine a few of the F<sub>1</sub> flies and record your observations and conclusions in the table for your practical report.
5. Discard the remaining F<sub>1</sub> flies after ensuring you have viable flies for the P<sub>2</sub> generation.

| What I observed | What I thought |
|-----------------|----------------|
|                 |                |

## **PRACTICAL 4: CHICK EMBRYOLOGY & DIHYBRID CROSS PART 4**

### ***CHICK EMBRYOLOGY***

#### **Objectives:**

At the end of this practical:

- Students should be able to identify and label main morphological structures (somites, neural tube, notochord, primitive streak etc) on sections through chick embryos of selected stages.
- By referring to the information pack supplied students should be able to identify at which level of an embryo a section was taken.

#### **Material required:**

(Per group of students):

- 1 microscope
- 1 slide of chicken embryo development (24 hr incubation, serial sections)

#### **EXPERIMENTAL PROCEDURE:**

- 1) You are supplied with a slide, containing serial head-to-tail sections through a 24hr (HH8) chicken embryo. Three of the sections are marked out for you to view. Referring to the information pack, draw and label the 3 sections in your report.

PLEASE BE VERY CAREFUL WHEN ADJUSTING THE FOCUS OF THE MICROSCOPE, BECAUSE IF YOU MOVE THE OBJECTIVE TOO LOW AND IT HITS THE SLIDE, NOT ONLY WILL THAT DESTROY AN EXPENSIVE SLIDE, IT WILL ALSO DAMAGE THE OBJECTIVE OF A VERY EXPENSIVE MICROSCOPE!!! Please ask for help if you are not sure.

- 2) Answer all questions in the practical report template below. Submit the report to your TAs at the end of the session for marking.

Name \_\_\_\_\_ Student number \_\_\_\_\_

**REPORT**

**(29 marks)**

(PLEASE SUBMIT THIS TO YOUR TA AT THE END OF THE PRACTICAL).

**Section 1: (8 marks)**

In the space below, draw a labelled diagram of the section No 1 (24hr embryo).

**Section 2: (10 marks)**

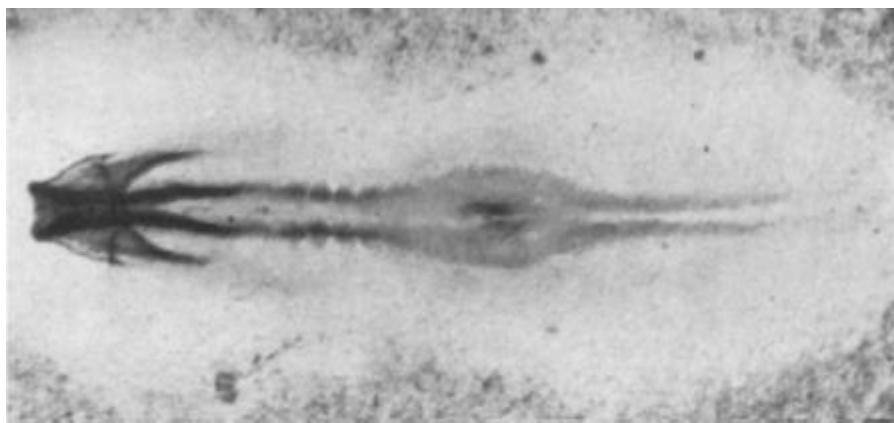
In the space below, draw a labelled diagram of the section No 2 (24hr embryo). Where appropriate, indicate the germ layer of origin.

Name \_\_\_\_\_ Student number \_\_\_\_\_

**Section 3: (8 marks)**

In the space below, draw a labelled diagram of the section No 3 (24hr embryo). Where appropriate, indicate the germ layer of origin.

On the photograph of the whole-mount embryo shown below, draw lines to indicate the levels of the three sections above (3 marks).



#### **DIHYBRID CROSS PART 4**

The adult flies in your culture bottle are the males and females you put in there last week. By now they should have mated and laid eggs, and the offspring will still be in the larval stage. These offspring are the F<sub>2</sub> generation. You must now discard the parental generation, so that they do not interfere with the final analysis of your cross next week.

#### **PRACTICAL COMPONENT**

1. Moisten the lid of your etherising jar with ether.
2. Shake all flies from your culture bottle into your etherising jar. Make certain that you discard ALL adult flies.
3. Record the date diagonally across the label on the culture bottle.
4. Replace the lid and return the culture bottle to the basket for incubation.

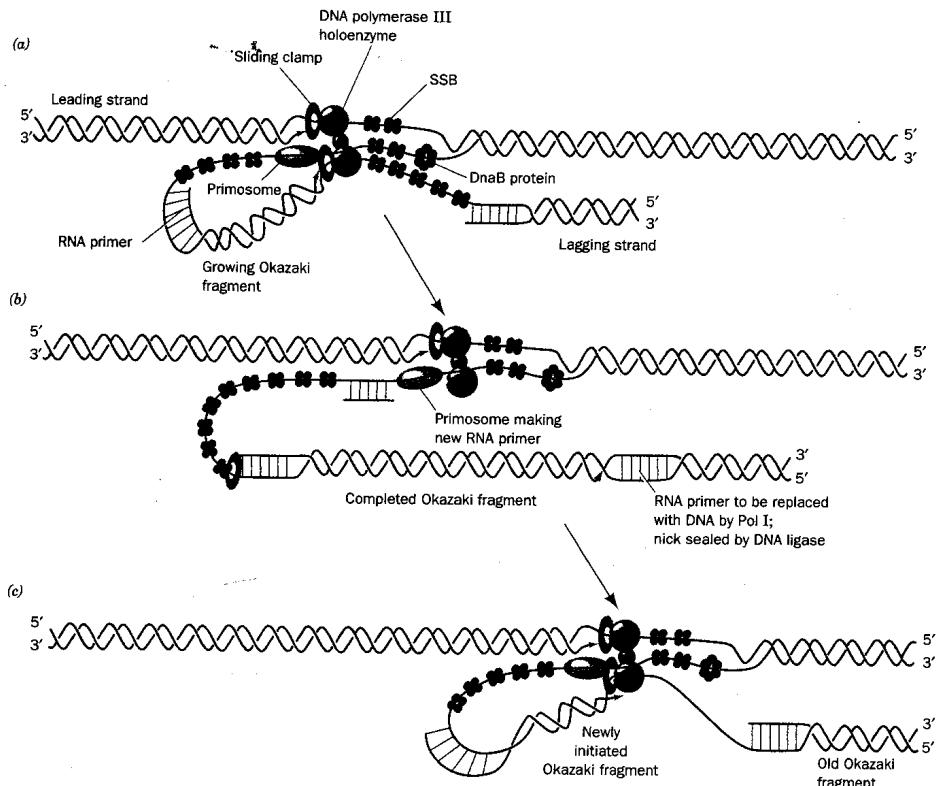
| What I observed | What I thought |
|-----------------|----------------|
|                 |                |

## PRACTICAL 5: DNA REPLICATION & DIHYBRID CROSS PART 5

### **DNA REPLICATION**

#### **Objectives:**

1. By the end of this practical students should understand the essential features of DNA replication in prokaryotes.
2. Students will obtain practice in writing simple instructions and in following written instructions.
3. Students will use critical thinking to evaluate the kit or game of another group.



### The Replication of *E. coli* DNA

*(Taken from Voet and Voet 3e. 2004; p1155)*

- (a) The *E. coli* DNA replisome, which contains two DNA polymerase III holoenzyme complexes, synthesizes both the leading and the lagging strands. The lagging strand template must loop around to permit the holoenzyme to extend the primosome-primed lagging strand.
- (b) The holoenzyme releases the lagging strand template when it encounters the previously synthesized Okazaki fragment. This possibly signals the primosome to initiate the synthesis of lagging strand RNA primer.
- (c) The holoenzyme rebinds the lagging strand template and extends the RNA primer to form a new Okazaki fragment. (In this model, leading strand synthesis is always ahead of lagging strand synthesis).

## **PREPARATION**

Look at the diagram above and read (and make sure you understand) the descriptions of prokaryote DNA replication in your textbook. Consult your partner/s and collect the materials you'll need for the practical session.

## **PRACTICAL COMPONENT**

1. **Design and make** a kit or game which could be used in a first year Biology practical to enable students to understand the process of DNA replication in bacteria. Your kit or game should address issues such as unwinding the DNA double helix, supercoiling, leading and lagging strand replication, and the action of the various enzymes and proteins involved in prokaryotic DNA replication. Your kit or game should include instructions. Write these! (They may include diagrams). You will be given a maximum of 1.5 hours to complete this part of the exercise.
2. Swap with another group on your practical bench and test their kit (or game) by following the instructions given.
3. **Write a critical evaluation of the other kit or game and the instructions.** Your evaluation should reflect on which aspects you think will be the most valuable as a teaching tool and whether you think the game could have been improved. **Your kit (or game), its instructions, and your evaluation of the other kit must be handed to your TA before you leave the laboratory.**

**See the marking criteria which follow. These should guide you in your kit or game design and in writing your critical evaluation.**

**DNA Replication marking guidelines**

|  | Strongly<br>agree<br>4 | Agree<br>3 | Neutral<br>2 | Disagree<br>1 | Strongly<br>disagree<br>0 |
|--|------------------------|------------|--------------|---------------|---------------------------|
| It was obvious that the pair understands the process of replication      |                        |            |              |               |                           |
| The kit or game makes the process of replication clear                   |                        |            |              |               |                           |
| The process of replication as introduced in the kit or game was accurate |                        |            |              |               |                           |
| The kit or game would make a good teaching tool                          |                        |            |              |               |                           |
| The rules were clearly written and easy to follow                        |                        |            |              |               |                           |
| The kit or game would be fun to use or do                                |                        |            |              |               |                           |
| Innovation /creativity rating  | 6                      | 5          | 4            | 3             | 2                         |
| TOTAL / 30   |                        |            |              |               |                           |

**DNA REPLICATION REPORT:**    Student Name: \_\_\_\_\_

**Instructions for your kit or game:**

## Critical evaluation of the kit (or game) made by another group

**Marking guidelines for the critical evaluation of another kit or game**

|  | Strongly<br>agree         | Agree             | Neutral                     | Disagree                  | Strongly<br>disagree |
|--|---------------------------|-------------------|-----------------------------|---------------------------|----------------------|
| The other kit or game has been well described                                  | 8                         | 6                 | 4                           | 2                         | 0                    |
| A clear argument has been developed  | 8                         | 6                 | 4                           | 2                         | 0                    |
| The argument has been well supported with appropriate evidence                 | 8                         | 6                 | 4                           | 2                         | 0                    |
| The writing flowed and ideas were well linked and presented in a logical order | 8                         | 6                 | 4                           | 2                         | 0                    |
| Cognitive level  | Extended<br>abstract<br>8 | Relational<br>6-7 | Multi-<br>structural<br>3-5 | Uni-<br>structural<br>1-2 | Pre-structural<br>0  |
| TOTAL / 40   |                           |                   |                             |                           |                      |
| General comments   |                           |                   |                             |                           |                      |

## **DIHYBRID CROSS PART 5**

After much anticipation, the F<sub>2</sub> generation of flies has hatched. You are now faced with the arduous but important task of analysing the results of this cross. Since you are probably shaking with excitement at this prospect, read the practical component below and answer the questions. These must be written down, along with your thoughts and observations, and handed to your TA before you leave. The results as well as the combined results off all the same crosses (including reciprocal crosses) are needed for your writing assignment, where you will compare the results of the Chi-square test of your flies as well as a Ch-square test for the total number for that cross.

### **PRACTICAL COMPONENT**

1. Using your notes and your cross number as a guide, give the genotypes and phenotypes of the flies used in this cross.
2. Indicate, using the correct symbols, the actual cross, starting with the P<sub>1</sub> generation, through to the F<sub>2</sub> generation.
3. State the phenotypic classes you expect to see in the F<sub>2</sub> generation.
4. Moisten the lid of the etherising jar with ether and empty ALL the flies from your cross into the jar. Be careful not to allow any of the flies to escape. Now separate the flies into the phenotypic classes and count the number of flies in each group. Record your results.
5. Using the Chi-square test, determine whether your results differ significantly from the expected phenotypic ratios.
6. What can you conclude from the results of this cross?
7. Which of Mendel's laws, which would not be demonstrated by a monohybrid cross, was demonstrated here?

**Task:** During the study break write a 3-5 page practical report on the five week *Drosophila melanogaster* dihybrid cross. A hard copy, as well as an electronic copy, must be submitted into the concepts box in the Gate house foyer on the due date (Tuesday 3 April) - see lecturing schedule for details.

**Marking Guideline (TOTAL/100):**

|                     | <b>Assessment Criteria</b>  | <b>Mark Awarded</b> | <b>Out of:</b>                          |
|---------------------|---|---------------------|---|
| Title               | <ul style="list-style-type: none"> <li>• Is the title informative?</li> <li>• Is the title concise?</li> </ul>  |                     | 2,5<br>2,5<br>(5)                       |
| Abstract            | <ul style="list-style-type: none"> <li>• Has the abstract summarized all aspects of the report?</li> <li>• Is the abstract written succinctly?</li> <li>• Did it read well?</li> </ul>  |                     | 6<br>2<br>2<br>(10)                     |
| Introduction        | <ul style="list-style-type: none"> <li>• Has the relevant information been included and written in the student's own words?</li> <li>• Is there background information about the organism that was studied?</li> <li>• Is there background information about Mendelian Genetics?</li> <li>• Does the student understand the concept of transmission genetics?</li> <li>• Does the student introduce their specific cross?</li> <li>• Is it evident that the student understands what the hypothesis or research question is?</li> <li>• Has the student written the introduction in such a way that it starts with the broad concepts and ends with a focused aim?</li> </ul> |                     | 2<br>3<br>3<br>5<br>2<br>3<br>2<br>(20) |
| Materials & Methods | <ul style="list-style-type: none"> <li>• Has the student explained clearly and without copying the practical manual, how the experiments were performed?</li> <li>• Is there evidence that the student understands what was done and why?</li> <li>• Was this section written in the past tense/</li> </ul>   |                     | 5<br>4<br>1<br>(10)                     |
| Results             | <ul style="list-style-type: none"> <li>• Were the results presented appropriately?</li> <li>• Were the results well described in the text?</li> <li>• Is the student able to make the distinction between male and female <i>Drosophila melanogaster</i>?</li> <li>• Does the student know the mutant features involved in their cross?</li> <li>• Does the student adequately describe the phenotypes of the F1 and F2 generations?</li> <li>• Was a table given to calculate the <math>\chi^2</math> and P values? (Must be done for the individual</li> </ul>  |                     | 3<br>3<br>2<br>2<br>2<br>5              |

|                            |  |  |                                    |
|----------------------------|--|--|------------------------------------|
|                            | <p>group as well as all students with that cross – therefore 2 tables)</p> <ul style="list-style-type: none"> <li>• Are the calculations correct?</li> </ul>   |  | 3<br>(20)                          |
| Discussion                 | <ul style="list-style-type: none"> <li>• Does the student adequately discuss the results which have been presented?</li> <li>• Is it clear that the student understands exactly what was done and why the experiment had been designed as it had?</li> <li>• Does the student offer explanations for their results?</li> <li>• Can the student offer suggestions as to why the expected results may not have been achieved and suggest ways of improving the experiment (Is there any difference between the results for the two different sample sizes and why)?</li> <li>• Does the student come to the correct conclusion and make it clear what that is?</li> <li>• Does the student know which of Mendel's Laws has been demonstrated in the experiment?</li> </ul> |  | 3<br>3<br>2<br>3<br>2<br>2<br>(15) |
| References                 | <ul style="list-style-type: none"> <li>• Does the student have at least three good references (Textbook or Journal Article)? And no websites!</li> <li>• Has the student cited the references correctly in the text?</li> </ul>  |  | 2,5<br>2,5<br>(5)                  |
| Context,<br>Focus, Writing | <ul style="list-style-type: none"> <li>• Was the writing clear, concise and did it capture the essence of what the student wanted to say?</li> <li>• Did the writing flow and were ideas linked so that the reader was aware of the central theme?</li> <li>• Was the report written for an appropriate audience (scientific)?</li> <li>• Were there no or minimal spelling and grammatical errors?</li> </ul>   |  | 4<br>4<br>4<br>3<br>(15)           |

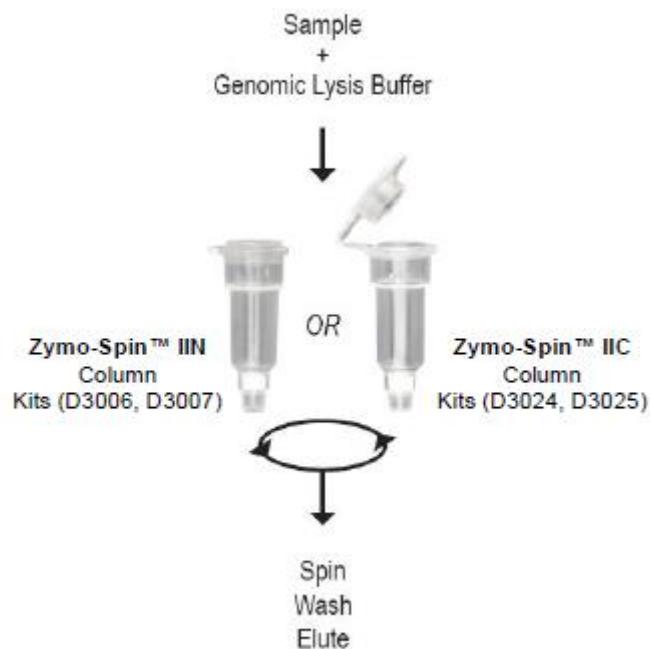
## **PRACTICAL 6: DNA EXTRACTION**

**OBJECTIVES:** At the end of the practical the student should be able to:

1. Extract DNA from a buccal swab.
2. Explain the principles underlying DNA extraction.

### **INTRODUCTION**

The extraction of DNA from cells can essentially be divided into three main steps. First, the cell wall and/membrane have to be disrupted to release the DNA. Secondly, DNA-protein complexes need to be dissociated and lastly, the DNA has to be separated from other soluble cellular components. To ensure that the extracted DNA is kept intact, it is important that the *in vitro* pH supports the phosphodiester linkages in the backbone and glycosidic bonds of the DNA structure. The ionic strength of the buffers will influence the hydrogen bonds between bases and intracellular DNases released during cell disruption must be inhibited. In today's practical, you will use the Quick-gDNA™ MiniPrep (Zymo Research) to extract DNA from buccal swabs. The kit employs spin-column technology where your DNA is bound to the column matrix while impurities are removed (Figure 1.)



**Figure 1.** A schematic diagram illustrating the basic principles of the Quick-gDNA™ MiniPrep kit.

**RECOMMENDED READING:** Boyer R. (2006) Biochemistry Laboratory: Modern Theory and Techniques, Second Edition. Pearson Education: New Jersey, p 275-277

### **AIM**

To extract DNA from a buccal swab.

