Protein Analysis/Proteomics

The following section covers buffers used in protein analysis and investigation of protein–protein interactions. This section is presented in two parts: (1) basic methods in protein analysis for proteomics and (2) advanced protocols in identifying protein–protein interactions for proteomics. Areas covered include peptide sequencing, analysis of peptide/DNA complexes, and two-hybrid and library screening.

> ***Caution:*** See Cautions Appendix for appropriate handling of materials marked with ☣.

Basic Methods in Protein Analysis for Proteomics

Chromatography

The following sections include solutions used in various chromatographic purification protocols.

■ *Size Exclusion Chromatography*

Denaturing Buffer Containing Urea PUR

8 M urea ☣
5 mM dithiothreitol ☣
150 mM NaCl
50 mM Tris-Cl (pH 7.5)

Warm the solution to 40°C to dissolve the urea. Adjust the pH to 7.5 if necessary.

Denaturing Buffer Containing Guanidine Hydrochloride PUR

6 M guanidine hydrochloride ▼
5 mM dithiothreitol ▼
50 mM Tris-Cl (pH 7.5)

Warm the solution to 40°C to dissolve the guanidine hydrochloride. Adjust the pH to 7.5 if necessary.

Peptide Elution Buffers PUR

20% acetonitrile ▼ /0.1% TFA ▼ in H_2O
0.1% TFA in H_2O
or
0.1% acetic acid ▼ in H_2O

■ *Hydrophobic Interaction Chromatography*

High-density Phenyl-substituted Matrix Column PUR

Suitable columns include the POROS HP2 (M) chromatography column (Applied Biosystems), SOURCE 15 PHE PE (100 × 4.6 mm), or Bio-Gel TSK Phenyl-5PW HIC column (75 × 7.5 mm I.D. 1000 Å) (Bio-Rad).

Dimethyl Pimelimidate (DMP) Solution PUR

Prepare 10 ml of 20 mM DMP (Pierce) in 0.1 M triethanolamine ▼ (pH 9.5; the pH of the solution decreases to ~8.0 due to the acidity of DMP). This reagent must be prepared fresh immediately before use.

Column Regeneration Buffer PUR

10 mM Tris-HCl (pH 7.6)
1 mM EDTA
2.5 M NaCl
1% Nonidet P-40 (NP-40)

NP-40 will never completely dissolve. Swirl just before use.

Column Storage Buffer PUR

10 mM Tris-HCl (pH 7.6)
1 mM EDTA
0.3 M NaCl
0.04% sodium azide ▼

Add sodium azide fresh from a 4% stock solution just before use.

HEMGN Buffer PUR

25 mM HEPES (K^+, pH 7.6)
0.1 mM EDTA
2.5 mM $MgCl_2$ ▼
10% glycerol
0.025% Nonidet P-40
1 mM dithiothreitol (DTT) ▼

Add DTT fresh immediately before use. Store the buffer at 4°C.

■ *Immobilized Metal-ion Affinity Chromatography*

Supplemented Buffer P PUR

50 mM sodium phosphate (pH 7.4) ▼
0.5 M NaCl
0.5 mg/ml lysozyme ▼
1 mM PMSF ▼
1.7 units/ml Benzonase (Merck)
1 mM $MgCl_2$ ▼

Add the lysozyme, PMSF, and Benzonase just before use.

Binding Buffer PUR

20 mM sodium phosphate (pH 7.4) ▼
0.5 M NaCl
20 mM imidazole ▼

Elution Buffer PUR

20 mM sodium phosphate (pH 7.4) ▼
0.5 M NaCl
500 mM imidazole ▼

Inclusion Body Wash Buffer PUR

2 M urea ☣
500 mM NaCl
2% Triton X-100 ☣
20 mM Tris-HCl (pH 8.0)

Refolding Buffer PUR

20 mM imidazole ☣
500 mM NaCl
1 mM 2-mercaptoethanol ☣
20 mM Tris-HCl (pH 8.0)

Urea Binding Buffer PUR

For preparation of buffers containing urea, weigh the denaturant, add 2 M imidazole ☣, 1 M Tris-HCl (pH 8.0), and H_2O to 90% of the final volume. Heat the container, which is cooled by the solubilization process of the denaturant, to room temperature in a water bath at 45°C while stirring. Avoid high temperatures. The denaturant will dissolve within minutes. Alternatively, stir overnight at room temperature. Adjust the pH, add H_2O to the final volume, and filter the solution. The pH adjustment must be done at room temperature. Add 2-mercaptoethanol ☣ immediately before use.

■ *Chromatofocusing*

The following section includes a solution used in purifying proteins by chromatofocusing.

Acid Solutions PUR

2 M acetic acid ☣
2 M HCl ☣
iminodiacetic acid (saturated solution) ☣

These solutions are used for adjusting the pH of start and elution buffers.

■ *Two-dimensional Gel Electrophoresis*

The following section includes solutions for preparation and visualization of proteins by two-dimensional (2D) electrophoresis.

Thiourea/Urea Lysis Buffer PUR

2 M thiourea V
7 M urea V
4% (w/v) CHAPS V
1% (w/v) DTT V
2% (v/v) carrier ampholytes (pH 3–10)
10 mM Pefabloc proteinase inhibitor

To prepare 50 ml of thiourea/urea lysis buffer, dissolve 22.0 g of urea (GE Healthcare) in deionized H_2O, add 8.0 g of thiourea (Sigma-Aldrich), and adjust the volume to 50 ml with deionized H_2O. Add 0.5 g of Serdolit MB-1 mixed-bed ion-exchange resin (Serva), stir for 10 minutes, and filter. To 48 ml of the urea solution add 2.0 g of CHAPS, 1.0 ml of Pharmalyte (pH 3–10; GE Healthcare), 0.5 g of DTT, and, immediately before use, 50 mg of Pefabloc proteinase inhibitor (VWR).

Urea Lysis Buffer PUR

9.5 M urea V
1% (w/v) DTT V
2% (w/v) CHAPS V
2% (v/v) carrier ampholytes (pH 3–10)
10 mM Pefabloc proteinase inhibitor

To prepare 50 ml of urea lysis buffer, dissolve 30.0 g of urea (GE Healthcare) in deionized H_2O and adjust the volume to 50 ml. Add 0.5 g of Serdolit MB-1, stir for 10 minutes, and filter. Add 1.0 g of CHAPS, 0.5 g of DTT, and 1.0 ml of Pharmalyte (pH 3–10) to 48 ml of the filtered urea solution. If necessary, add 50 mg of Pefabloc proteinase inhibitor immediately before use.

IMPORTANT: Lysis buffer must be freshly prepared. Alternatively, make 1-ml aliquots and store at –80°C for up to several months. Do not refreeze lysis buffer that has already been thawed once! Never heat urea solutions above 37°C. Otherwise, protein carbamylation may occur.