Revision Questions

and tentative sample answers

One mark each

- Explain the purpose of following chemical reagents for SDS-PAGE.
- a) SDS

it is used to denature and provide negative charge to the proteins.

- c) Coomassie Blue

it is used to stain the proteins in polyacrylamide gel

Differentiate between agarose gel electrophoresis and SDS-PAGE based on-buffer systems used. (2 marks)

- In agarose gel, single buffer is used for running of the gel. Whereas in SDS-PAGE discontinuous buffer system is used. In a discontinuous system a nonrestrictive large pore gel, called a stacking gel, is layered on top of a separating gel and buffers at different pH are used for separating gel and resolving gel.

Explain use of the following during cell culturing:

a. Hemocytometer-

an instrument used for counting cells.

b. CO_2 incubator –

to maintain humidity and pH of the solution when used with bicarbonate containing media.

Confluency refers to: (Circle one) **(1mark)**

a) the area that each cell occupies.

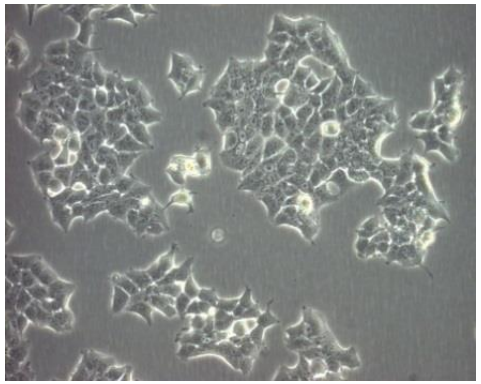
b) the viable cells per ml.

c) the ratio of area occupied by the cells and the total area available.

d) the area below the line of a growth curve.

e) the solute concentration in cell culture media.

Identify and name the morphology : **(1 mark)**



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What is cell viability and how it is calculated? (3 marks)

- Give formula and example

What is BSL4 laboratory?

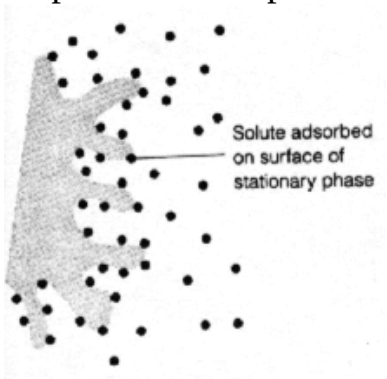
- Give example of organisms that can be cultured
- Safety signs outside door
- Bench work
- Safety measures- which ones?

Why stacking gel is used?

- Role of glycine and chloride ions, pH of buffers, sandwich proteins etc.

What is adsorption chromatography? Explain principle of separation and its disadvantages. (4 marks)

Separates solutes based on their adsorption to solid particles (stationary phase).



- Mobile phase: liquid or gas
- Stationary phase: adsorbs solute onto its surface- can be polar or non-polar

Disadvantages–

1. very strong retention of some solutes
2. may cause catalytic changes in solutes
3. solid support may have a range of chemical and physical environments → non-symmetrical peaks and variable retention times

Methods of protein purification?

(4 marks)

- Chromatography → types
- Electrophoresis → types