## METHODS

1. Thoroughly rinse mouth out before isolating cells.
2. Brush the inside of the cheek with a buccal swab for 15 seconds (approximately 20 brushes). Make sure you cover the entire area of the inner cheek.
3. Rinse the brush into a microcentrifuge tube with 500 µl of Genomic Lysis Buffer.
4. Vortex 4-6 seconds.
5. Incubate at room temperature for 10 minutes.
6. Transfer the mixture to a Zymo-Spin™ Column in a Collection Tube.
7. Centrifuge at 10 000 x g for 1 minute.
8. Discard the Collection Tube with the flow through.
9. Transfer the Zymo-Spin™ Column to a new Collection Tube.
10. Add 200 µl of DNA Pre-Wash Buffer to the spin column.
11. Centrifuge at 10 000 x g for 1 minute.
12. Add 500 µl of g-DNA Wash Buffer to the spin column.
13. Centrifuge at 10 000 x g for 1 minute.
14. Transfer the spin column to a clean microcentrifuge tube.
15. Add 20 µl DNA Elution Buffer to the spin column.
16. Incubate 5 minutes at room temperature.
17. Centrifuge at 10 000 x g for 30 seconds to elute the DNA.
18. The eluted DNA will be stored -20 °C for practical 8.

**Student Number:** \_\_\_\_\_ **Name:** \_\_\_\_\_

**Locker number:** \_\_\_\_\_ **TA:** \_\_\_\_\_

**Mark awarded :** \_\_\_\_\_ / 20

**PRAC 8 REPORT:**

**DNA extraction**

**This must be handed to your teaching assistant before you start the practical.**

1. What is the function of the lysis and why is it vital for your experiment to work? (2)

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2. What chemical reagents do you expect to find in your lysis buffer? Explain why these reagents are essential and how they work. (4)

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3. After extracting the DNA, you quantify your sample and realize that you were unsuccessful and obtained no DNA. At which 3 steps in the protocol do you think things might have gone wrong? Explain your answer. (6)

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4. How could you determine whether the extracted DNA is intact? (2)

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5. Explain how you could determine the concentration of the extracted DNA? (2)

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6. Name the two cellular components that are most likely to contaminate your DNA sample. (2)

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7. Explain how you can use spectrophotometry to assess the purity of the extracted DNA? (2)

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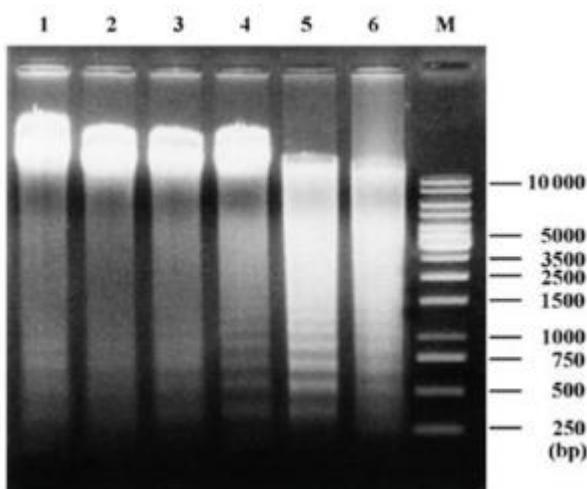
## **PRACTICAL 7: VISUALISATION OF NUCLEIC ACIDS; AGAROSE GEL ELECTROPHORESIS**

**OBJECTIVES:** At the end of the practical the student should be able to:

1. Perform agarose gel electrophoresis.
2. Explain the principles underlying nucleic acid electrophoresis.
3. Visualize nucleic acids on an agarose gel.
4. Use agarose gel electrophoresis as a semi-quantitative method for estimating the concentration of DNA.

### **INTRODUCTION**

Electrophoresis is an analytical tool used to separate macromolecules (i.e. proteins and nucleic acids) under the influence of an applied electrical field. The migration of the molecules under the influence of the electric field is based on the size, shape and charge of the molecules. Agarose gels, prepared by dissolving agarose (a linear polymer of galactopyranose derivatives extracted from seaweed) in an electrophoresis buffer (TAE/TBE buffer), is the preferred solid medium for nucleic acid electrophoresis. Following electrophoresis, gels stained with ethidium bromide can be viewed under UV light. Since ethidium bromide is a fluorescent dye that will intercalate between stacked bases in the nucleic acid, orange-red bands will appear on the gel where nucleic acids are present (Figure 1). This technique can be used to validate the integrity of the extracted DNA, as intact genomic DNA will present itself as a high molecular weight band at the top of the gel. Moreover, the technique can be used as a semi-quantitative method where the intensity of the band is directly proportional to the amount of nucleic acid present.



**Figure 1.** High molecular weight bands (lane 1-4) indicate intact DNA, while smearing (lane 5-6) indicate degraded DNA.

**RECOMMENDED READING:** Boyer R. (2006) Biochemistry Laboratory: Modern Theory and Techniques, Second Edition. Pearson Education: New Jersey, p 165-167, 177-178.

### **AIM**

To visualize extracted DNA on an agarose gel.

## METHODS

You will be pouring a 1% agarose gel which has been partially prepared for you. The following steps have already been done (i.e. you don't need to repeat these steps):

1. Weigh out 1 g of agarose powder.
2. Prepare 100 ml of 1X Tris borate EDTA (TBE) buffer.
3. Mix the agarose powder into the TBE buffer.
4. Dissolve the agarose by heating gradually in the microwave until it is bubbling and all powder has dissolved.
4. Allow the liquid agarose to cool down.
5. The liquid gel is now ready to pour.

### **You will start here:**

1. Assemble the gel casting apparatus as demonstrated by your TA.
2. Remove a bottle of liquid gel mixture from the 50 °C waterbath.

#### **CAUTION: THE BOTTLE IS HOT. HANDLE WITH CARE!**

3. Add 5 µl GR green dye per 50 ml gel.
4. Pour 50 ml gel into the gel casting tray and allow it to set (minimum of ½ an hour).
5. Make a 10X, 100X and 1000X dilution of your extracted DNA solution.
6. On a piece of parafilm, mix 1 µl of 6X loading dye with 5 µl of DNA sample. Do this for your 1X, 10X, 100X and 1000X DNA.
7. Load 5 µl of the DNA-loading mix into the set agarose gel.
8. Allow the gel to electrophoresis at 100 V for 30 min.
9. Remove the gel from the chamber. Check under UV light or blue light transilluminator.
10. Draw the band patterns you observe.

Student Number: \_\_\_\_\_ Name: \_\_\_\_\_

Locker number: \_\_\_\_\_ TA: \_\_\_\_\_

Mark awarded : \_\_\_\_\_ / 30

**PRAC 7 REPORT:**

**VISUALISATION OF NUCLEIC ACIDS; AGAROSE GEL ELECTROPHORESIS**

**This must be handed to your teaching assistant before you leave the laboratory. Question 1-3 should be done as preparation before you come to the practical session.**

1. Explain the principles underlying agarose gel electrophoresis of nucleic acids. (5)

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4. Describe how you would prepare 100 ml TBE buffer (pH 8.0) consisting of 89 mM Tris ( $M_r = 121.14$  g/mol), 89 mM Boric acid ( $M_r = 61.83$  g/mol) and 50 mM EDTA ( $M_r = 292.24$  g/mol). Show all calculations. (5)

5. Describe how you will prepare the 10X, 100X and 1000X dilution of your DNA sample. Show all calculations. (6)

6. Write a paragraph describing your results (as you would for a scientific report). (5)

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7. Draw a schematic diagram representing the results you obtained. Provide a descriptive figure legend for your results (as you would for a scientific report). (5)

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8. What can you conclude from the results obtained? (2)

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9. Explain how you could use the results obtained to quantify the amount of extracted DNA? (2)

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## **PRACTICAL 8: CHROMATOGRAPHY OF A MIXTURE OF AMINO ACIDS**

**OBJECTIVES:** At the end of the practical the student should be able to:

1. Draw the structures of the amino acids
2. Explain and apply the principles of paper and thin layer chromatography.
3. Justify why in terms of their side chain structure, amino acids show preferential affinity for polar or non-polar solvents.
4. Apply the principles of experimental design and use these to design an experiment to identify the amino acids in an unknown mixture of amino acids using chromatography.
5. Perform paper and HPTLC chromatography and calculate  $R_f$  values.

### **INTRODUCTION**

Amino acids have distinct physical and chemical properties which are conveyed by their side chains. Because of this, the presence of an amino acid residue at a particular position in a protein will influence the properties of that protein, particularly when it comes to the three-dimensional shape of the protein. In general, the hydrophobic amino acid residues cluster towards the interior of the protein, whereas the more polar amino acid residues tend to lie on the protein's surface. It is, therefore, interesting to investigate how various amino acids partition themselves in different solvents.

Chromatography can be used to separate the individual constituents in a mixture of amino acids on the basis of physical characteristics such as size, shape, solubility or adsorptivity. Chromatographic techniques involve partitioning of substances between a stationary phase and a mobile phase. Those substances that interact strongly with the stationary phase will be retarded to the greatest extent, while those that interact little with the stationary phase, but have an affinity for the mobile phase, will move faster, or travel further, through the system.

Movement of substances in partition chromatography is characterised by an  $R_f$  value.

The  $R_f$  value is the ratio of the distance moved by the sample to that moved by the solvent, both measured from the origin line. That is,

$$R_f = \frac{\text{distance moved by sample}}{\text{distance moved by solvent}}$$

The  $R_f$  value is generally proportional to the solubility of the sample in the mobile phase.

Thin layer chromatography is an important separation technique. It is based on the adsorption of solutes to materials such as silica gel and the ability of various solvents to elute the solutes from the silica in a differential fashion depending on the properties of the solute, e.g. ionic solutes will not readily elute in organic solvents, whereas non-polar molecules will.

Samples to be analysed are spotted on to a plate covered with silica gel (the stationary phase) and solvent (the moving phase) is allowed to flow by capillary action across the silica gel sheet. Some solutes will be carried towards the front of the moving solvent, whereas others will move comparatively little - hence a separation is achieved.

Very fine silica gel coating leads to high performance thin layer chromatography (HPTLC).

Paper chromatography differs in that the stationary phase is water, which is adsorbed on to the cellulose fibres of the paper, and the mobile phase is organic.

## MATERIALS

1. HPTLC plates coated with 0.2 mm Silica gel 60 with fluorescent indicator UV254 (Machery-Nagel Brand from Merck)
2. Chromatography paper
3. 1 mg/ml Standard amino acids in 0.1 M HCl
4. Unknown mixture of 3 amino acids, 1 mg/ml in 0.1 M HCl
5. Propanol
6. 0.2%  $\alpha$  Naphthol in 0.5 M NaOH. Prepare fresh daily.
7. 5% Aqueous sodium hypochlorite
8. **Pauli/diazo Reagent**  
Mix equal volumes of 1% sulphanilic acid in 1 M HCl and 0.7% (w/v) aqueous sodium nitrite solution. Prepare fresh daily.
9. 10% Sodium carbonate
10. **Ninhydrin spray**  
200 mg Ninhydrin in 100 ml ethanol
11. Butanol
12. Acetic Acid

## METHODS

### Paper chromatography of amino acids

- **Prepare the following solvent: 12 ml butanol + 3 ml acetic acid + 5 ml water.**
- Line the sides of a 250 ml beaker with blotting paper saturated with this solvent. Pour the remaining solvent into the bottom of the beaker and cover with an airtight lid. Leave for about 10 minutes to allow the atmosphere to become saturated with solvent.
- Draw a faint pencil line about 0.5 cm from one edge of the paper. **Make sure that you do not get**

**finger prints all over the paper.** Mark, at a distance of approximately 0.5 cm apart, positions for the application of amino acid standards and an unknown sample. Apply 5  $\mu$ l quantities of the unknown amino acid mixture to one of the marked positions and similar quantities of the standard amino acids to the remaining positions. See Fig. 1 below. Allow the spots of application to dry by blowing gently with a hair dryer.

- Lower the paper carefully into the container so that the amino acid spots are just **above** the level of solvent at the bottom. Cover the beaker with a lid or aluminium foil.
- Develop the chromatogram by allowing the solvent to travel up the paper until it is almost at the top. Remove the paper, mark the solvent front and blow dry.
- Follow the instructions below for the HPTLC plates to locate the amino acids on the paper.

## 2. High Performance Thin Layer Chromatography of amino acids.

**NB:** **Be careful not to touch the surface of the HPTLC plate as this will damage the thin layer.**

- Prepare the following solvent: 17 ml propanol + 8 ml water.
- Line the sides of a 250 ml beaker with blotting paper saturated with this solvent. Pour the remaining solvent into the bottom of the beaker and cover with an airtight lid. Leave for about 10 minutes to allow the atmosphere to become saturated with solvent before placing the plate in the beaker.
- Draw a faint pencil line about 1.0 cm from one edge of the thin layer plate. Mark at a distance of approximately 0.5 cm apart, positions for the application of amino acid standards and an unknown sample. Apply 5  $\mu$ l quantities of the unknown amino acid mixture to one of the marked positions and similar quantities of the standard amino acids to the remaining positions. Please do not touch the plates with your fingers – hold it on the edges. Be careful not to make holes in the thin layer or to scratch it. See Fig. 1 below. Allow the spots of application to dry by blowing gently with a hair dryer.

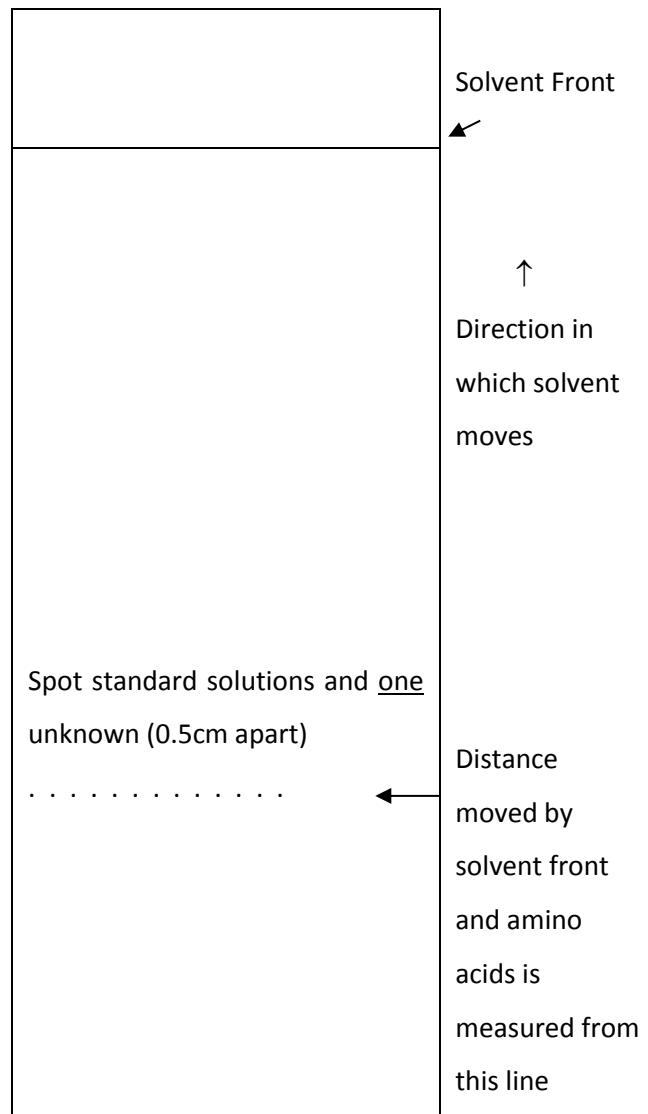


Fig. 1 TLC plate

Lower the plate carefully into the container so that the amino acid spots are just above the level of solvent at the bottom. Cover the container with a lid or aluminium foil.

Develop the chromatogram by allowing the solvent to travel up the plate. Remove the plate, mark the solvent front and blow dry.

**Instructions for both paper chromatograms and thin layer chromatographic plates.**

Locate the amino acids by spraying the plate or paper with Ninhydrin reagent and placing it in an oven at 105 °C for a few minutes. Ring the spots lightly with pencil.

Measure and record the distance moved from the origin by each spot and calculate the  $R_f$  values. Identify the amino acids in your unknown by comparison with the  $R_f$  values of the known standards.

Draw a pencil line down the middle of two different filter paper discs.

Apply 5  $\mu$ l of the unknown amino acid mixture to the one half of the two different filter paper discs.

On the other half of each filter paper disc spot one disc with arginine and the other with histidine.

Spray one of these with the test for arginine and the other with Pauli spray.

**3. Test for Arginine**

This method of detection is specifically for arginine.

Spray first with a 0.2% solution of  $\alpha$  Naphthol in 0.5 M NaOH.

Wait until excess moisture has evaporated and then spray with a 5% solution of sodium hypochlorite. The red colour forms immediately.

**4. Pauli Spray**

Histidine is readily detected by means of the Pauli/diazo reagent, with which it gives an intense cherry red colour. Spray with freshly-prepared reagent, allowing paper to lose excess moisture in a current of air for a few minutes. Respray the area with 10% sodium carbonate. The characteristic colour appears immediately if histidine is present.



Student Number: \_\_\_\_\_ Name: \_\_\_\_\_

Locker number: \_\_\_\_\_ TA: \_\_\_\_\_

Mark awarded : \_\_\_\_\_ / 40

**PRAC 8 REPORT:**

**CHROMATOGRAPHY OF A MIXTURE OF AMINO ACIDS**

This must be handed to your teaching assistant before you leave the laboratory. Some of these questions should be done as preparation before you come to the practical session. You may also be required to complete the preparation questions on Sakai and obtain a passing mark before you will be allowed to do the practical. You will be assigned a mark for your practical report.

