

## Application Guide for Sample Preparation of Biological Mixtures

i.e. Proteins, Nucleic Acids, Carbohydrates	Chromato-graphic	Dialysis	Equilibrium Dialysis	ElectroPrep	Liposomes	CoZap
Acrylamide Removal	•					
Affinity Purification	•					
Antibody Production					•	
Buffer Exchange		•		•		
Cancer Therapy					•	
Carbohydrate Purification	•					
Cell-Cell Interactions					•	
Cosmetics					•	
CsCl Removal				•		
Destaining Gels						•
Detergent Removal	•	•		•		
Diagnostics					•	
DNA Binding Assays			•			
Drug Delivery					•	
Drug Entrapment Studies					•	
Dye Removal	•	•		•		
Electrophoresis				•		
Enzyme Replacement Therapy					•	
Extraction from Gels				•		
Gene Therapy					•	
Glycoprotein/Glycopeptide Purification	•					
Immunoblotting				•		
In Vitro Cell-Liposome Interaction Studies					•	
Ligand Binding Assays			•			
Lipid Purification	•					
Mediated Delivery of Macromolecules					•	
Metal Chelation Therapy					•	
Nick Translation	•					
PCR Cleanup	•			•		
Peptide Removal	•	•		•		
Plasmid Purification	•					
Primer Removal	•			•		
Protein Binding Assays			•			
Protein Purification for HPLC/HPCE/GC	•	•		•		
Protein Purification for Mass Spectroscopy (MALDI, GC/MS, MS-NMR, ESI-MS)	•	•		•		
Protein-Drug Binding Assays			•			
Protein-Protein Interactions			•			
Purification of Samples with Unknown Isoelectric Points				•		
Pyridoxal-5-Phosphate Removal				•		
Radiolabel Removal	•	•				
Radiopharmaceutical Marker Tracing	•	•				
Receptor Binding Assays			•			
Reconstitution Experiments (for Ion Transport)					•	
Salt Removal	•	•		•		
Sample Concentration	•	•		•		
SDS Removal	•	•		•		
Silicate Removal after Chromatography				•		
Size Fractionation	•			•		
Small Molecule Removal	•	•		•		
Time-Dependent Release Studies					•	

## Technology Selection Guide

### Chromatography Methods (i.e., Tip Columns, PrepTips, ProTips and Spin Columns)

Physical separation methods, such as:

**Hydrophilic** - Molecules are bound to a functional group on a matrix in the organic phase and eluted in the aqueous phase;

**Hydrophobic** - Molecules are bound to a functional group on a matrix in the aqueous phase and eluted in the organic phase;

**Gel filtration** - The smaller molecules diffuse into the pores of the support material making their path and elution time longer than the larger molecules that are excluded on the outside of the particle. Hence the larger molecules elute first and the smaller ones elute later;

**Ion Exchange** - Molecules are bound to an ionic functional group on a matrix by charge.

### Dialysis

A physical separation method in which small molecules pass through the pores of a size selective membrane while the larger molecules are retained in the dialysis bag or chamber. In order to drive the equilibrium mechanism the volume on the outside must be about 200 x the volume of the retained volume and devoid of the small molecules of interest.

### Electro Dialysis

A physical separation method in which the movement of the molecules through a semi-permeable membrane is accelerated by an electric field so that molecules can be separated quickly by charge and size. This technique is useful in separation, collection and fractionation of large and small ionic molecules.

### Equilibrium Dialysis

A specific application of dialysis which is used to measure the amounts of ligand bound to macromolecules and the free ligand present in two compartments separated by a dialysis membrane. The concentrations of ligand provide information on various binding parameters such as binding constants binding capacity and the number of binding sites.

	Absorption	Size exclusion	Ion exchange			
1 Methods development time	Moderate to Considerable	Minimal	Moderate to Considerable	Minimal	Minimal in dialysis, size only mode; moderate to complex in size and charge mode	Minimal
2 Average time/speed of separation	5 minutes	5 minutes	5 minutes	1 to 24 hrs	3 to 5 minutes	3 to 24 hrs
3 Ability to collect specific sample fractions-small molecules	Excellent	Good	Excellent	Poor	Excellent	Fair
4 Ability to collect specific sample fractions-large molecules	Good	Good	Good	Good	Good	Good
5 Ability to separate more than one set of components simultaneously	Excellent	Fair	Excellent	Poor	Excellent	Poor
6 Sample Volumes (non flow thru versions)	a few microliters to > 1 ml	a few microliters to > 5 ml	a few microliters to > 5 ml	a few microliters to > 5 ml	a few microliters to > 1 ml	96 well 50 to 200 µl
7 Number of Samples run simultaneously	1 to 96	1 to 96	1 to 96	1 to 96	1	1 to 96
8 Materials compatibility for maximum activity recovery	Medium to high	Medium to high	Medium to high	Medium to high	Medium to high	Medium to high
9 Auxiliary equipment	centrifuge, pipettor	centrifuge, pipettor	centrifuge, pipettor	Stirrer, temperature controller	200 VDC, 100 mA power supply, rotator	200 VDC, 100 mA power supply, rotator
10 Sterilizable	CHEMICALLY	CHEMICALLY	CHEMICALLY	CHEMICALLY	CHEMICALLY	CHEMICALLY
11 Suitable for organic & aqueous solutions	YES	YES	YES	YES	YES	YES
12 Suitable for high throughput screening (HTS)	YES	YES	YES	YES	YES	YES

### Recommended Literature

#### Dialysis

James B. Ames, Alexander M. Dizhoor, Mitsuhiro Ikura, Krzysztof Palczewski, and Lubert Stryer, "Three-dimensional Structure of Guanylyl Cyclase Activating Protein-2, a Calcium-sensitive Modulator of Photoreceptor Guanylyl Cyclases", The Journal of Biological Chemistry, Vol. 274, No. 27, pp. 19329-19337, 1999.

#### Electro Dialysis

1) Zuo, Xun & Speicher, David; "A Method for Global Analysis of Complex Proteoms Using Sample Prefractionation by Solution Isoelectrofocusing Prior to Two-Dimensional Electrophoresis"; Analytical Biochemistry, Vol. 284, pp. 266-278, 2000. 2) ElectroPrep™ User Manual.

#### Equilibrium Dialysis

1) Kariv, Hong, & Oldenburg; "Development of a High Throughput Equilibrium Dialysis Method"; Journal of Pharmaceutical Sciences; Vol. 90, No. 5, pp. 580-587, 2001. 2) Guide to Equilibrium Dialysis, ED-96 Users Manual.

Footnote: Membranes are used in Dialysis, Electro Dialysis and Equilibrium Dialysis and can be damaged by high temperature.