Polyfuge

DoubleGene

Educational Lab Packet

Important Safety Information

***Warning*:** Centrifuges are versatile tools, but can be **dangerous** when handled incorrectly. Please closely read through the “Important Safety Information” section in the Polyfuge User’s Guide before continuing.

Disclaimer

We are not responsible for damages, injuries, deaths, or other ill effects arising from proper or improper use of or assembly of the Polyfuge components. To the fullest extent permissible by the applicable law, we hereby disclaim any and all responsibility, risk, liability, and damages arising out of death or personal injury resulting from assembly of or operation of this kit.

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What is biotechnology?

Biotechnology is about using living systems to make improvements to the world. From engineering fluorescent plant tissues to creating pharmaceutical drugs for treating diseases, biotechnology is an exciting interdisciplinary study that uses cells and proteins to accomplish various important tasks.

What will we be doing?

In this packet, we will be using centrifugation to extract various molecular substances using household reagents. By doing so, we will learn about the various chemical and biological principles behind common lab protocols!

Why is this important?

In biotechnology, isolation protocols are extremely useful for chemically analyzing particular substances or even obtaining genomic data. In reverse transcription quantitative polymerase chain reaction (RT-qPCR) for example, RNA is extracted, converted to cDNA, and then analyzed with a program to measure relative gene expression.

How do we start?

To start off, make sure you read through the Polyfuge User's Guide for the safety information and operation instructions. Afterwards, grab a few friends and get started!

DNA Isolation Lab

Protocol Goal

Extract DNA from any sample!

Concepts to Learn

- DNA Isolation principles
- Function of salt in DNA isolation
- Function of alcohol in DNA isolation
- Chemical properties of DNA
- Structural properties of DNA

Introduction

We often associate our physical characteristics to our DNA, but what exactly is DNA?

Deoxyribonucleic acid (DNA) belongs to a major class of macromolecules known as nucleic acids. Their major function is to store genetic information in the form of base pair sequences.

DNA is composed of a chain of nucleotides, which each consist of a nitrogenous base (Adenine, Guanine, Thymine, or Cytosine), a sugar (deoxyribose), and a phosphate group.

Two DNA strands form a structure known as a double-helix: each strand is connected to another strand through hydrogen bonds between the nitrogenous bases, forming a base pair. In a cellular context, DNA provides the blueprints needed to construct proteins - the machinery of life.

Instruments & Reagents

- Polyfuge
- A sample to extract DNA from (ex. Strawberry, Mouthwash solution, etc.)
- Table salt
- Dish soap
- Cold rubbing alcohol
- Glass container
- 1.5 mL microcentrifuge tubes

Protocol

1. First, we need to prepare a sample in liquid form. For the purpose of this protocol, we will be using a piece of strawberry (you can use any form of sample, as long as it contains DNA).
2. Next, we need to break up the sample in order to release the DNA inside. There are many ways to do this: blending, homogenization, or a simple mortar and pestle. Whichever method you choose, make sure that the sample is sufficiently broken up.
3. If the sample is too dry, add some distilled water to dilute the sample.
4. Place the sample solution into a small glass beaker.
5. Add 2-3 drops of dish soap.
6. Add a “pinch” of table salt.
7. Add a thin layer of cold rubbing alcohol (3-4 drops) to the surface. DNA should appear at this step with white and cloudy appearance.
8. Draw off the 1.5 mL of supernatant and place into a separate tube/container. Extracted DNA should be included within this liquid.
9. Spin down supernatant using 1.5 mL microcentrifuge tube (with snap cap). Spin on high for around 1 minute. When done, you should see highly concentrated “pellet” at the bottom of an otherwise clear liquid. That’s the DNA!