1. Why is it important to saturate the atmosphere in the tank before running a chromatogram? (2)

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2. On the basis of their structure predict which amino acids (of the 20 commonly found in proteins) would be likely to be found on the surface of a globular protein. Fill in the table below (20)

| Amino Acid | Structure | Probable position in a globular protein | Justify your choice |
|------------|-----------|---|---------------------|
|            |           | Interior/Surface                        |                     |
|            |           | Interior/Surface                        |                     |

|            |           | Interior/Surface                        |                     |
|------------|-----------|---|---------------------|
| Amino Acid | Structure | Probable position in a globular protein | Justify your choice |
|            |           | Interior/Surface                        |                     |

|            |           | Interior/Surface                        |                     |
|------------|-----------|---|---------------------|
| Amino Acid | Structure | Probable position in a globular protein | Justify your choice |
|            |           | Interior/Surface                        |                     |

| Amino Acid | Structure | Probable position in a globular protein | Justify your choice |
|------------|-----------|---|---------------------|
|            |           | Interior/Surface                        |                     |

3. Present your results in a table comparing the  $R_f$  values obtained for the various amino acids by thin layer chromatography and paper chromatography. If the solvent had been allowed to run 7 cm instead of the distance run in your chromatogram on the thin layer plates, how far would the amino acids on your chromatogram have moved in this solvent? What would their  $R_f$  values be? Fill in the tables below. (6)

**TLC**

| Amino acid | Distance travelled by amino acid | Distance travelled by solvent front | $R_f$ value | Distance amino acid would have travelled if solvent front travelled 7 cm |
|------------|----------------------------------|-------------------------------------|-------------|--|
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |

**Paper chromatography**

| Amino acid | Distance travelled by amino acid | Distance travelled by solvent front | $R_f$ value | Distance amino acid would have travelled if solvent front travelled 7 cm |
|------------|----------------------------------|-------------------------------------|-------------|--|
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |
|            |                                  |                                     |             |  |

4. Identify the amino acids present in the unknown mixture. (4)

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5. What can you conclude about  $R_f$  values? (1)

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6. *"The amino acid with the lowest  $R_f$  value is the least soluble in the chromatography solvent and the most strongly absorbed on to the silica gel particles."* Explain this statement. (2)

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7. With reference to the previous question, which amino acid was the least soluble amino acid of those you tested? (1)

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8. With reference to its structure and the nature of the solvent explain why. (1)

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9. Which amino acid moved the greatest distance on the thin layer plates? (1)

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10. In terms of its structure explain why this was the case. (1)

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11. In terms of its structure, justify why the amino acid that travelled the least on paper was attracted most strongly to the stationary phase. (1)

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## **PRACTICAL 9: PROPERTIES OF PROTEINS & DETERMINATION OF PROTEIN CONCENTRATION**

### **OBJECTIVES:**

At the end of the practical the student should be able to:

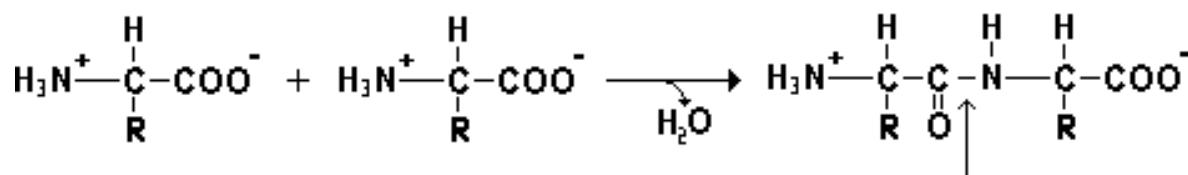
- Identify the factors affecting protein solubility and in terms of their structure be able to explain why proteins are affected by the various factors investigated.
- Draw the structure of a peptide bond and understand the principle behind the Biuret test for protein determination.
- Construct and use a standard curve to determine the concentration of various protein solutions of unknown concentration.
- Explain what a blank solution is and why it was necessary to include a blank solution in the calibration curve.
- Dilute a stock solution to prepare various amounts of solution with different concentrations.
- Apply the principles of experimental design to design an experiment to test for the presence of protein in a solution.
- 

### **BACKGROUND**

#### **1. Proteins**

Proteins are the most abundant organic molecules within cells. They are fundamental to all aspects of cell structure and function.

In protein molecules, the successive amino acid residues are covalently bonded together by **peptide** bonds, which arise by the elimination of the elements of water from the carboxyl group of one amino acid and the amino group of the next.



Such polymers, which are called **polypeptides**, may contain hundreds of amino acid units and there may be more than one polypeptide chain in a protein molecule. Proteins are not merely random polymers of varying length: each type of protein molecule has a specific composition, molecular weight and sequential order of its amino acid building blocks.

## **2. Factors affecting protein solubility**

### **(a) Precipitation of proteins as salts**

Most proteins can be completely precipitated from aqueous solution by the addition of certain acids, such as trichloroacetic acid, which form acid-insoluble salts with the proteins. Proteins may, in a similar manner, be precipitated by heavy cations such as  $Zn^{2+}$  and  $Pb^{2+}$ .

### **(b) Separation of proteins by solubility differences**

Globular proteins vary considerably in their solubility in aqueous systems; these differences may be used to effect separation of mixtures of proteins. There are four important variables influencing protein solubility:

#### **(i) pH**

Nearly all globular proteins are least soluble at their isoelectric pH because at this pH the molecule has no net charge and thus no electrostatic repulsion exists between the neighbouring protein molecules. At pH values above or below the isoelectric point all the protein molecules will have a new charge of the same sign. They will, therefore, repel each other, and prevent aggregation of molecules to form insoluble precipitates.

Since different proteins have different isoelectric points, they can be separated from one another by **isoelectric precipitation**. When the pH of a protein mixture is adjusted to the isoelectric pH of one of its components, most of that component will precipitate, thus making isolation of that protein possible.

#### **(ii) Salt Concentration**

Neutral salts, like ammonium sulfate, have pronounced effects on the solubility of globular proteins. At low concentrations they increase the solubility of many proteins, a phenomenon called salting-in.

**Salting-in** is a function of the ionic strength of the salt. In addition, the process shows a peculiar sensitivity to the nature of the cation. Salting-in effects are caused by changes in the tendency of dissociable groups on the protein to ionise, thus increasing the solubility of the protein. The net result is a decrease in the activity coefficient of the protein. When the concentration of neutral salts is greatly increased, the solubility of proteins begins to decrease again and at a very high concentration a protein may be completely precipitated. This phenomenon is called **salting-out**. The physical-chemical basis of salting-out is complex. Salting-in and salting-out are useful procedures in the separation of protein mixtures, since different proteins vary in their response to salt concentrations.

**(iii) Solvent**

The addition of organic solvents such as ethanol and acetone decreases the solubility of most proteins in water to such an extent that they will precipitate out of solution. Protein solubility is a function of the dielectric constant of the medium and of the tendency of the added solvent to decrease hydration of ionic groups. Ethanol has a lower dielectric constant than water. Since a decrease in dielectric constant increases the attractive force between two opposite charges, ethanol decreases the ionisation of proteins and thus promotes their coalescence. Mixtures of proteins can thus be separated on the basis of quantitative differences in their loss of solubility with increasing ethanol or acetone concentration.

**(iv) Temperature**

Within a limited range, most proteins will increase in solubility with increasing temperature. Above 40 °C to 50 °C, most proteins become unstable and begin to denature with a loss of solubility.

**3. Biuret Test**

One colour reaction of peptide and proteins which is not given by free amino acids is the Biuret reaction. The name of the test comes from the compound Biuret which typically gives a positive reaction. (Biuret is the substance formed when urea is heated) The reaction depends on the presence of 2 or more peptide bonds. Treatment of a peptide or proteins with CuSO<sub>4</sub> and alkali yields a purple complex of Cu<sup>++</sup> and the peptide. The colour is apparently caused by the coordination complex of the copper atom and four nitrogen atoms, 2 from each peptide chain. The colour will vary with the size of the protein molecule, small peptides giving a pink colour.

The intensity of the colour formed is proportional to the concentration of protein and the Biuret reaction can therefore be used in the colourimetric determination of protein.

**MATERIALS**

1. Protein solution to demonstrate effect of various treatments on protein solubility  
(Part A)
2. 10% TCA
3. Alcohol 96%
4. Acetone
5. Solid ZnSO<sub>4</sub>
6. Solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

7. 0.5 M Ba(OH)<sub>2</sub> - heat water to dissolve and cool slowly
8. **Standard casein:** 20 mg casein/ml in 0.5 M NaOH
9. Unknown casein dissolved in 0.5 M NaOH
10. 1% Gelatin
11. Egg albumin diluted 15 x
12. 0.5% Protamine sulfate
13. **Biuret Reagent**

(This will have been prepared for you by dissolving 90 g sodium potassium tartrate + 10 g copper sulfate + 10 gm potassium iodide in 0.2 M sodium hydroxide and made up to 2 litres).

## **METHODS**

### **PART A**

1. **Protein Solubility:** Use 1 to 8 dilution of the protein solution provided for this part of the practical

Using **2 ml** of diluted protein solution for a, b, c, d and e, observe the effect of the following protein precipitants. Do each test singly.

- a). **Trichloracetic Acid (TCA):** You will be provided with a centrifuge tube which has been treated as follows: TCA (2 ml of 10%) was added to the protein solution in the centrifuge tube which was centrifuged for 5 minutes at 5000 x g. Remove 1 ml of the supernatant using a pipette. Carry out the Biuret test (Part B) on this 1 ml of the supernatant to establish whether all the protein precipitated or whether there is still some present in the supernatant  
(unknown vi)
- (b) **Salt:** Add solid ammonium sulfate gradually to saturation.
- (c) **Organic Solvents:** Add 3 volumes (6 ml) of acetone. Into a second tube, add 5 volumes (10 ml) of alcohol.
- (d) **Heavy Metal Salts:** Add a small amount of 0.5 M ZnSO<sub>4</sub> gradually. Into a second tube, add 2 ml of 0.5 M Ba(OH)<sub>2</sub> solution.
- (e) Heat: Boil.

## **PART B**

### **COLOURIMETRIC ESTIMATION OF PROTEIN USING THE BIURET METHOD**

#### **Standard Solutions**

You are provided with a solution containing 20 mg casein per ml in 0.5 M NaOH.

Prepare 4 ml of a series of dilutions from this stock casein solution. Mix well before use. **These are the standard solutions from which you will withdraw some solution on which to perform the Biuret test. DO NOT ADD ANYTHING TO THESE TUBES!**

| Conc.<br>(mg/ml) | Stock Casein<br>(ml) | 0.5 M NaOH<br>(ml) |
|------------------|----------------------|--------------------|
| 0                |                      |                    |
| 5                |                      |                    |
| 10               |                      |                    |
| 15               |                      |                    |
| 20               |                      |                    |

#### **Construction of a calibration (standard) curve**

In duplicate withdraw 1 ml from each of the 5 protein concentrations and place in 10 clean tubes. (Always work from the most dilute to the most concentrated solutions).

#### **Determination of the protein concentration in the solutions of unknown concentration**

Pipette 1 ml of each of the following solutions in duplicate into a clean test tube - (You will now have 10 more tubes + 1 tube (vi) from the TCA precipitate from part A – i.e. a total of 21 tubes on which to perform the Biuret test):

- (i) An unknown concentration of casein - mix well before use
- (ii) Protein solution used for part A, diluted 1 to 10.  
(Remember it has already been diluted 1 to 8).
- (iii) Gelatin solution, dilute 1 to 2
- (iv) Egg albumin diluted 1 to 30
- (v) Protamine sulfate

(vi) Supernatant from TCA precipitation from PART A

**PERFORM the BIURET test on the 1 ml of the standard solutions and on the 1 ml of the unknown solutions as follows - NB: TREAT THE UNKNOWN AND STANDARD SOLUTIONS IDENTICALLY.**

Add 5 ml Biuret reagent to the 10 standards, 10 unknown tubes and 1 tube from Part A. Mix and allow the tubes to stand for 30 minutes. Measure the absorbance of the solutions in a spectrophotometer at 550 nm against a water reference. Be sure to zero the spectrophotometer properly - **USING WATER.**

**Correct for the blank by subtracting the blank reading from all other readings.**

Plot a graph of absorbance (y) versus concentration (x) and determine the concentration of the unknown solutions from this graph.

Draw a flow diagram showing how you will be carrying out part B of this practical.

Show this to your TA before you start the practical session.

**Solubility of proteins** - Describe and give an explanation for what you observed in Part A of the practical. (If the protein did not behave as you expected, attempt to explain (in the “what I thought” column why this could have been the case).

|   | What I expected | What I observed | What I thought |
|---|-----------------|-----------------|----------------|
| (1) TCA   |                 |                 |                |
| (2) $(\text{NH}_4)_2\text{SO}_4$                |                 |                 |                |
| (3) acetone                                     |                 |                 |                |
| (4) alcohol                                     |                 |                 |                |
| (5) $\text{ZnSO}_4$<br>$\text{BA}(\text{OH})_2$ |                 |                 |                |
| (6) Heat  |                 |                 |                |



Student Number: \_\_\_\_\_ Name: \_\_\_\_\_

Locker number: \_\_\_\_\_ TA: \_\_\_\_\_

Mark awarded: \_\_\_\_\_ / 40

**PRAC 9 REPORT**

**This report must be hand to your TA at the start of the next practical. Some of the questions should be completed before you enter the laboratory.**

1. This practical is divided into 2 parts. What does part A investigate? (1)

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2. What is meant by "*isoelectric precipitation*"? (2)

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3. "*Organic solvents decrease protein solubility in water by lowering the dielectric constant*".

Explain what this means. (2)

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4. What is the purpose of carrying out the Biuret test on the supernatant obtained after TCA precipitation and subsequent centrifugation? (1)

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5. What type of chemical bond is a peptide bond? (1)

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6. Draw the structure of the peptide bond formed between alanine and leucine. (3)

7. What is the basis of the Biuret Reaction? (2)

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8. Explain fully how the Biuret reaction gives an indication of protein concentration. (2)

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9. Would glycylalanine give a positive Biuret reaction? \_\_\_\_\_ Justify your answer.

(2)

10. Why is the absorbance read at 550 nm after addition of Biuret reagent to the protein solutions?

(1)

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11. What is meant by a “blank solution”?

(1)

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12. List the components of the blank solution in this practical

(2)

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13. Why must the “blank” be subtracted from the other readings?

(1)

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14. What protein was used to prepare the standard solutions in order to construct the standard curve in this practical? \_\_\_\_\_

(1)

15. Show how you calculated how to dilute the 20 mg stock solution of casein to prepare 4 ml of 15 mg/ml casein solution. (2)

16. If you were given an unknown solution and told that it probably contained protein, briefly describe how you could confirm whether this was the case. (Do not forget to describe what you would use as controls (i.e. both positive and negative). (6)

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## The Biuret Reaction

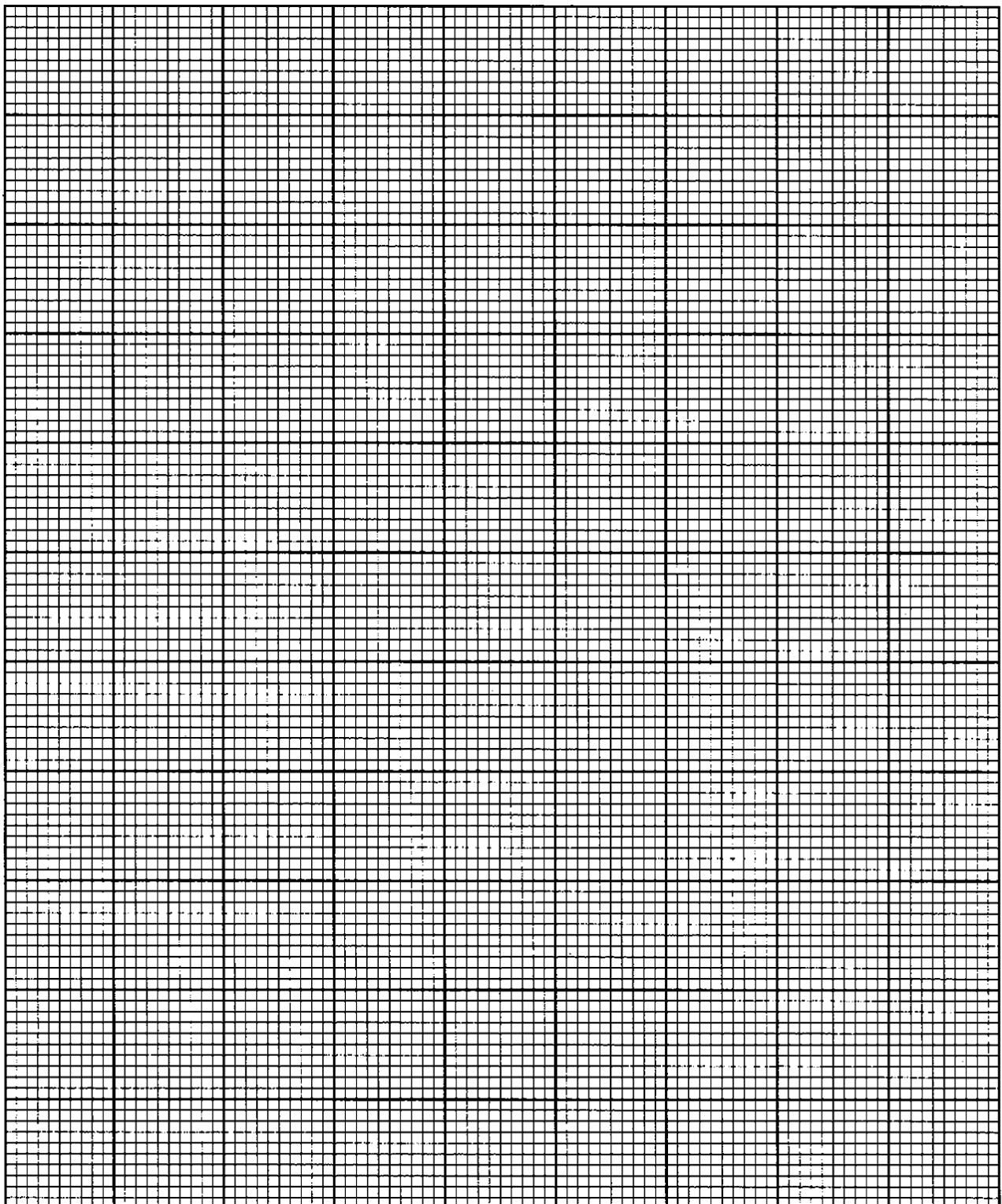
(10)

Organise your data into tables and plot a standard curve.

Table 1 should contain the data collected in order to plot a standard curve.

Table 2 should contain the data necessary for determining your unknowns.

**NB: Remember to give your tables the appropriate headings and to write an adequate legend for your graph.**



Calculate the undiluted concentrations (mg/ml) – To do this you will have to read the value from the calibration curve and then take the dilution factor into account

Casein unknown \_\_\_\_\_

Plasma (1 to 10) \_\_\_\_\_

Gelatin \_\_\_\_\_

Egg albumin \_\_\_\_\_

Protamine sulfate \_\_\_\_\_

TCA treated protein solution from part A (1 to 8) \_\_\_\_\_

## **PRACTICAL 10: EXPLORING BIOLOGICAL DATABASES**

### **INTRODUCTION**

You have been provided with a text file, containing a single accession number (found on the course website). Today, you will use the databases and resources provided by the European Bioinformatics Institute (EBI) at <http://www.ebi.ac.uk> to describe the characteristics of the gene corresponding to this accession number and the protein(s) it encodes.

### **OBJECTIVES**

By the end of this practical, students should be able to:

1. Search biological databases using accession numbers.
2. Select the appropriate database in order to obtain specific biological information.



