Name: **Enrolment No:**	

UPES

End Semester Examination, May 2025

Course: Bioseparation and Biochemical Analysis **Semester : VI**

Program: B.Tech Biotechnology **Duration : 3 Hours**

Course Code: HSBT3009 **Max. Marks: 100**

Instructions:

S. No.	Section A Short answer questions/ MCQ/T&F (20Qx1.5M= 30 Marks)	Marks	COs
Q 1	The technique used to separate a protein mixture based on molecular size is called _____	**1.5**	**CO1**
Q 2	Precipitation by ammonium sulfate exploits differences in protein solubility.	**1.5**	**CO1**
Q 3	Which of the following methods is most suitable for separating proteins based on their charge? a) Gel filtration b) SDS-PAGE c) Ion exchange chromatography d) Affinity chromatography	**1.5**	**CO1**
Q 4	Which component plays a key role in the selective binding of proteins in affinity chromatography? a) Ligand b) Salt c) pH buffer d) Detergent	**1.5**	**CO1**
Q 5	Which of the following gives a blue color with proteins? a) Benedict's reagent b) Biuret reagent c) Molisch reagent d) Ninhydrin	**1.5**	**CO2**
Q 6	Lowry's method is more sensitive than the Biuret assay. (True/False)	**1.5**	**CO2**
Q 7	The DNS assay is used for estimating the amount of _____.	**1.5**	**CO2**

Q 8	Absorbance of nucleic acids is generally measured at: a) 540 nm b) 280 nm c) 260 nm d) 420 nm	**1.5**	**CO2**
Q 9	Which of the following filtration methods is based on constant pressure? a) Depth filtration b) Cross-flow filtration c) Dead-end filtration d) Constant rate filtration	**1.5**	**CO3**
Q 10	A filtration process with high cake resistance will require higher pressure to maintain flow. **(True/False)**	**1.5**	**CO3**
Q 11	The driving force for ultrafiltration across a membrane is: a) Concentration gradient b) Osmotic pressure c) Pressure gradient d) Temperature gradient	**1.5**	**CO3**
Q 12	Which type of filtration involves passage through a compressible cake layer? a) Microfiltration b) Cake filtration c) Tangential flow d) Reverse osmosis	**1.5**	**CO3**
Q 13	Buffers are used in protein separation to stabilize pH during the process. **(True/False)**	**1.5**	**CO4**
Q 14	A good buffer should have a pKa value close to: a) The ambient temperature b) The ionic strength c) The pH of the solution d) The buffer volume	**1.5**	**CO4**
Q 15	Hollow fiber membrane modules provide the highest surface area among common module designs. **(True/False)**	**1.5**	**CO4**
Q 16	Prefiltration is mainly used to: a) Concentrate the product b) Remove dissolved ions c) Eliminate suspended particles d) Elute the protein	**1.5**	**CO4**
Q 17	Chromatographic scale-up only involves increasing column diameter. **(True/False)**	**1.5**	**CO5**
Q 18	Which unit operation is best for recovering intracellular proteins?	**1.5**	**CO5**

	a) Foam fractionation b) Homogenization c) Ultrafiltration d) Dialysis		
Q 19	The final step in industrial protein purification usually aims to achieve high _____ purity.	**1.5**	**CO5**
Q 20	Which analytical technique is often used for identity verification of a therapeutic protein? a) UV-Vis spectroscopy b) Western blotting c) TLC d) ELISA	**1.5**	**CO5**
Section B (4Qx5M=20 Marks)			
Q 1	What role does ammonium sulfate play in protein precipitation? Give one example.	**5**	**CO1**
Q 2	Explain the Biuret test and how it indicates the presence of proteins.	**5**	**CO2**
Q 3	What is cross-flow filtration? How is it different from dead-end filtration?	**5**	**CO3**
Q 4	Define buffer capacity. What factors influence the buffering action?	**5**	**CO4**
Section C (2Qx15M=30 Marks)			
Q 1	SDS-PAGE is commonly used for analyzing protein size and purity. Explain the principle of SDS-PAGE and why SDS is used. **(5 marks)** Describe the role of stacking and resolving gels in the separation process. **(5 marks)** How can the results from SDS-PAGE help in assessing the success of a purification step? **(5 marks)**	**15**	**CO3**
Q 2	You are planning to purify a recombinant protein that includes a Maltose-Binding Protein (MBP) tag to aid purification. What is the purpose of tagging a protein with MBP in downstream purification? **(5 marks)**	**15**	**CO4**

	List the steps involved in purifying an MBP-tagged protein using affinity chromatography. **(5 marks)** Mention two precautions that must be taken during scale-up to prevent product loss. **(5 marks)**		
Section D **(2Qx10M=20 Marks)**			
Q 1	Thin Layer Chromatography (TLC) is used to separate and identify compounds in a mixture. What is the principle behind TLC? **(3 marks)** List the major components used in a basic TLC setup. **(3 marks)** Explain how you would calculate the Rf value and interpret results **(4 marks)**	**10**	**CO5**
Q 2	A researcher is isolating DNA from cultured cells for downstream molecular applications. List the key steps involved in isolating and purifying nucleic acids. **(5 marks)** Explain how the A260/A280 and A260/A230 absorbance ratios are used to assess nucleic acid purity. **(5 marks)**	**10**	**CO2**