Notes on Mechanism

Breaking Cells Open

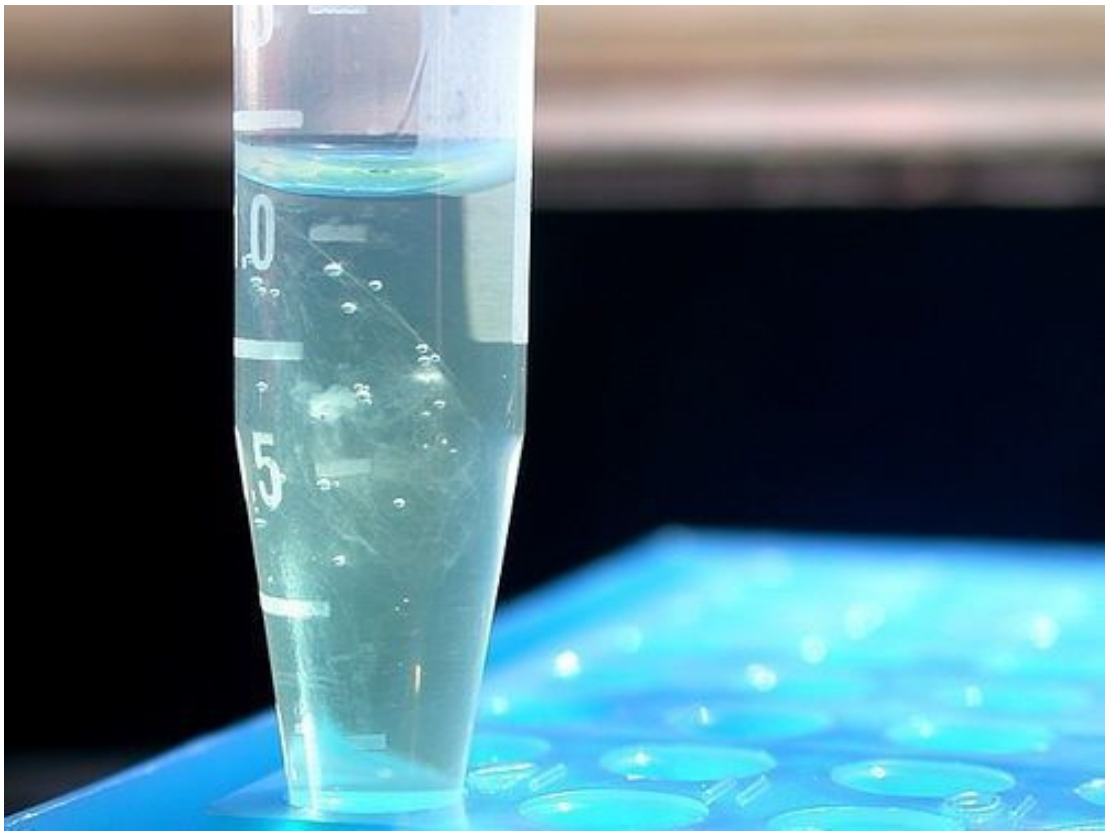
The cells in a sample are separated from each other, often through physical means like grinding or vortexing, and then put into solution containing salt. The salt functions to neutralize the charge of the phosphate groups on the DNA backbone, essentially protecting negatively charged phosphate groups that run along the backbone of the DNA. A detergent is then added. The detergent breaks down the lipids in the cell membrane and nuclei. DNA is released as these membranes are disrupted.

Separating DNA

To get a clean sample of DNA, it's necessary to remove as much of the cellular debris as possible. This can be done by a variety of methods. Often a protease (protein enzyme) is added to degrade DNA-associated proteins and other cellular proteins. Alternatively, some of the cellular debris can be removed by filtering the sample.

Precipitating DNA

Finally, ice-cold alcohol (either ethanol or isopropanol) is carefully added to the DNA sample. DNA is soluble in water but insoluble in the presence of salt and alcohol. By gently stirring the alcohol layer with a sterile pipette, a precipitate becomes visible and can be spooled out. If there is lots of DNA, you may see a stringy, white precipitate. Alcohol precipitates nucleic acids because it is much less polar than water, meaning that Na^+ will be more inclined to interact with the negative phosphate portions of the DNA backbone (PO_3^-).



Casein Precipitation Lab

Protocol Goal

Precipitate casein from a milk sample!

Concepts to Learn

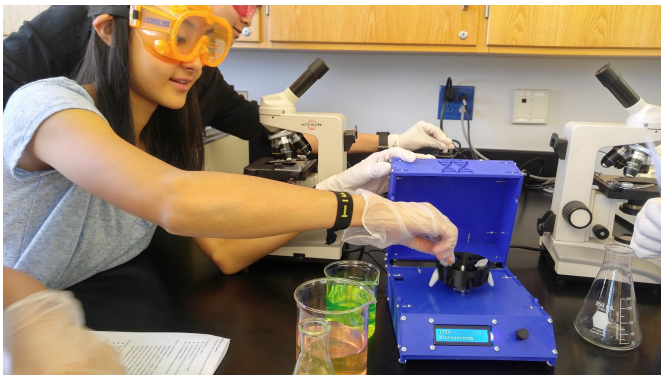
- Chemical contents of milk
- Function of acids in casein precipitation
- Isoelectric point
- Principles behind cottage cheese and cream cheese manufacturing

Introduction

Proteins are a class of macromolecules composed of chains of amino acids known as polypeptides. They are responsible for many different functions within cells including enzymatic activity, structural support, cell signaling, and transport.

Instruments & Reagents

- Polyfuge
- Milk
- Vinegar (White distilled vinegar is best for this)
- 1.5 mL microcentrifuge tube



Protocol

1. Add 400-500μL of milk sample into microcentrifuge tube.
2. Add 400-500μL white distilled vinegar into microcentrifuge tube.
3. Shake the tube vigorously or vortex if you have one!
4. Centrifuge at 1560 speed for 3 minutes.
5. Aspirate supernatant. The remaining white substance at the bottom of the tube is your precipitated milk protein!

Notes on Mechanism

Milk Proteins

Milk protein consists of 80% casein and 20% whey protein. There are four major types of casein molecules: alpha-s1, alpha s2, beta, and kappa.

Isoelectric Point

Milk, in its natural state, is negatively charged. The negative charge permits the dispersion of casein in the milk. When an acid is added to milk (vinegar), the H^+ concentration neutralizes the negatively charged casein micelles (a submicroscopic aggregation of molecules, as a droplet in a colloidal system).

When milk is acidified to pH 4.7, the isoelectric point (the point at which all charges are neutral) of casein, an isoelectric precipitate known as acid casein is formed.

Industrial Applications

Cottage cheese and cream cheese manufacture involves an acid precipitation of casein with lactic acid or lactic acid-producing microorganisms. Acid casein is used in the chemical industry and as a glazing additive in paper manufacturing.