**Student Number:** \_\_\_\_\_ **Name:** \_\_\_\_\_

**TA:** \_\_\_\_\_

**Mark awarded:** \_\_\_\_\_ / 15

**PRAC 10 REPORT**

1. Write down the full HGNC approved name of this gene. (1)

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2. What is the official HGNC ID of this gene? (1)

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3. List one of the synonyms for this gene. (1)

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4. On which human chromosome is this gene located? (1)

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5. List one of the paralogues of this gene. (1)

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6. This gene has an orthologue in *Bos taurus*. What is its name and on which chromosome can it be found? (2)

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7. This gene produces multiple mRNA transcripts through alternative splicing. What is the length (in bp) of the shortest transcript produced by this gene? (1)

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8. What is the primary function of the full length protein encoded by this gene? (1)
- 
- 
9. What is the length (in amino acids) and molecular weight (in kDa) of the protein encoded by the “canonical” sequence associated with this gene? (1)
- 
10. List a conserved domain present in this protein. (1)
- 
11. Does this protein bind any metal ions? If yes, list them. (1)
- 
12. Which method was used to resolve the 3D structure of this protein? (1)
- 
13. List the PDB accession number associated with the 3D structure of this protein. (1)
- 
14. Which expression system was used to produce the 3D structure of this protein? (1)
-

## **PRACTICAL 11: BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST)**

### **INTRODUCTION**

Basic Local Alignment Search Tool (BLAST) is a suite of bioinformatics tools that allow query sequences to be aligned against those contained in selected primary and secondary sequence databases. While multiple web-based implementations of BLAST are available, today you will explore the BLAST tools provided by the National Center for Biotechnology Information (NCBI). Use the tools provided at <http://blast.ncbi.nlm.nih.gov/> to identify the sequence contained in the file **unknown.fa** (provided on the course website) and answer the following questions:

### **OBJECTIVES**

By the end of this practical, students should be able to:

1. Select the appropriate BLAST tool with which to identify a given sequence.
2. Select an appropriate BLAST algorithm for the given problem.
3. Select an appropriate sequence database against which to search.
4. Interpret the output provided by BLAST.



**Student Number:** \_\_\_\_\_ **Name:** \_\_\_\_\_

**TA:** \_\_\_\_\_

**Mark awarded:** \_\_\_\_\_ / 15

**PRAC 11 REPORT**

1. You have been provided with a text file containing an unidentified sequence (unknown.fa).
  - (a) Is your sequence a nucleotide sequence or a protein sequence? (1)  
\_\_\_\_\_
  - (b) In which file format has your sequence been provided? (1)  
\_\_\_\_\_
2. Given the type of sequence you have been provided with, which Basic BLAST program will you use to identify your sequence?  
(1)  
\_\_\_\_\_
3. In order to identify your sequence, you will have to select a database against which to BLAST.
  - (a) Which database will you use to identify your sequence? (1)  
\_\_\_\_\_
  - (b) Why did you select this database? (2)  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_
4. The BLAST program you have chosen requires you to select an appropriate algorithm to aid in identifying your unknown sequence.
  - (a) Which algorithm will you choose to identify your sequence? (1)  
\_\_\_\_\_

(b) Why did you select this algorithm? (1)

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5. The algorithm you have selected has a number of alterable parameters. Today, you will perform your BLAST search using the default parameters.

(a) What is the default word size used by the BLAST algorithm you have selected? (1)

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(b) Which substitution matrix is used by default by the BLAST algorithm you have selected? (1)

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6. Based on the results of your BLAST search:

(a) What type of molecule does your sequence encode? (1)

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(b) Which organism is your sequence derived from? (1)

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(c) Along which chromosome can the gene encoding your sequence be found? (1)

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7. Has the crystal structure of the molecule encoded by your sequence been resolved? How did you arrive at this conclusion? (2)

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### **Essay writing rubric - MIND diet**

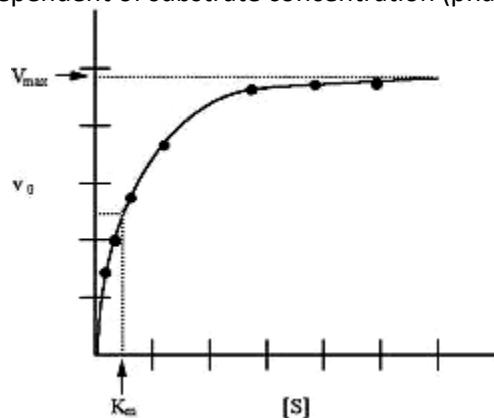
| Essay writing rubric – <b>The MIND diet</b>   | <b>Strongly agree</b> | <b>Agree</b> | <b>Neutral</b> | <b>Disagree</b> | <b>Strongly disagree</b> |
|---|-----------------------|--------------|----------------|-----------------|--------------------------|
| The student has written the document in the format of an argumentative essay  | 5                     | 3            | 2              | 1               | 0                        |
| The student has written the essay for the correct audience (scientific writing).  | 6                     | 4            | 3              | 2               | 0                        |
| The language, grammar and spelling are good; the essay is succinct.   | 6                     | 4            | 3              | 2               | 0                        |
| The student has produced a clear argument for/against the eating plan. The paragraphs are linked so that the argument flows easily.   | 9                     | 7            | 5              | 2               | 0                        |
| The student has provided <u>reasons</u> supporting the argument that are justified by specific <u>evidence</u> from the literature.   | 8                     | 6            | 4              | 2               | 0                        |
| The student has referred to appropriate metabolic pathways and has made an attempt to link these pathways to weight and health.   | 10                    | 8            | 6              | 3               | 0                        |
| The conclusion has linked/tied all the ideas introduced in the essay and has arrived at the correct/appropriate conclusion for the argument.  | 8                     | 6            | 4              | 2               | 0                        |
| The student has correctly referenced the information in the report.   | 4                     | 3            | 2              | 1               | 0                        |
| There is no evidence of plagiarism in any part of the work presented by the student (plagiarism of the entire document or copying from another student's work will result in a mark of 0 being awarded for the essay) | 4                     | 3            | 2              | 1               | 0                        |
| Total: 60 marks.  |                       |              |                |                 |                          |

## PRACTICAL 12: MICHAELIS CONSTANT OF THE HYDROLYSIS OF SALICIN BY $\beta$ - GLUCOSIDASE

**Objectives:** By the end of this practical students should be able to:

1. Perform an enzyme assay
2. Determine the Michaelis constant and  $V_{\max}$  from a Lineweaver-Burk plot
3. Convert data so that a straight-line plot is obtained from a hyperbolic plot
4. Identify the factors which affect the reaction rate of an enzyme.

Michaelis *et al* in the early part of the 20<sup>th</sup> century reasoned correctly that an enzyme-catalysed reaction at varying substrate concentrations is biphasic; that is, at low substrate concentrations the active sites on the enzyme are not saturated by substrate and, thus, the reaction rate varies with substrate concentration (phase I – first order). As the number of substrate molecules increases, the sites are covered to a greater degree until at saturation no more sites are available and the enzyme is working at full capacity and the rate is independent of substrate concentration (phase II – zero order).



The mathematical equation that defines the quantitative relationship between the rate of an enzyme reaction and the substrate concentration and, thus, fulfils the requirement of the hyperbolic curve is the Michaelis-Menten equation:

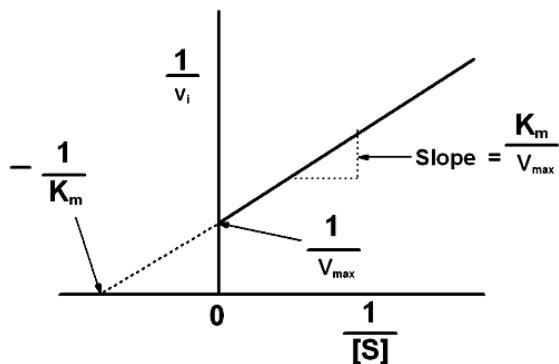
$$v_0 = \frac{V_{\max} [S]}{K_M + [S]}$$

In this equation,  $v_0$  is the observed velocity at a given substrate concentration  $[S]$ ;  $K_M$  is the Michaelis constant expressed in units of concentration (moles / litre); and  $V_{\max}$  is the maximum velocity at saturating concentrations of substrate. For a derivation of this equation see your prescribed text. The constant  $K_M$  is important since it provides a valuable clue to the mode of action of an enzyme-catalysed reaction.

In 1934 Lineweaver and Burk showed that if the reciprocal of each side of equation (1) were taken, then

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

which is equivalent to the straight-line equation  $y = mx + c$



Therefore, if we plot  $1/v_0$  on the ordinate and  $1/[S]$  values on the abscissa ("a double-reciprocal plot), a straight line relation exists from which  $K_M$  can easily be evaluated.

#### DETERMINATION OF MICHAELIS CONSTANT

In this practical you will determine the Michaelis constant of the hydrolysis of salicin by  $\beta$ -glucosidase.

$\beta$ -Glucosidase readily hydrolyses the artificial substrate salicin to form glucose and salicyl alcohol. The reaction is followed by measuring the amount of salicyl alcohol produced by the enzyme in a defined time interval. Salicyl alcohol is measured by the colour produced on reaction with the Folin-Ciocalteu reagent.

In this experiment you will determine  $K_M$ , the substrate concentration at which half the maximal velocity of reaction is attained. The reaction velocity of a particular enzyme preparation is measured at several different substrate concentrations and from the results the  $K_M$  may be determined graphically as described earlier.

#### MATERIALS

##### 1. Citrate - phosphate buffer pH 5.0

0.2 M  $\text{NaH}_2\text{PO}_4$  adjusted with 0.2 M tri-sodium citrate to pH 5.0

##### 2. 10 mM Salicin

##### 3. Folin - Ciocalteu reagent

##### 4. 14% Sodium carbonate

5.  $\beta$ -Glucosidase (35  $\mu\text{g}$  / ml).

## EXPERIMENTAL

### **1. Preparation of Substrate concentrations:**

$\beta$ -Glucosidase activity (rate of reaction) will be determined at **six** different substrate concentrations. These are prepared from the stock **10 mM salicin solution** as indicated in the table. Prepare each substrate concentration **in duplicate** (12 tubes total).

Notice that the final concentrations you must prepare are given as the number of  $\mu\text{moles}/3 \text{ ml}$  i.e. you must prepare **3 ml** of each concentration.

| Tube | Stock salicin<br>(ml) | Water<br>(ml) | Salicin<br>( $\mu\text{mol}/3 \text{ ml}$ ) |
|------|-----------------------|---------------|---|
| 1    |                       |               | 0   |
| 2    |                       |               | 5   |
| 3    |                       |               | 10  |
| 4    |                       |               | 15  |
| 5    |                       |               | 20  |
| 6    |                       |               | 30  |

Get your teaching assistant to check your table **BEFORE** you start the enzyme assay.

**MAKE SURE THAT YOU UNDERSTAND HOW TO DO THE DIULTIONS.**

### **2. Enzyme Assay**

To each of the above tubes add 1 ml of citrate-phosphate buffer pH 5.0. Start the reaction with 1 ml enzyme and incubate for exactly 10 minutes at room temperature. Stop the reaction by adding 2.5 ml of Folin-Ciocalteu reagent. After 3 minutes, add 2.0 ml of 14% sodium carbonate. Mix well. Place the tubes in a boiling water bath for exactly 5 minutes.

Cool, and if solutions appear cloudy, filter. You can fold the filter paper circles into funnel shapes and place these directly (without actually using glass funnels) onto the top of the tubes. Position the filter paper funnels so that the filtrate runs into the tubes and not down the sides. Use the tube without substrate (tube 1) to set zero absorbance on the spectrophotometer at 760 nm. Read the absorbance of all your tubes and record your results in the "Report" section.

## CALCULATIONS

### 1. Determining $1/v_o$ :

To determine  $K_M$  by the Lineweaver-Burk method, you should plot  $1/v_o$  on the y-axis and  $1/[S]$  on the x-axis.

In this experiment, the velocity of the reaction can be expressed as the concentration of salicyl alcohol produced in each tube in 10 minutes. Since the absorbance of the reaction solution is directly proportional to the concentration of salicyl alcohol, the absorbance readings can be regarded as representative of the reaction velocity.

Thus  $1/v_o$  can be calculated from  $1/\text{absorbance}$

### 2. Determining $\frac{1}{[S]}$

The substrate concentration at which the enzyme is reacting is that concentration in the **final** reaction mixture i.e. substrate + buffer + enzyme in a total of 5 ml.

For example, in tube 2 the amount of salicin was 5  $\mu\text{mol}$  in 3 ml. In the final reaction, therefore, the concentration would be  $5 \mu\text{mol} / 5 \text{ ml} = 1 \mu\text{mol/ml}$  or a 1 mM solution.

Calculate the concentrations of salicin in tubes 3 to 6 and then take the reciprocal of each concentration of salicin,  $\frac{1}{[S]}$



Student Number: \_\_\_\_\_ Name: \_\_\_\_\_

Locker number: \_\_\_\_\_ TA: \_\_\_\_\_

Mark awarded: \_\_\_\_\_ / 30

**PRAC 12: Report**

**Some of these questions should be done (as preparation) before you enter the laboratory. This must be signed by your teaching assistant before you leave the laboratory.**

1. Define  $K_M$  (2)

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2. What are the units of  $K_M$ ? (1)

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3. Why is it useful to know the  $K_M$  of an enzyme? (2)

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4. Draw a flow diagram showing how you will be carrying out the experimental procedure in this practical. (3)

5. Write out the reaction of the hydrolysis of salicin by  $\beta$ -glucosidase. (1)

6. Which product reacts with Folin-Ciocalteu reagent? (1)

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7. If you were asked to prepare 3 ml of a solution containing 5  $\mu\text{mol}$  Salicin from a 10 mM stock salicin solution, how many ml of the stock solution would you use? (3)

8. What are the advantages of the Lineweaver-Burk plot for the determination of  $K_M$ ? (2)

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**1. K<sub>M</sub> Determination**

1. Present your experimental data and results in tables in the space below. (4)

2. Construct a graph of  $v_o$  against [S]

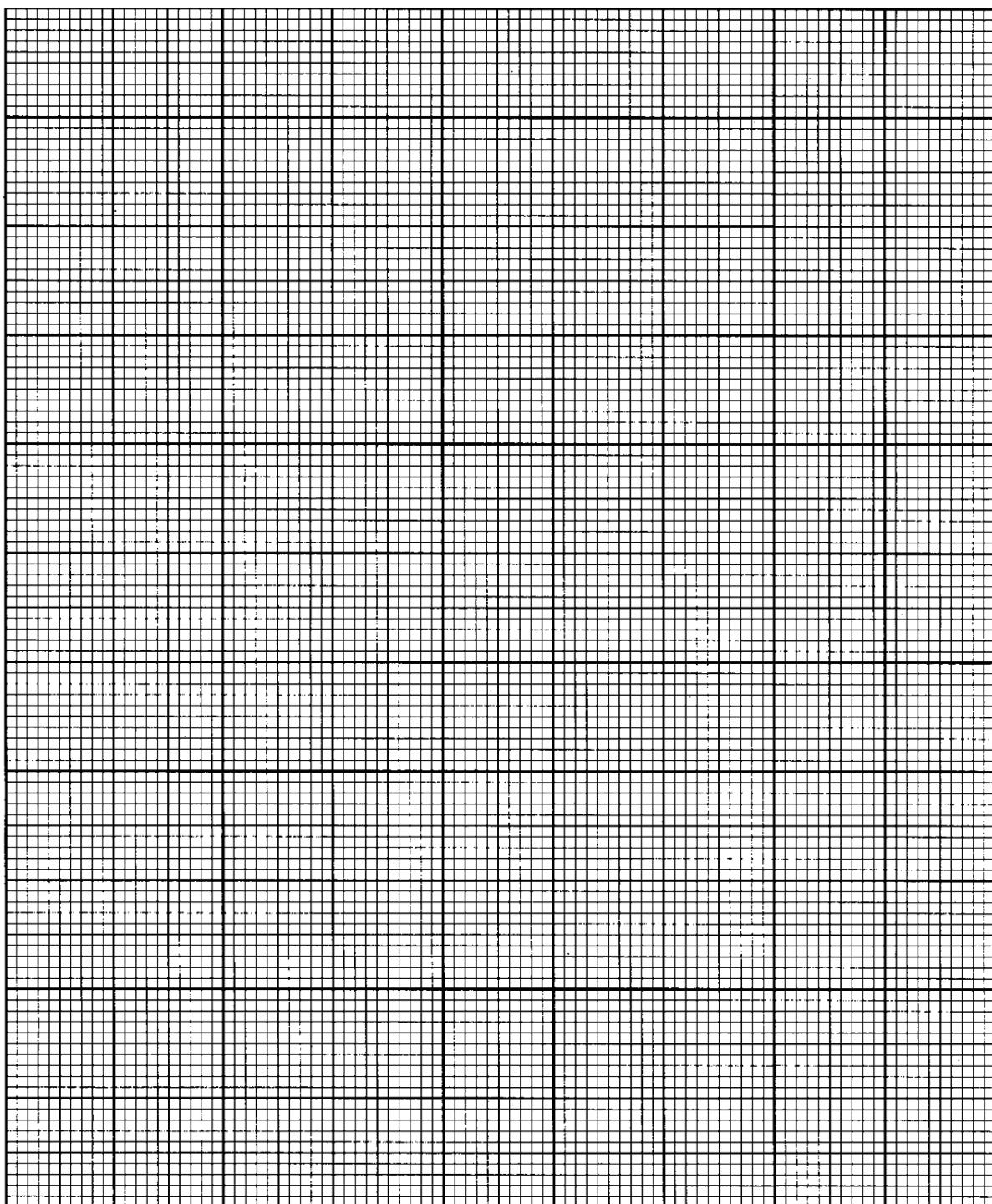
(1)

a) The  $K_M$  for salicin from this graph is \_\_\_\_\_

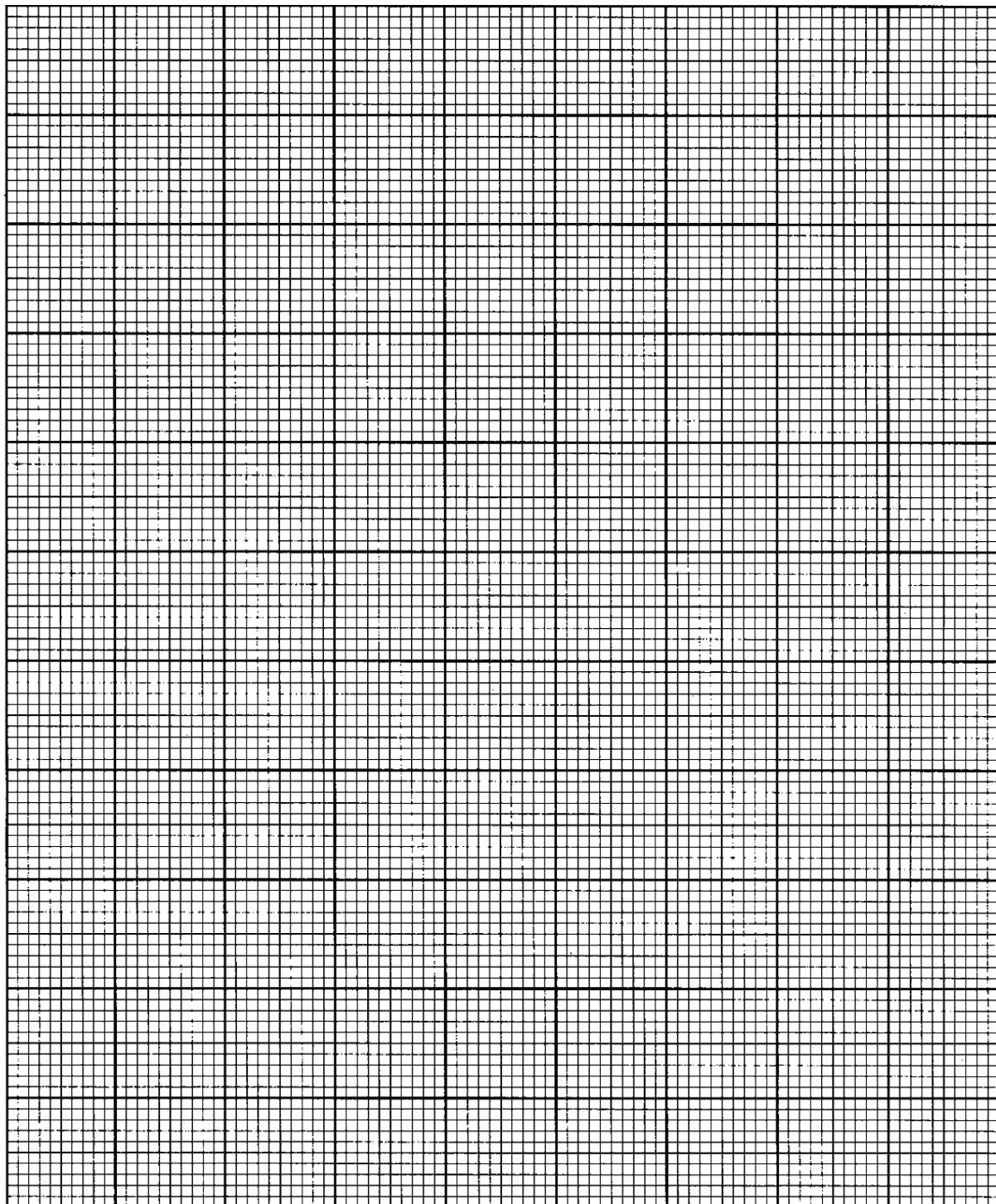
(1)

b)  $V_{max}$  is \_\_\_\_\_

(1)



3. Construct a graph of  $v_o$  against  $[S]$ . (Hint: you might like to use the graph paper sideways). (1)
- a) From this graph the  $K_M$  is \_\_\_\_\_ (1)
- b)  $V_{max}$  is \_\_\_\_\_ (1)





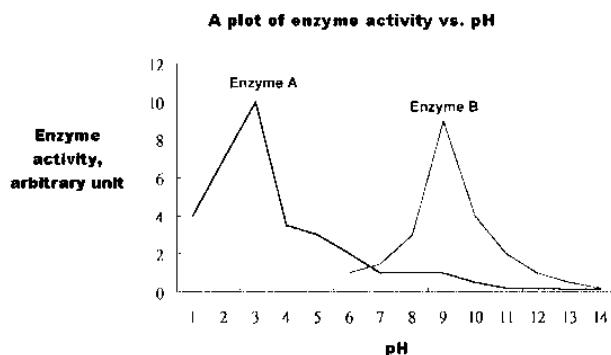
## **PRACTICAL 13: THE IMPORTANCE OF pH IN THE FUNCTIONING OF DIGESTIVE ENZYMES**

### **OBJECTIVES**

At the end of the practical students will be able to:

- 1.Determine the amount of a substance from a calibration curve
- 2.Perform an enzyme assay
- 3.Experimentally determine the optimum pH of an enzyme
- 4.Explain and interpret experimental results

Since enzymes are proteins, pH changes will profoundly affect the ionic character of the amino and carboxylic acid groups on the protein and will, therefore, markedly affect the catalytic site and conformation of an enzyme. In addition to the purely ionic effects, low or high pH values can cause considerable denaturation and hence, inactivation of the enzyme protein. These effects are probably the main determinants of a typical enzyme activity-pH relation. Thus, a bell-shaped curve is obtained with a relatively small plateau and with sharply decreasing rates on either side. The plateau is usually called the optimal pH. All enzymes tend to have pH optima, that is, they exhibit maximal activity in solutions of a characteristic pH.



In this experiment you will be determining the pH optimum of  $\alpha$ - amylase.

#### **1. Assay of $\alpha$ -amylase**

The reducing sugars (aldehyde containing molecules) which are liberated during starch hydrolysis are measured by their reduction of 3,5-dinitrosalicylic acid. The reduced acid has a reddish-brown colour, which can be measured at 540 nm.

#### **2. Determination of pH Optimum**

In this practical you will be provided with starch substrate at different pH values within the range of 3.0 to 7.0 at 0.5 pH unit increments. You will determine the enzyme activity at each pH value. You will also construct a standard curve of absorbance against the amount of reducing sugar, with a maltose standard. This may be used to relate the absorbance you obtained at each pH value to the amount of reducing sugar (maltose equivalents) formed.

## MATERIALS

1. **Enzyme:** You are provided with a solution of  $\alpha$ - amylase/diastase in water at a concentration of 1 mg/ml.
2. **Buffer-substrate solutions:** You will be provided with the solutions of buffered-substrate from pH 3 to pH 7 at 0.5 pH unit increments. The buffered substrate contains 0.2 M sodium acetate, 1% starch and 6 mM NaCl, and the pH was adjusted with concentrated HCl. Therefore the prepared buffer solutions contain the substrate and activator (NaCl) for the reaction.
3. **Sumner Reagent:** this will have been prepared for you by dissolving 30% sodium potassium tartrate in 0.4 M NaOH, then adding 1% dinitrosalicylic acid and heating to dissolve.
4. 7 mM Standard maltose solution.

## METHODS

### 1. Standard Curve

Prepare maltose solutions containing 0, 1, 2, 3, 4, 5, 6, 7  $\mu$ mol maltose/ml by appropriate dilution of the standard maltose solution. Prepare 3.5 ml of each standard solution.

| Tube No. | $\mu$ mol Maltose ( $\mu$ mol/ml) | Maltose standard (ml) | Water (ml) |
|----------|-----------------------------------|-----------------------|------------|
|          |                                   |                       |            |
|          |                                   |                       |            |
|          |                                   |                       |            |
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Withdraw 0.5 ml of each of the maltose concentrations in duplicate and place in 16 test tubes. To each test tube add 0.5 ml Sumner reagent and place in a boiling water bath for 5 minutes. Cool and then add 5 ml water. Mix and read the absorbance at 540 nm and set the spectrophotometer against distilled water.

Record your results in the report section.

**Plot the standard curve as the amount (i.e. not concentration) of maltose (in  $\mu\text{mol}$ ) vs absorbance of maltose.**

2. **Enzyme Assay**

**In a water bath set at 37 °C**, prepare 3 test tubes (2 experimental, one blank) for each pH value, each containing 0.25 ml substrate (27 tubes total). To the first tube, at each pH, add 0.25 ml water (this is the blank without enzyme). To the other two experimental tubes, add 0.25 ml enzyme serially at 10 second intervals (consult your TA about how to do this) and incubate each tube for 5 minutes at 37 °C. Stop the reaction by serially adding 0.5 ml Sumner reagent to all test tubes. Mix and place in a boiling water bath for 5 minutes, cool and add 5 ml water. Read the absorbance at 540 nm as before, setting the instrument against distilled water as for the standard curve. Using the standard curve, calculate the amount of reducing sugar liberated at each pH, ( $\mu\text{mol}$  maltose). From this, calculate the units of enzyme activity at each pH. **One unit of enzyme activity liberates 1  $\mu\text{mol}$  product (reducing units determined as maltose) per minute at 25 °C.** Tabulate your results in the report section. Determine the pH optimum of  $\alpha$ -amylase from a graph of  $\alpha$ -amylase activity vs pH.



Student Number: \_\_\_\_\_ Name: \_\_\_\_\_

Locker number: \_\_\_\_\_ TA: \_\_\_\_\_

Mark awarded: \_\_\_\_\_ / 30

**PRAC 13:**      **Report**

**This must be shown to your TA before you leave the laboratory. Some of the questions should be done before you enter the laboratory.**

1. If you wished to assay an enzyme that you know nothing about, what types of experiments should you do to establish optimum conditions? (3)

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2. Describe how you would carry out these experiments. (3)

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3. What is meant by "*the pH optimum of an enzyme*"? (1)

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4. What factors contribute to an enzyme functioning best at a particular pH? (3)

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5. What factors contribute to an enzyme functioning best at a particular temperature? (2)

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6. Write down the reaction catalysed by  $\alpha$  - amylase. (1)

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7. Explain how you will be measuring the amount of product formed in the reaction. (1)

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8. Draw a flow diagram showing how you will be carrying out the experiment. (5)

9. Explain why Sumner reagent can be used to stop the reaction.

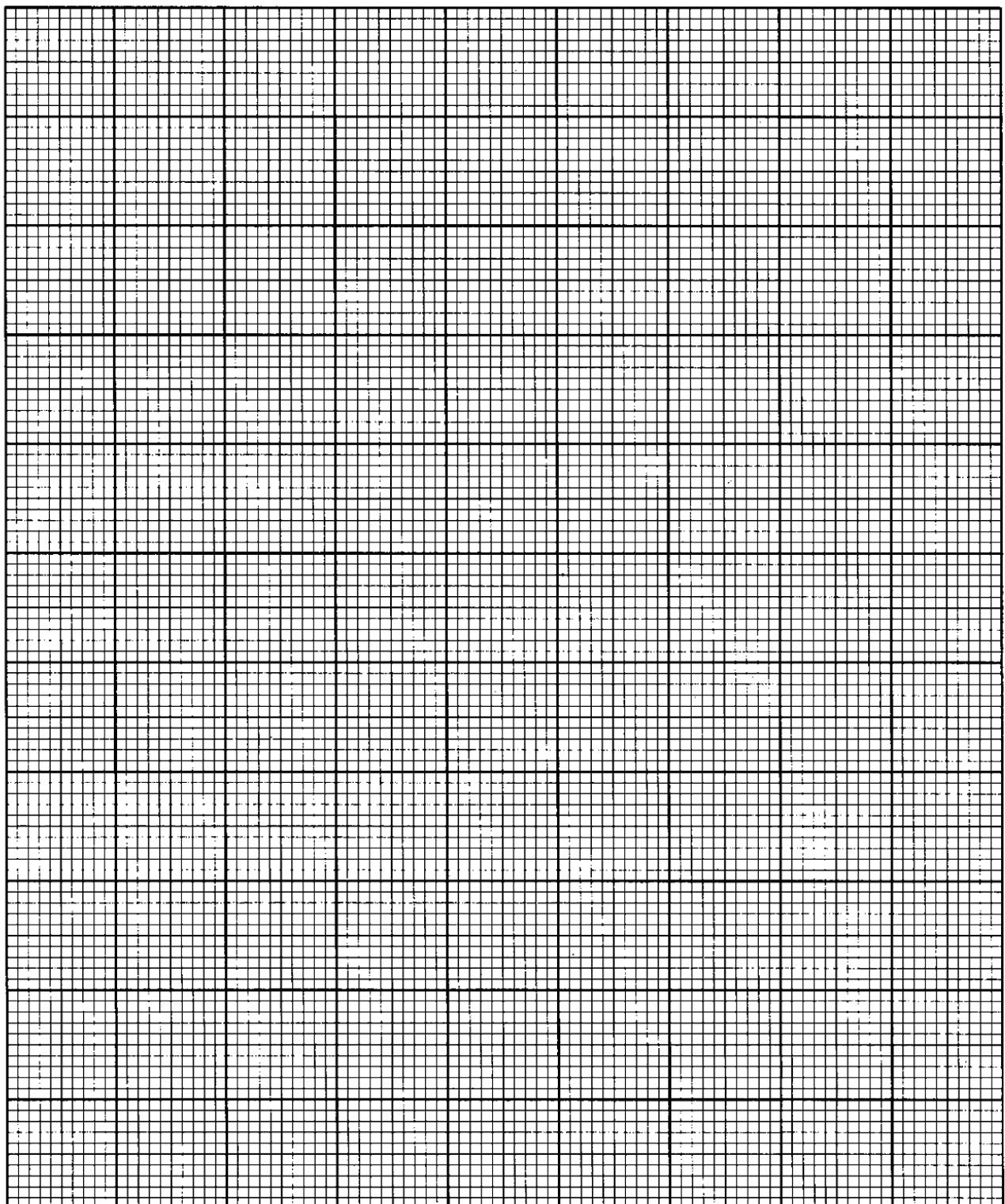
(1)

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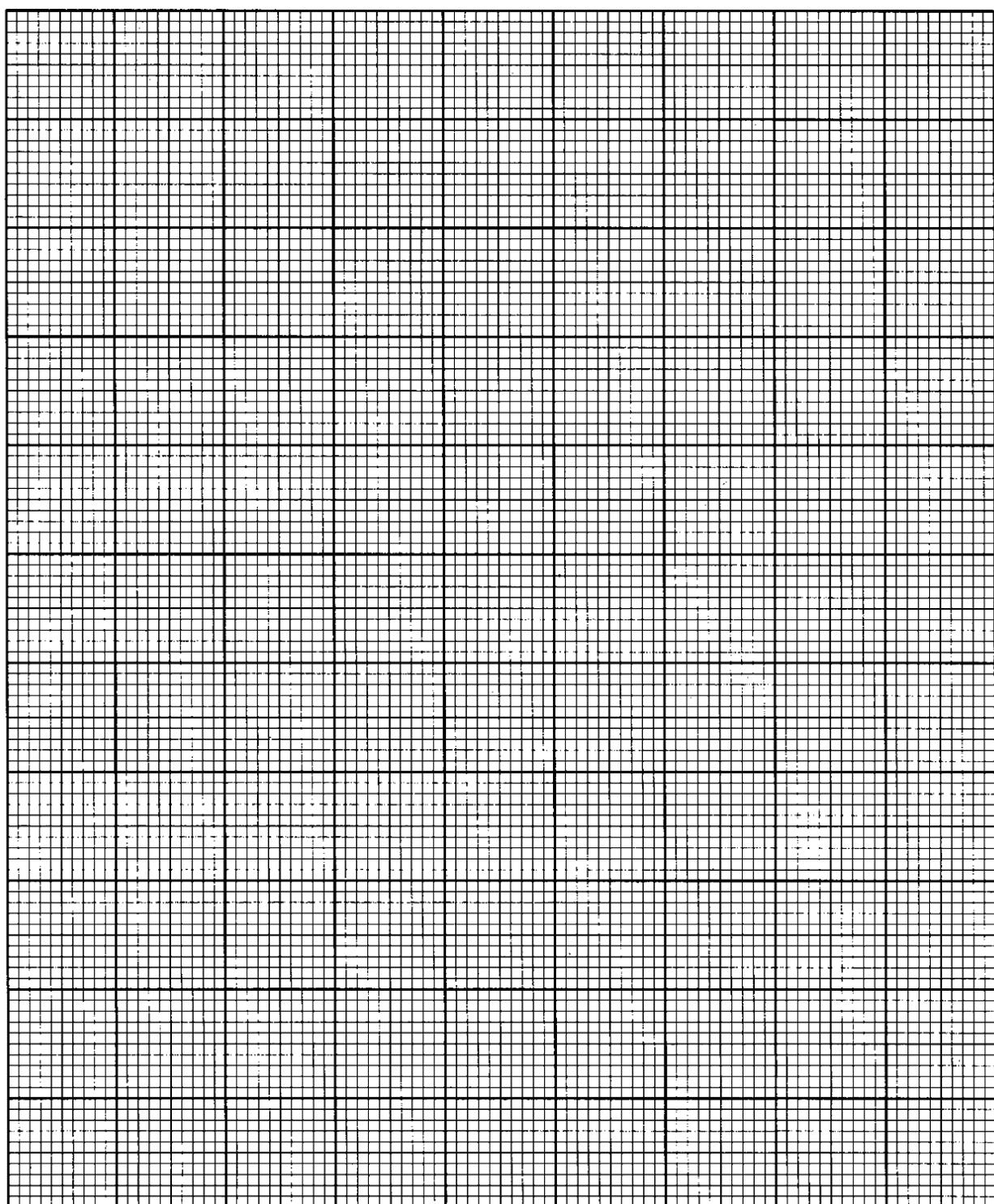
10. Present the data obtained for the Maltose Standard Curve in a table and draw the standard curve for maltose. Use it to determine the amount of maltose formed in each assay mixture. (5)



2cm/2mm squares

11. Draw a graph showing the pH optimum of  $\alpha$  – amylase.

(5)



2cm/2mm squares



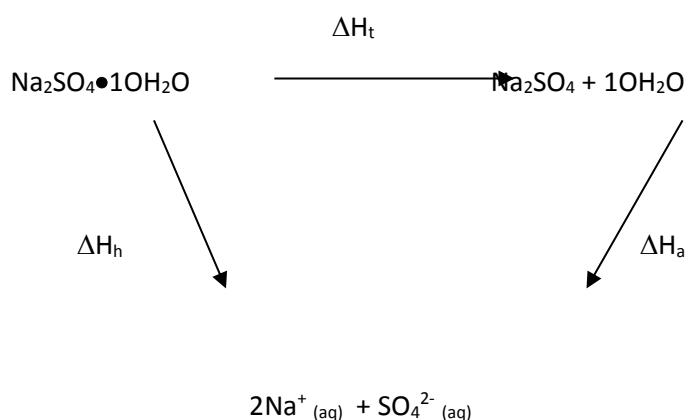
## **PRACTICAL 14: THE CALORIMETRIC DETERMINATION OF ENTHALPY AND ENTROPY CHANGES**

### **BACKGROUND**

The process under investigation is the thermal decomposition of sodium sulfate decahydrate:



The enthalpy of this process may be calculated from knowledge of the enthalpies of solution of the hydrated and anhydrous species by applying Hess' Law. Thus:



The enthalpies of solution of the hydrated salt,  $\Delta H_t$ , can be measured by simple calorimetry. Then,

$$\Delta H_t = \Delta H_h - \Delta H_a$$

Assuming  $\Delta H_t$  is independent of temperature over the range concerned, the entropy of the transition can be evaluated from  $\Delta H_t$  and the transition temperature (in kelvin not  $^\circ\text{C}$ ). The transition temperature ( $T_{\text{trans}}$ ) is obtained by plotting a cooling curve of the system as it passes from one form to another.

$$\Delta S_{\text{trans}} = \frac{\Delta H_{\text{trans}}}{T_{\text{trans}}}$$

## MATERIALS

1. Sodium sulfate decahydrate - solid
2. Sodium sulfate anhydrous. (Heated to 110 °C and cooled in a desiccator overnight).

## PROCEDURE

### **(i) Transition Temperature of Sodium Sulfate Decahydrate**

Secure a boiling tube in a retort stand and in the tube place an accurate thermometer and about 5 g of sodium sulfate decahydrate. Gently heat the tube with a small Bunsen flame (**NB: STIR WITH A GLASS ROD - NOT THE BULB OF THE THERMOMETER**) until the crystals melt and the temperature reaches about 50 °C. Remove the heat and record the temperature every three minutes. (The time and temperature should be tabulated in your report). At around 32 °C the temperature should become constant and remain constant for some time. (NB: The temperature may continue to fall below 32 °C without becoming constant. If this happens, wait until the temperature reaches 29 °C, add a few crystals of the decahydrate and continue recording the temperature. Record all your observations and give a full explanation in your discussion section. When the temperature does become constant (say, for more than ten minutes) set the apparatus aside and periodically check the temperature (say every fifteen minutes, but record the exact time). This temperature is the transition temperature and must be recorded as accurately as your thermometer will permit. In the meantime, proceed with part (ii). Plot a graph of temperature **vs.** time and determine the temperature of the transition, which is represented by the flat portion of the curve.

### **(ii) Enthalpy of Transition**

Determine the water equivalent of your calorimeter (which consists of a glass beaker held in an insulated container) as follows. Weigh exactly 50 g ( $\pm 0.05$  g) of distilled water into a beaker and place the beaker in the insulated container. Pack some polystyrene pieces around the beaker to minimize heat losses. Record the temperature ( $t_1$  °C) of the water. Weigh another 50 g (again  $\pm 0.05$  g) of distilled water into a conical flask and heat this with constant stirring to about 35 °C. Remove the heat and record the temperature ( $t_2$  °C). Pour this water immediately into the calorimeter (taking care not to splash any), stir rapidly and record the temperature ( $t_3$  °C) when steady. Empty and dry the apparatus.

Weigh 100 g ( $\pm 0.1$  g) of distilled water at room temperature into a beaker and assemble the calorimeter. Record the temperature. Add about 4 g (it need not be precisely 4.00 g, but the actual weight must be known exactly i.e.  $\pm 0.01$  g) of sodium sulfate decahydrate to the calorimeter, stir rapidly with a glass rod and record the temperature as soon as it becomes steady. Empty and dry the apparatus.

Repeat the procedure described above by using: 2 g **AND** 1 g of sodium sulfate decahydrate.

Now repeat the above procedure using: 1 g, 1.5 g **AND** 2 g of anhydrous sodium sulfate.

**Repeat** the solution enthalpy determinations **three times** using different weights of solids, and check that your results are consistent.

## **RESULTS**

The water equivalent of the calorimeter is a measure of the heat required to bring the calorimeter itself to thermal equilibrium with its contents. It is conveniently expressed in calculations as an extra amount of water that requires heating. In this determination, the heat lost by the warm water must equal the heat gained by the cold water plus the heat gained by the calorimeter. Assuming that the specific heat of water is  $4.184 \text{ J g}^{-1}\text{K}^{-1}$  and that the calorimeter is equivalent to a mass, w, of water:

$$50 \times 4.184 \times (t_2 - t_3) = w \times 4.184 \times (t_3 - t_1) + 50 \times 4.184 \times (t_3 - t_1)$$

where w is the water equivalent of the calorimeter in grams.

The left hand side of the equation represents the heat loss by the warm water (mass x specific heat x change in temperature) and the right hand side is the heat gained by the calorimeter plus the heat gained by the cold water.

Using the principles above, calculate the heat evolved ( $\Delta H$ ) in joules **per gram** of solid, and then the enthalpy of solution in joules **per mole** for each of the sodium sulfate species.

Calculate the standard deviation and the **standard error** of your mean result for  $\Delta H$ .

Remember to use the mass of the **solution** and not just the mass of water in your calculations. That is, the heat evolved or absorbed (take care with the + and - signs) will equal the mass of solution x specific heat x temperature change plus w x specific heat x temperature change.

You may assume that the specific heat of the solution is the same as that of water. Determine also the mean temperature at which you have determined the enthalpy of solution.

From your results calculate the molar enthalpy change and the molar entropy change of the transition. Assuming  $\Delta H_t$  and  $\Delta S_t$  are constant over the temperature concerned; calculate the free energy change of the process at 10 °C, the **transition temperature**, and at 50 °C.

## **DISCUSSION AND CONCLUSIONS**

Critically assess the experiment you have performed and comment on any improvements that could be

made. Explain as far as you can all your observations and such procedures as the necessity for accurate weighing in some cases part (ii) and not others part (i). Why is it necessary to dry the anhydrous sodium sulfate by heating and cooling in a desiccator? Comment on the free energy changes at different temperatures and explain what the results mean in terms of the stability of the two species.

## **QUESTIONS**

1. Define the following terms:

State function

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Closed system

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Isobaric process

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2. Anhydrous sodium sulfate is often used to dry organic liquids. Explain why you think it is effective and discuss any limitations it may have in this respect.

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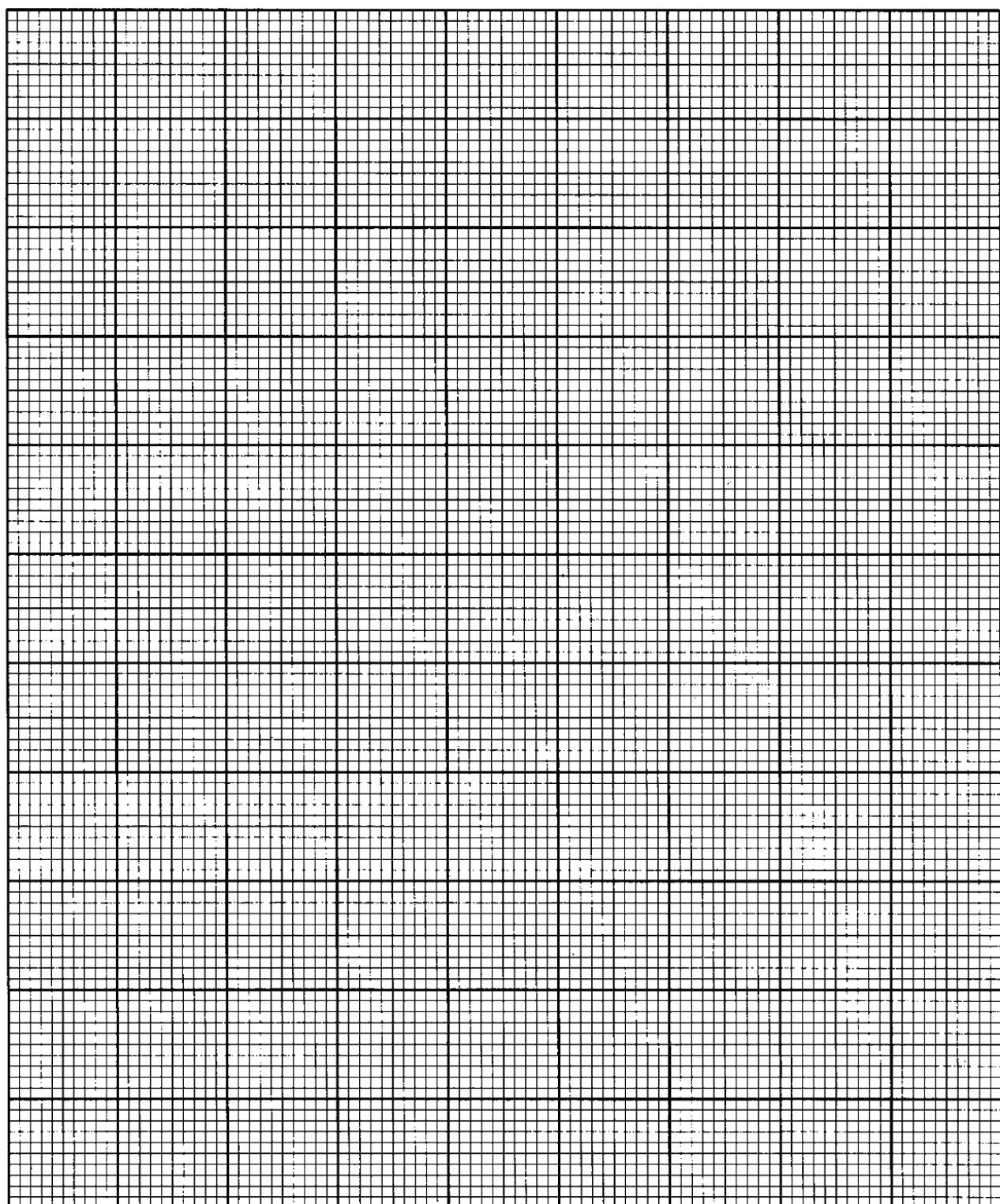
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3. Plot a graph of your calculation of  $\Delta G$  at 10 °C and 50 °C showing standard error bars for the two points. Confirm that the straight line between your two points passes through  $\Delta G = 0$  at the transition temperature.



2cm/2mm squares

## **PRACTICAL 15: ALCOHOLIC FERMENTATION**

### **References:**

1. Matthews and von Holde (1996) in Biochemistry (2e), Benjamin/Cummings Pub, CA.
2. Dickley, J (1997) in Laboratory Investigations for Biology (Customized for University of Saint Thomas), Addison-Wesley Inc

### **Objectives:**

At the end of this practical students should be able to:

1. Describe the process of alcoholic fermentation, and be able to list the enzymes, co-enzymes, substrates and products involved.
2. Describe how substrates other than glucose enter the glycolysis pathway.
3. Draw from memory the reactions involved in glycolysis, as well the regulatory sites of this pathway.
4. Discuss the principles behind the measurement of alcoholic fermentation.
5. Apply the principles of experimental design to design and perform an experiment to investigate a factor that affects alcoholic fermentation.

### **Introduction:**

Glycolysis is a multi-step pathway that converts one molecule of glucose to 2 molecules of pyruvate, with the concomitant generation of 2 molecules of ATP and reducing equivalents in the form of NADH. Because storage polysaccharides or oligosaccharides are broken down to glucose, other hexose sugars, or sugar phosphates, which are all able to enter the glycolytic pathway, the initial substrate does not have to be glucose *per se*.

Glycolysis is an ancient metabolic pathway that was probably used by the earliest known bacteria, under strictly anaerobic conditions. If the pathway is to operate anaerobically, NADH must be re-oxidised to  $\text{NAD}^+$  by transferring electrons to an electron acceptor, so that a steady state is maintained. The most straightforward route is simply to use NADH to reduce pyruvate to lactate by the enzyme lactate dehydrogenase. This reaction is carried out by the lactic acid bacteria when milk sours and in the manufacture of cheese (1).

**Fermentation is defined as an energy yielding metabolic pathway that involves no net change in oxidation state** (1). Therefore, glycolysis, operating anaerobically, can be considered to be fermentation. Another important fermentation, which is carried out by yeasts, occurs when pyruvate is converted to acetaldehyde and  $\text{CO}_2$ , and the acetaldehyde is then reduced to ethanol by alcohol dehydrogenase. This fermentation is obviously useful when making alcoholic beverages, but is also used in baking, when the  $\text{CO}_2$  causes the bread to rise and the ethanol evaporates during baking. For many years, glycolysis was exploited when brewing, or using yeasts to bake bread, but it was only understood in the 20<sup>th</sup> century.

However, Pasteur's demonstration in 1856, that fermentations are carried out by micro-organisms, can be considered a milestone in the history of science (1).

You will be investigating the fermentation performed by yeast, using corn syrup, which contains sucrose and fructose, as a substrate. The rate of alcoholic fermentation will be measured by collecting CO<sub>2</sub> at intervals after fermentation has begun.

#### Apparatus:

The apparatus used to collect the CO<sub>2</sub> as well as how to set it up is shown in the figures below.

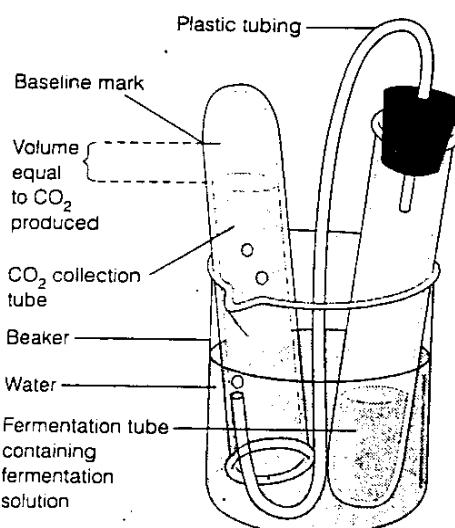


Fig 1: Apparatus for measuring CO<sub>2</sub> production in alcoholic fermentation. (Taken from 2)

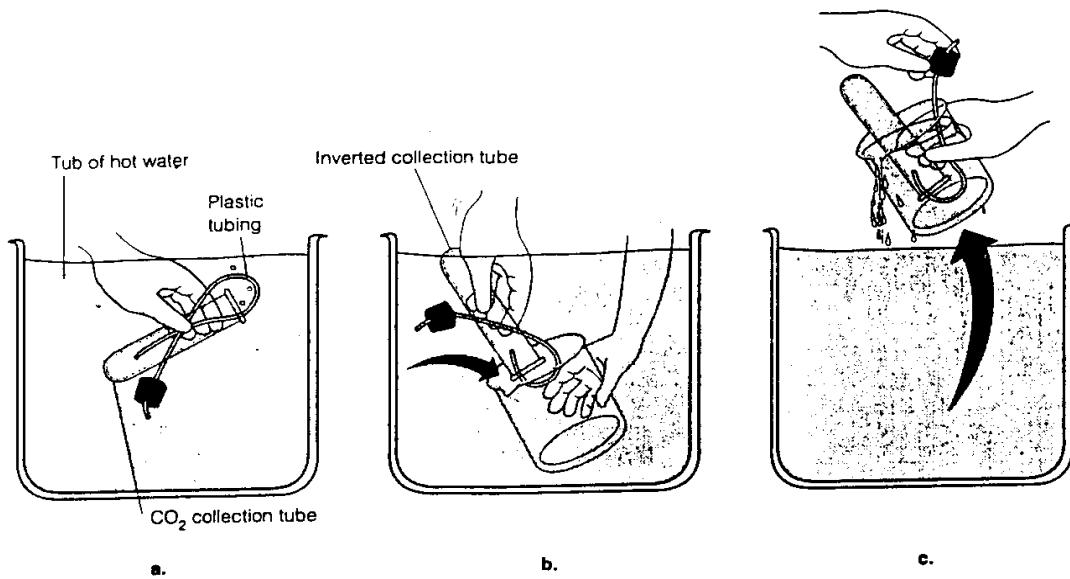


Fig 2: Setting up the CO<sub>2</sub> collection tube. (Taken from 2)

Fill a tub or sink with hot water (50 °C – 60 °C). Insert the end of the plastic tubing into one of the test tubes. This tube will be the CO<sub>2</sub> collection tube. Submerge the collection tube and plastic tubing in the tub of hot water. Submerge the beaker. Place the collection tube in the beaker in an inverted position. Bring the beaker out of the water. One end of the plastic tubing should still be inserted in the collection tube. Hold the other end of the tubing (the one with the rubber stopper on it) so that the water won't be

siphoned out. Pour some water out of the beaker so that the water level is at least 1 cm from the top of the beaker. *Check the tubing for kinks.*

**Procedure:**

1. Set up, as shown above, 2 sets of apparatus for measuring the CO<sub>2</sub> produced by fermentation.
2. In a test tube, mix together (by gently swirling the test tubes), 3 ml of yeast suspension (5 g in 500 ml) and 4 ml of corn syrup (6 g sugar in 12 ml H<sub>2</sub>O).
3. Set up a control test tube for your experiment.
4. Place one test tube in each beaker.
5. Put rubber stoppers in the fermentation tubes. This will force most of the water out of the tubing.
6. After the air bubbles from inserting the stopper have cleared the tubing (half a minute to a minute), mark the water level on each collection tube with a wax pencil. This marks the baseline for your experiment.
7. At 5 minute intervals, measure, in mm, the distance from the baseline mark to the water level. Continue taking data for at least 20 minutes.
8. Plot a graph showing the rate of CO<sub>2</sub> formation in fermentation.
9. Repeat the experiment, using it to investigate one factor, which you think could affect the fermentation rate. (Hint: As fermentation is carried out by enzymes, any factor which affects the performance of an enzyme should theoretically affect the fermentation rate.) If you require any additional apparatus or materials, check with the laboratory technician if these will be available.



## **PRACTICAL 15: PREPARATION**

1. Draw a diagram of the glycolysis pathway from glucose to pyruvate, showing the enzymes which catalyse each reaction and the structures of the key intermediates.  
(NB: Make sure that you know this diagram and any subsequent diagrams **before** you enter the laboratory).

2. Write down the reactions, carried out by yeasts, in which pyruvate is converted to ethanol and  $\text{CO}_2$ . Show the structures of the substrates and products and show which enzymes catalyse the reactions.

3. List the enzymes catalysing regulatory points of glycolysis.

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4. Show the positions of the 6 glucose carbons in the two lactate molecules formed by anaerobic glycolysis.

5. Show (by means of a diagram) how fructose enters the glycolytic pathway in liver and in muscle and adipose tissue.

6. Write a balanced chemical equation for the fermentation of one glucose molecule in starch to ethanol, with the initial cleavage involving  $\alpha$ -amylase.
7. Suppose it were possible to label glucose with  $^{14}\text{C}$  at any position or combination of positions. For yeast fermenting glucose to ethanol, which form/forms of labeled glucose would give the most radioactivity in  $\text{CO}_2$  and the least in ethanol?

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8. Draw up tables for data collection.

**REPORT PRACTICAL 15:**

**This report must be signed by your teaching assistant before you leave the laboratory.**

1. What was your hypothesis when investigating a factor that could affect the rate of fermentation?

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2. What was the dependent variable?

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3. What was the independent variable? Explain why you think this independent variable will affect alcoholic fermentation.

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4. What was your control?

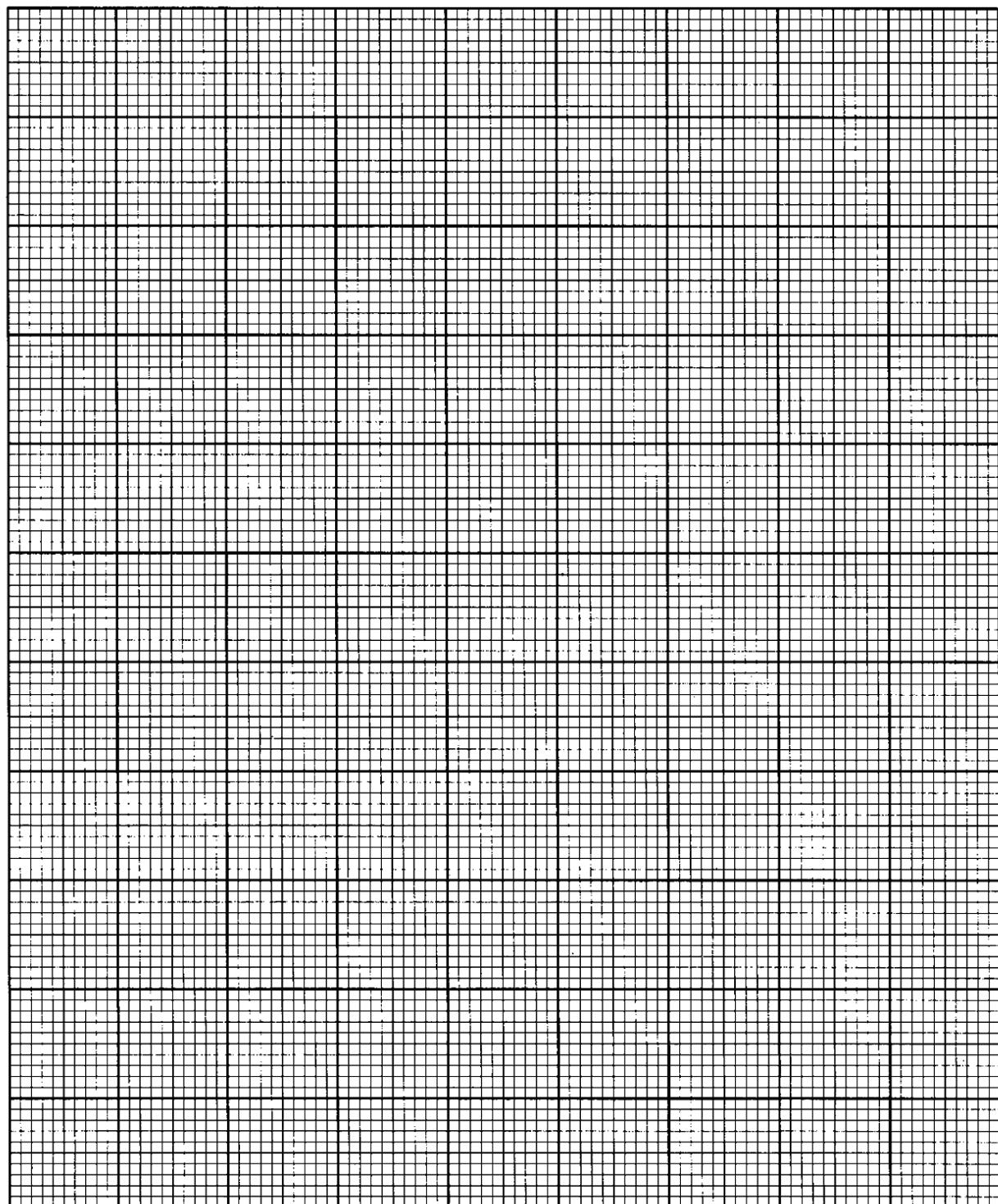
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5. Present the results of your experiments in a table.

6. Draw a graph showing how the rate of CO<sub>2</sub> formation in fermentation is affected by the factor you investigated.



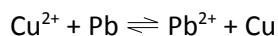
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## PRACTICAL 16 : THE USE OF REDOX TO DETERMINE THERMODYNAMIC PARAMETERS

### **BACKGROUND**

The thermodynamic constants for the reaction:



can conveniently be measured using an electrochemical cell. At constant pressure, the cell voltage (E) is related to the free energy change ( $\Delta G$ ) of the reaction by the expression:

$$\Delta G = -n \times F \times E$$

where F is Faraday's constant and n is the number of moles of electrons transferred in the cell reaction.  
The change in entropy ( $\Delta S$ ) is related to free energy by:

$$\Delta S = \left[ \frac{\delta G}{\delta T} \right] p$$

where T is the absolute temperature in kelvin (K). Thus,

$$-\Delta S = \left[ \frac{\delta(nFE)}{\delta T} \right] p$$

$$\Delta S = nF \left[ \frac{\delta E}{\delta T} \right] p$$

So, if the voltage of the cell is measured at different temperatures, the entropy change of the reaction, and hence the free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ) and equilibrium constant (K) may be calculated. The pressure, which is atmospheric, is assumed constant throughout the experiment.

## **MATERIALS**

### **Experiment A:**

1. Agar
2. Potassium nitrate - solid
3. 0.5 M Lead nitrate
4. 0.5 M Copper sulfate
5. 0.25 M Lead nitrate/0.5 M Potassium nitrate

### **Experiment B:**

1. Agar
2. Potassium nitrate - solid
3. 0.25 M Lead nitrate
4. 0.25 M Copper nitrate

## **PROCEDURE**

A simple cell may be constructed using Pb/Pb<sup>2+</sup> and Cu/Cu<sup>2+</sup> half-cells connected by a salt bridge. In practice, a third compartment is employed to prevent the sulfate ions, which migrate from the copper sulfate solution, precipitating the lead in the other half of the cell.

Prepare the salt bridges as follows. Dissolve 0.25 g of agar and 2.5 g of potassium nitrate in 20 ml of water and heat with stirring until the mixture thins, thickens, and thins again. Pour the solution into each of the two U-tubes, which should first be rinsed with hot water. Make sure no air bubbles are trapped and that the tubes are completely full, in fact, with a convex meniscus. Set aside to cool.

Clean the lead and copper electrodes thoroughly with abrasive paper. It is essential that fresh metal be exposed or the cell will not work.

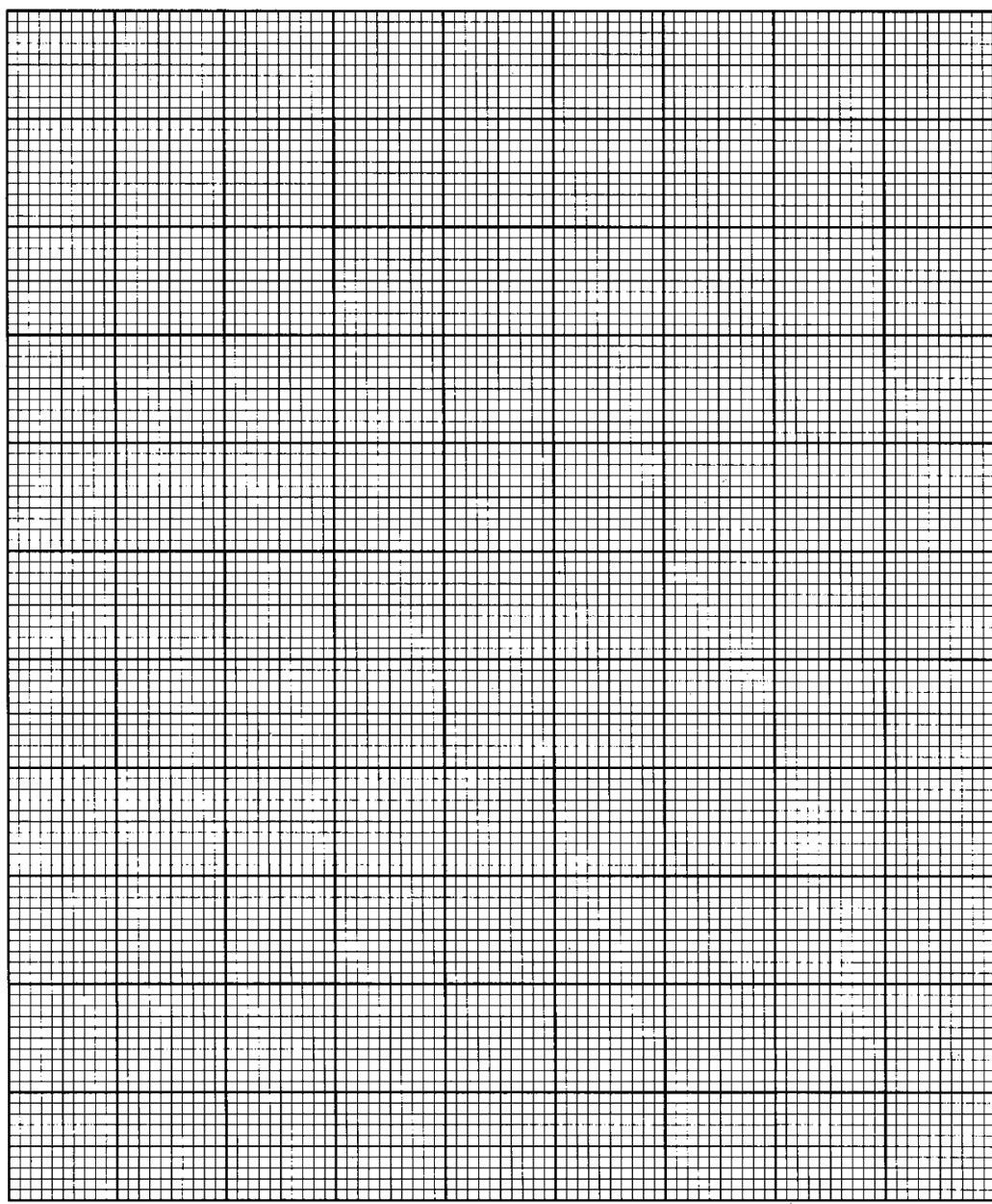
Pour 20 ml of 0.5 M lead nitrate solution into the first compartment of the cell and put in the lead electrode. Into the second compartment, place the copper electrode and 20 ml of 0.5 M copper sulfate solution. Into the third compartment, place 20 ml of the 0.25 M lead nitrate/0.5 M potassium nitrate solution. Place the salt bridges across the first and third, and second and third, compartments. The two half-cell compartments must not be connected directly.

Place the cell in a beaker containing ice-water and allow the temperature to stabilise. Measure the cell temperature by inserting a thermometer into the third compartment of the cell. Connect the electrodes to the milli-voltmeter. Read and record the voltage, and disconnect the electrodes. Heat the water with a Bunsen burner and record the voltage at several temperatures up to approximately 70 °C. Make sure the temperatures are constant when voltage readings are taken.

**Repeat** the experiment using 0.25 M lead nitrate and 0.25 M copper nitrate.

## RESULTS

Plot a graph of voltage **vs.** temperature (in kelvin, K), which should be a straight line, and from it calculate the entropy change of the reaction. Read off the voltage at 298 K which will be the standard e.m.f. ( $E^\circ$ ) of the cell. Hence, calculate  $\Delta G^\circ$ ,  $\Delta H^\circ$  and the equilibrium constant ( $K_{eq}$ ) for the reaction.



## **DISCUSSION AND CONCLUSION**

Discuss the thermodynamic parameters you have obtained in terms of the cell reaction, i.e. the favourable direction, point of equilibrium etc. Compare your results with accepted values given in physical chemistry texts. Explain why  $E^\circ$  can be read directly from the graph. Does the choice of anion make a difference, and does change in concentration make a difference?

## **QUESTIONS**

What would happen if

- (i) powdered lead were added to copper nitrate solution?

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## **PRACTICAL 17: THE EFFECT OF DETERGENTS ON PROTEIN DETERMINATION IN CELL EXTRACTS.**

### **INTRODUCTION**

The development of techniques and methods for the separation and purification of biological macromolecules such as proteins has been an important prerequisite for many of the advancements made in bioscience and biotechnology over the past three decades. However, not all problems in protein purification are solved by the acquisition of sophisticated laboratory equipment. Difficulties still remain in finding optimum conditions for protein extraction and sample pre-treatment as well as in choosing suitable methods for monitoring protein and biological activity. In most cases, one has to extract the desired activity from a tissue or a cell. This means that a considerable number of contaminating molecular species are set free and that proteolytic activity will make the preparation work more difficult. The extraction of a particular protein from a solid source often involves a compromise between recovery and purity. Optimisation of extraction conditions should favour the release of the desired protein leaving the contaminants behind. Of particular concern is to find conditions under which the already extracted protein is not degraded or denatured while more is being released.

To arrive at a suitable composition for the extraction medium one must first study conditions where the protein of interest is stable and, secondly, where it is most efficiently released from the cells or tissue. The following factors have to be taken into consideration:

- i) *pH*. Normally the pH-value chosen is that of maximum activity of the protein. However, it should be noted that this is not always the pH that gives the most efficient extraction, nor is it necessarily the pH of maximum stability.
- ii) *Buffer salts*. Most proteins are maximally soluble at moderate ionic strengths, 0.05-0.1, with these values being chosen if the buffering capacity is sufficient.
- iii) *Detergents*. In many extractions the desired protein is bound to membranes or particles, or is aggregated due to its hydrophobic character. In these cases, one can reduce the hydrophobic interactions by using a detergent. Some of the commonly used detergents are listed in the table below. Several of them do not denature globular proteins or interfere with their biological activity. Others, such as SDS, will do that. Quite often it is not necessary to continue using a detergent in the buffer after the first step(s) in the purification. However, in other cases, it is necessary to use a detergent at all times.

| Detergents used for solubilisation of proteins |                 |                     |
|--|-----------------|---------------------|
| Detergent                                      | Ionic character | Effect on protein   |
| Triton X-100                                   | non-ionic       | mild non-denaturing |
| Nonidet P 40                                   | non-ionic       |                     |
| Lubrol PX                                      | non-ionic       |                     |
| Octyl glucoside                                | non-ionic       |                     |
| Tween 80                                       | non-ionic       |                     |
| Sodium deoxycholate                            | anionic         |                     |
| Sodium dodecyl sulfate (SDS)                   | anionic         |                     |
| CHAPS  | zwitter-ionic   | strong denaturing   |

Detergents are known to cause interference with certain assay procedures, in particular assays designed to measure protein concentration in extracted cells or tissues. The commonly used methods of assaying protein are listed in the following table.

| Comparison of commonly used methods for assaying protein |                       |                           |
|--|-----------------------|---------------------------|
| Method   | Chemical interference | Protein/protein variation |
| Lowry  | Great                 | Significant               |
| Biuret   | Moderate              | Low                       |
| Kjeldahl   | Moderate              | Low                       |
| Bradford   | Slight                | Significant               |
| Absorbance at 280 nm                                     | Moderate              | Significant               |

The Bradford protein assay is a dye-binding assay based on the differential colour change of a dye in response to various concentrations of protein. This assay is often the method of choice for several reasons but nevertheless may still suffer from interference by detergents. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs.

## AIM

In this practical you will determine the effect that different concentrations of sodium dodecyl sulfate (SDS) have on the assay of proteins extracted from NRK cells.

## MATERIALS AND METHODS

Each group will be provided with a selection of the following:

1. - a solution of known protein concentration (casein)
  2. - a solution of protein extracted from NRK cells in buffer (20 mM Tris-HCl; pH 7.6) only
  3. - a solution of protein extracted from NRK cells in buffer containing 0.05% SDS
  4. - a solution of protein extracted from NRK cells in buffer containing 0.1% SDS
  5. - a solution of protein extracted from NRK cells in buffer containing 0.5% SDS
  6. - a solution of protein extracted from NRK cells in buffer containing 1.0% SDS
- Spectrophotometer capable of measurement at 595 nm
  - Cuvettes 1 cm path length cuvettes. Polystyrene cuvettes are recommended.
  - Test tubes
  - Vortex mixer
  - Appropriate micropipettes

Use the stock casein solution (0.5 mg/ml) to set up a four point (1 ml final volume) standard curve by completing the following table.

| Final concentration of casein | Volume of casein stock | Volume of buffer | Absorbance reading |
|-------------------------------|------------------------|------------------|--------------------|
| Blank                         |                        |                  |                    |
| 0.1 mg/ml                     |                        |                  |                    |
| 0.075 mg/ml                   |                        |                  |                    |
| 0.05 mg/ml                    |                        |                  |                    |
| 0.025 mg/ml                   |                        |                  |                    |

MAKE SIMILAR DILUTIONS OF EACH OF THE CELL EXTRACTS USING THE CORRECT SDS-CONTAINING BUFFER

### Assay procedure:

- Place 0.1 ml of each standard and appropriately diluted samples in clean, dry test tubes
- Place 0.1 ml sample buffer in "blank" test tube
- Add 2.9 ml diluted dye reagent - NB!! Handle the dye reagent carefully as it contains phosphoric acid
- Vortex (avoid excess foaming); or mix several times by gentle inversion of the test tube
- Wait 10 minutes for the colour reaction to develop fully
- Measure the OD<sub>595</sub> versus the reagent blank

**NB:** Add your standard curve readings to the class spreadsheet and average the readings.



**Answer the questions in the space provided. The completed questions must be handed to your lecturer/TA at the next practical. It is your duty to make sure that your submission is recorded by the lecturer/TA.**

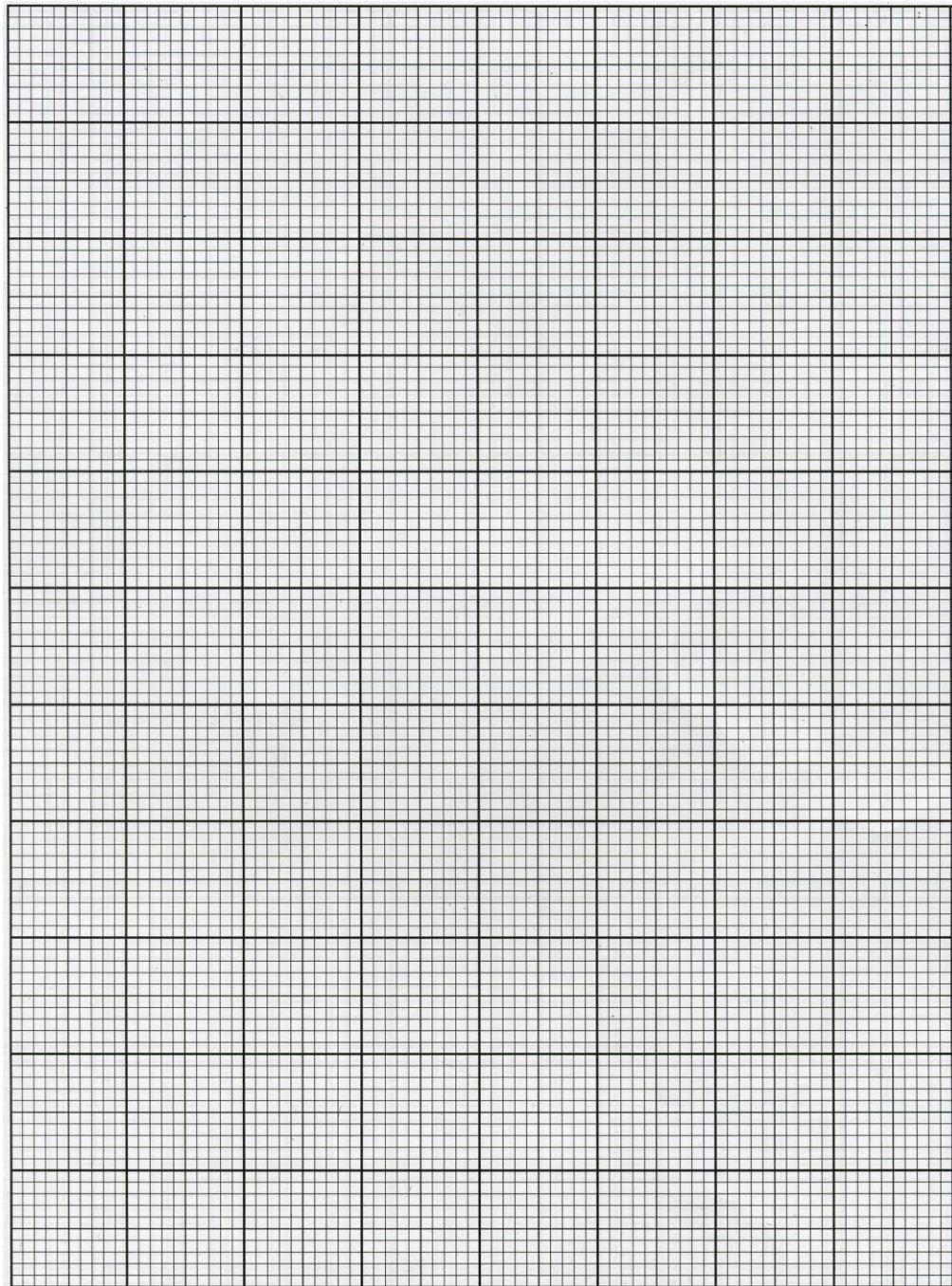
**REPORT PRACTICAL 17: The effect of detergents on protein determination in cell extracts.**

Name: .....

Student No: .....

1) Plot OD<sub>595</sub> versus protein concentration

[5]



- 2) Explain the interaction that SDS has with protein. How does this differ for a non-ionic detergent e.g. Triton X-100? [5]

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- 3) Is the standard curve linear over the concentrations tested? [1]

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- 4) Explain how the graphs of the data identify whether SDS has an effect on the Bradford method of protein determination? What is this effect? [4]

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- 5) Explain how the concentration of SDS influences the determination of protein by the Bradford method. [3]

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6) What is the max SDS concentration you would advise researchers to use in a determination of this nature? [1]

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7) Some groups did not get a clear-cut difference in the colour reaction. Provide a suggestion for the reason behind this. [3]

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## **PRACTICAL 18: ADHESION OF EUKARYOTIC CELLS**

### **INTRODUCTION**

All cells must interact with molecules in their environment, in most cases using cell surface adhesion proteins to bind these molecules. Multicellular organisms are particularly dependent upon adhesion of cells to each other and the extracellular matrix (ECM). During development, a carefully regulated programme of cell-cell and cell-matrix interactions specifies the architecture of each tissue and organ. The advantages of cultivating cells and tissues as model systems *in vitro* have long been recognised. Depending on their origin, animal cells grow either in suspension or as an adherent monolayer. Suspension cells - can survive and proliferate without being attached to a substrate. Haematopoietic cells, as well as some transformed cell lines and some cells derived from malignant tumours can be grown in suspension. Adherent cells - are anchorage dependent and propagate as a monolayer attached to the cell culture vessel. This attachment is essential for proliferation and many adherent cell cultures will stop proliferating once the surface of the culture vessel is filled with cells. Continuous cell lines are capable of unlimited proliferative potential and are thus generally easier to work with than primary or finite cell cultures. However, it should be remembered that continuous cell lines have usually undergone some genetic alteration and their behaviour *in vitro* may not represent the *in vivo* situation. In order to perpetuate continuous cell lines, the cells need periodically to be detached from the surface of the culture vessel thus permitting them to be subcultured. Cells in culture generally develop the same adhesive interactions as they would *in vivo*. That is, their capacity for adhesive interactions is defined by the selective expression of plasma membrane receptors called cell adhesion molecules (CAM's). In general, the several families of CAM's can be clustered into four groups - the cadherins, the integrins, the immunoglobulin family and the selectins. Since the cells that you will be dealing with in this practical exercise are rat kidney epithelial cells the first two groups of adhesion molecules are of particular importance. The cadherins strongly prefer to bind to themselves, thus promoting the adhesion of like cells (see your lecture notes for further details). The integrins bind a variety of ligands such as the matrix molecules collagen and fibronectin. Therefore, it is obvious that a critical step in the subcultivation of cells (or for counting or nucleic acid extraction) is their detachment from one another as well as from the surface. In the past, various enzymes and chelating agents have been used separately for this purpose, but more recently a combination of the two provides a quick and efficient method of cell detachment.

### **AIM**

In this practical you will make a comparison between using trypsin alone, ethylene diamine tetra- acetic acid (EDTA) alone and a combination of the two, to effect detachment of substrate-dependent epithelial cells.

## MATERIALS AND METHODS

Each group will be provided with 3 dishes of NRK epithelial cells -

- 1 to be used for trypsin alone
- 1 to be used for EDTA alone and
- 1 to be used for a combination of trypsin and EDTA

Treat each dish as follows :

- aspirate the medium and discard
- wash the cells with 2 ml phosphate buffered saline (PBS) by gentle swirling
- discard the PBS and repeat the above step
- discard the second PBS wash
- add 1 ml of pre-warmed trypsin or EDTA or trypsin/EDTA mix to dishes 1, 2 and 3, respectively
- the addition of these solutions is taken as time zero ( $T_0$ )
- incubate the dishes at room temperature on the desk top with occasional swirling
- carefully make a note of
  - i) the time it takes for the cells to become detached from the surface of the dish, for each of the three treatments.
  - ii) after 1 hour on the desk carefully attempt to assess the percentage detachment of the cells from the substrate.

Viewing the dish on a dark (preferably matt black) background will allow you to see detached cells floating in the medium.

### **IMPORTANT**

Add your data to class spreadsheet and calculate an average time-of-detachment for each of the treatments.

**Answer the questions over the page in the space provided. The completed questions must be handed to your lecturer/TA at the next practical. It is your duty to make sure that your submission is recorded by the lecturer/TA.**

**REPORT PRACTICAL 18: Adhesion of Eukaryotic cells**

Name: \_\_\_\_\_ Student No: \_\_\_\_\_

- 1) What is the fundamental basis to the phenomenon known as "contact inhibition of growth"? [5]

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- 2) Describe the differences in the way each of the treatments effected detachment (how did the cells detach from the substrate in each case)? [3]

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- 3) Relate the differences noted above to the mode of action of the treatment used. [3]

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4) Suggest 2 ways in which the process could be made even more rapid.

[2]

1. \_\_\_\_\_
2. \_\_\_\_\_

5) What dangers should a person be aware of when employing different detachment techniques for cell passaging?

[2]

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## **PRACTICAL 19: THE EFFECTS OF EXTRACELLULAR MATRIX (ECM) ON CELL ADHESION**

### **INTRODUCTION**

In addition to the distinctly cellular characteristics, most cells are also characterised by extracellular structures formed from materials that the cells transport outward across the plasma membrane. For animal cells, these structures are called the extracellular matrix (ECM) and consist primarily of protein fibres and proteoglycans. The extracellular matrix of animal cells forms a variety of structures, depending on the cell type. The primary function of the ECM is support, but the kinds of extracellular materials and the patterns in which they are deposited may regulate such diverse processes as cell motility and migration, cell division, cell recognition and adhesion, and cell differentiation during embryonic development. Despite this diversity of function, the ECM of animal cells almost always consists of the same three classes of molecules: (1) structural proteins such as collagens and elastins, which give the ECM its strength and flexibility and provide some sites for cell attachment; (2) protein-polysaccharide complexes called proteoglycans that provide the matrix in which the structural molecules are embedded; and (3) adhesive glycoproteins such as the fibronectins and laminins, which attach cells to the matrix. The considerable variety in the properties of the ECM in different tissues results not only from differences in the types of structural proteins and the kinds of proteoglycans present, but also from variations in the ratio of structural proteins (most commonly collagen), to proteoglycans and in the kinds and amounts of adhesive glycoproteins present.

### **AIM**

In this practical you will make a comparison between various ECM molecules with respect to their ability to enhance cell attachment in vitro.

### **MATERIALS AND METHODS**

Each group will be provided with the following:

- 1 dish of growing NRK epithelial cells
- 1 new untreated plastic cell culture dish
- 1 new plastic cell culture dish that has had the growing surface coated with one of the following  
ECM proteins either collagen, or fibronectin, or laminin.

Proceed as follows :

- trypsinise the dish of NRK cells by the following
  - aspirate the medium and discard
  - wash the cells with 2 ml phosphate buffered saline (PBS) by gentle swirling
  - discard the PBS and repeat the above step
  - discard the second PBS wash
  - add 500 µl of warmed trypsin solution to the dish of cells
  - incubate the dish at 37 °C for approximately 10 minutes or until most of the cells have detached from the surface of the dish (swirl to effect maximum detachment).
  
- swirl the trypsinised dish of cells to ensure an even distribution of detached cells
- pipette 200 µl of the cell suspension into the new untreated culture dish and similarly, 200 µl of the suspension into the new ECM treated culture dish.
- add 2 ml of fresh tissue culture medium to the new dishes and mix by swirling them gently
- label the culture dishes and place both of them in the 37 °C incubator for 1 hour.

After one hour treat both of the dishes as follows:

- gently aspirate the culture medium
- carefully rinse with 1 ml of PBS
- pour off the PBS
- add 1 ml of Coomassie Blue stain
- incubate at room temperature on the desk for 20 min
- aspirate the stain and return it to the bottle - DO NOT discard
- gently rinse the dishes with distilled H<sub>2</sub>O
- destain the dishes for 10 min in Destain Solution (mixture of methanol/acetic acid)

View the stained dishes over a white background and note the following:

- 1) any differences in cell attachment between the two dishes
  - if there are differences express them as a percentage difference in attachment.
  
- 2) any differences in the patterns of attachment between the two dishes
  - describe the difference in the patterns of attachment

**IMPORTANT:** Add your data to class spreadsheet and record the range of responses obtained for the different ECM proteins used during the practical exercise.

**Answer the questions over the page in the space provided. The completed questions must be handed to your lecturer/TA at the next practical. It is your duty to make sure that your submission is recorded by the lecturer/TA.**

**REPORT PRACTICAL 19: The effects of extracellular matrix (ECM) on cell adhesion**

Name: .....

Student No: .....

- 1) Does the coating of the tissue culture substrate with ECM protein enhance cellular attachment? [1]

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- 2) Suggest a simple way in which one might determine whether the substrate has been coated or not. [2]

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- 3) Are there any observable differences between the 3 ECM proteins used with respect to NRK cell attachment? [3]

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- 4) If you used a different cell line in the experiment would you expect to obtain the same result? Explain your answer. [4]

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5) How could one get a more quantifiable measure of the cell attachment from this experiment? [2]

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6) What are the primary integrin combinations responsible for attachment to - [3]

collagen \_\_\_\_\_

fibronectin \_\_\_\_\_

laminin \_\_\_\_\_

## **PRACTICAL 20: CELL PROLIFERATION ASSAY USING MTT (METHYLTHIAZOLETETRAZOLIUM)**

### **INTRODUCTION**

One of the most fundamental of all cellular processes, and one in which the cell culturist is particularly interested, is that of cell division. The growth rate of cultured cells is not necessarily always the same. It may vary from one cell strain to another but, in particular, it varies considerably with environmental conditions such as pH, temperature and osmotic pressure. All estimates of cell growth or survival are based on the measurement of the number of viable cells in a population. Therefore, it is not surprising that the accurate determination of cell number is a critical first step in the analysis of cell proliferation *in vitro*. Methods for cell number determination *in vitro* are required for many different biological assays. The MTT-cell proliferation assay is a quantitative colourimetric assay for measurements of cellular proliferation, viability, and cytotoxicity. The assay is based on cleavage of the yellow tetrazolium salt, MTT, which forms water-insoluble, dark blue formazan crystals. This cleavage only takes place in living cells by the mitochondrial enzyme succinate-dehydrogenase. The water-insoluble formazan can be solubilised using isopropanol or another organic solvent. The optical density of the dissolved material is measured spectrophotometrically, yielding absorbance as a function of concentration of converted dye, which directly correlates to the number of metabolically active cells in the culture. The assay is simple, rapid, and well suited for analysis of large number of samples but is suited for monolayer cultures only. The metabolic activity between different cell lines varies and, therefore, a MTT standard curve has to be performed for each new cell line to test for linearity and to measure the slope of the curve. As alternatives to the MTT substrate, there exists other kinds of tetrazolium salts (XTT, MTS, WST -1), which all yield water-soluble cleavage products. Dimethyl sulfoxide may be used instead of isopropanol as a solvent.

### **AIM**

In this practical you will ascertain the effect of epidermal growth factor (EGF) on the proliferation of NRK cells using the MTT-cell proliferation assay.

### **MATERIALS AND METHODS**

Each group will be provided with:

- 1 dish (3 cm) of growing NRK epithelial cells - untreated
- 1 dish (3 cm) of growing NRK epithelial cells that have been treated with epidermal growth

factor (EGF) for 24 hours prior to the practical session

- Cell culture medium (Dulbecco's Modified Eagle Medium DMEM) containing foetal calf serum (FCS) and MTT.
- MTT solution for standard curve determination. THIS IS DIFFERENT!!!!
- Phosphate-buffered saline (PBS)
- Eppendorf tubes
- Tissue culture dishes

**NB!!** Time is required for the cells to properly metabolise the MTT so DO NOT DELAY treating the cell cultures with the MTT solution as follows -

- aspirate the culture medium from the 2 dishes and discard
- wash the cells with 2 ml phosphate buffered saline (PBS) by gentle swirling
- discard the PBS
- add 600 µl of MTT- containing culture medium (0.25mg/ml) to each of the dishes
- incubate the dishes for 2 hr at 37 °C and 5% CO<sub>2</sub> in a humidified incubator

While the cells are incubating set up a MTT Standard Curve

In order to create a set of standards of a known concentration of MTT, the MTT must first be reacted with an enzyme solution containing succinate dehydrogenase (**this will have been done overnight for you before the day of the practical**). This will produce a standard curve capable of being read spectrophotometrically.

Use the stock MTT solution (0.5 mg/ml in isopropanol) to set up a four point (1 ml final volume) standard curve by completing the following table.

| Final concentration of MTT | Volume of MTT stock | Volume of isopropanol |
|----------------------------|---------------------|-----------------------|
| Blank                      |                     |                       |
| 0.1 mg/ml                  |                     |                       |
| 0.075 mg/ml                |                     |                       |
| 0.05 mg/ml                 |                     |                       |
| 0.025 mg/ml                |                     |                       |

- retrieve the cell cultures from the incubator
- discard the MTT-containing medium from the dishes and add 1 ml of isopropanol
- dissolve the formazan crystals by gentle swirling
- transfer the isopropanol solution to an Eppendorf tube for storage and to avoid evaporation of the isopropanol.

Using the correct spectrophotometer cuvettes measure the colour intensity of each of the standards, as well as the two experimental solutions at 570 nm

**NB:** Add your standard curve readings to the class spreadsheet and average the readings.

**Answer the questions over the page in the space provided. The completed questions must be handed to your lecturer/TA at the next practical. It is your duty to make sure that your submission is recorded by the lecturer/TA. -**

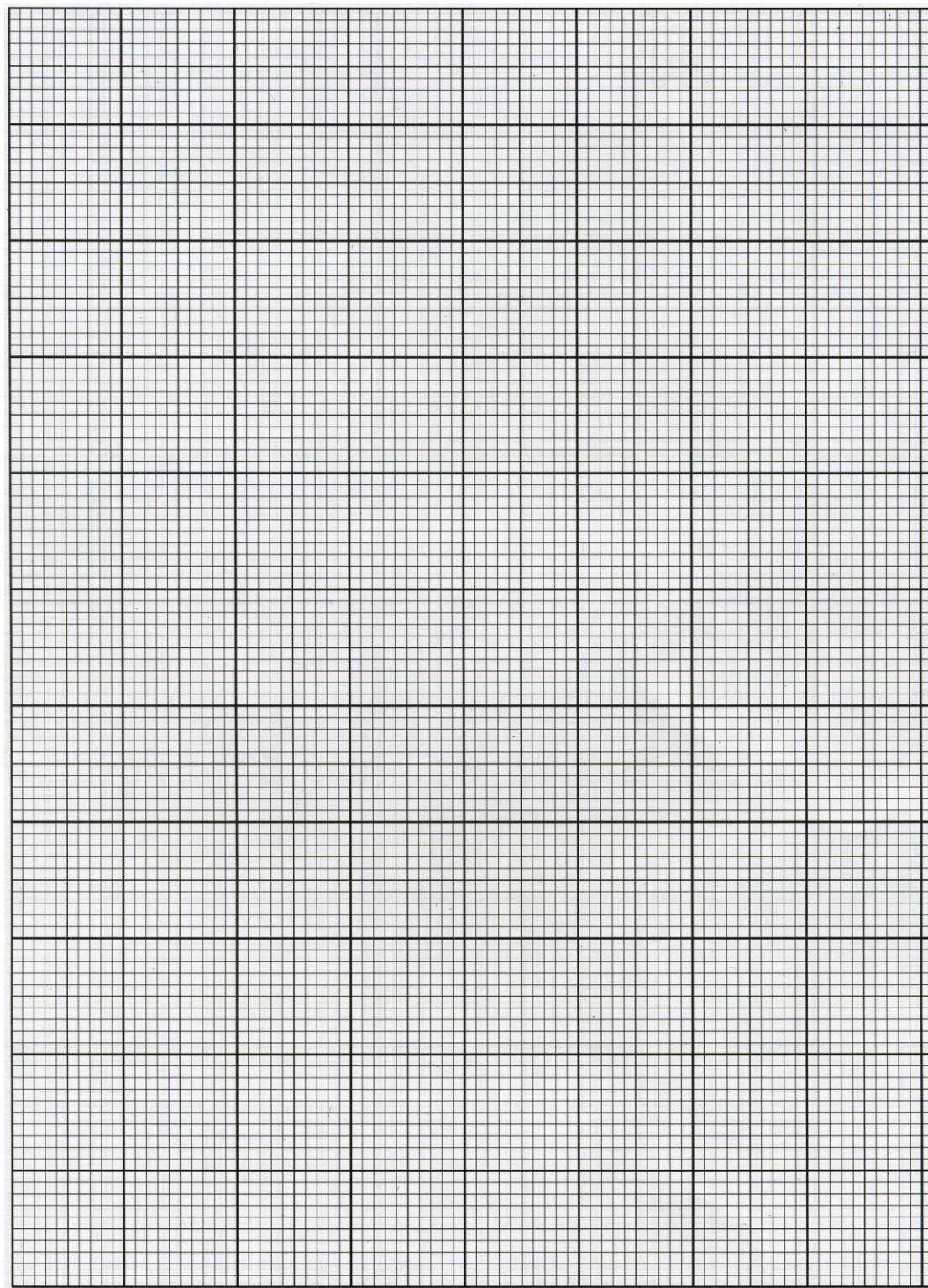


**REPORT PRACTICAL 20: Cell proliferation assay using MTT (Methylthiazoletetrazolium)**

Name: .....

Student No: .....

- 1) Plot the standard curve and determine the concentration of the two experimental samples. [5]



1a) Concentration of Untreated culture \_\_\_\_\_

1b) Concentration of EGF-treated culture \_\_\_\_\_

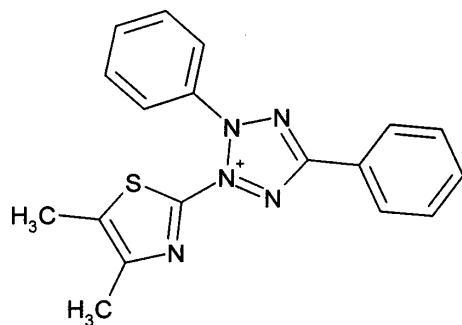
2) What type of enzyme is succinate dehydrogenase? [1]

3) What is the major difference in point of action of MTT, MTS, XTT and WST? [2]

4) What would you predict the outcome to be if the experiment was performed on live resting cells? [1]

5) Interpret the above concentration similarities/differences using the mechanism of action of EGF. [3]

6) Using the structure for MTT provided below show the reaction that results in the reduction (cleavage) of MTT. [4]



7) What is formazan? (Show the structure with your explanation)

[4]