

## ProteoSep™ Kit Lysis Protocol

### **Lysis buffer Contents:**

7.5M urea  
2.5M thiourea  
12.5% glycerol  
50mM Tris  
2.5% n-octylglucoside  
6.25mM TCEP(Tris-(carboxyethyl)phosphine hydrochloride)  
1.25mM of protease inhibitor in water solution.

### **12.5% Glycerol preparation (50 mL)**

1. Measure 6.25 mL Glycerol (Sigma, Part Number: G6279) into a 50 mL volumetric flask.
2. Add distilled water to volume.

### **Lysis buffer preparation (50 mL)**

1. Weigh the following into a 100 mL beaker:
  - 22.52 grams urea (Sigma, Part Number: U0631),
  - 9.52 grams thiourea (Sigma, Part Number: T7875),
  - 0.304 grams Tris (Fisher, Part Number: BP154-1, Molecular Biology Grade),
  - 1.25 grams n-octylglucoside (Sigma, Part Number: O8001),
  - 0.0896 grams TCEP (Sigma, Part Number: C4706),
  - 1.868 mL protease inhibitor (Sigma, Part Number: P2714).
2. Add 20 mL of 12.5% glycerol into the beaker. Stir thoroughly until all dissolved.
3. Transfer all solution to a 50 mL flask.
4. Add 12.5% glycerol to volume.
5. Dispense the above buffer solution into separate 2.0 mL vials, then store them at -20°C.

### **Bacterial cell lysing procedure**

1. Suspend the cell pellet in 0.4 mL of 50mM Tris (pH 7.8-8.2, pH adjusted with 10% HCl) and vortex rigorously, then sonicate with ultrasonicator with microtip in an ice bath. Repeat this procedure twice for 30 seconds each. This serves to break the cell membrane.

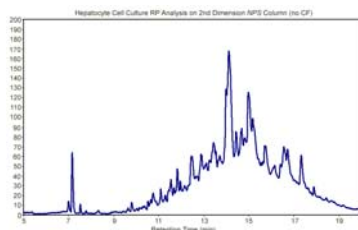
2. Mix the solution in 1.6 mL of lysis buffer. The final cell lysate consists of: 6M urea, 2M thiourea, 10% glycerol, 50mM Tris, 2% n-octylglucoside, 5mM TCEP, 1mM of protease inhibitor.
3. Centrifuge the solution at >20,000g (>15,000rpm) for 60 minutes.
4. Remove the supernatant from the cell debris. Store at –20°C before use.

### **Exchanging the cell lysate with the start buffer**

1. Add start buffer to above cell lysate for a final volume of 2.5 mL and vortex.
2. Equilibrate the PD-10 column (Code Number: 17-0851-01, Amersham Pharmacia Biotech) with approximately 25 mL of start buffer.
3. Load 2.5 mL sample onto PD-10 column. Discard the eluent.
4. Wash the PD-10 column with start buffer to elute the proteins and collect the first 3.5 mL fraction.
5. Inject 50 µL of exchanged sample onto the HPRP 2D Column to observe the detailed reversed phase elution profile for all the proteins in the sample prior to pI fractionation. If any sample preparation problems have occurred they will be readily identified after analysis of the chromatogram from this step.

**Note: Depending on the complexity and amount of proteins expected to be in the sample, a very “spikey or porcupine” looking chromatogram should be obtained at this point. Do not proceed to the CF fractionation step if you do not see a protein profile that makes sense for the sample you are analyzing!**

*Following please find an example of this type of spectra:*



6. The sample is now ready for injection on the HPCF 1D column. [See ProteoSep Protein Mapping Protocol.]

### **Human cell line lysing procedure**

1. Mix ~0.4 mL of cell line sample in 1.6 mL of lysis buffer and vortex vigorously. If cell is pellet, suspend cell pellet in 0.4 mL of 50mM Tris (pH 7.8-8.2 with 10%HCl) and vortex, then add 1.6 mL of lysis buffer, vortex vigorously.

The final cell lysate consists of: 6M urea, 2M thiourea, 10% glycerol, 50mM Tris, 2% n-octylglucoside, 5mM TCEP, 1mM of protease inhibitor.

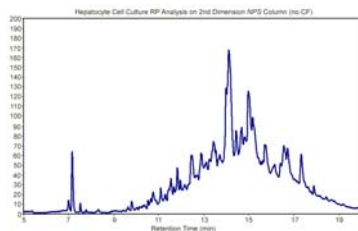
2. Centrifuge the lysate at >20,000g (>15,000 rpm) for 60 minutes.
3. Remove the supernatant from the cell debris. Store at –20°C before use.

## **Exchanging the cell lysate with the start buffer**

1. Add start buffer to above cell lysate for a final volume of 2.5 mL and vortex.
2. Equilibrate the PD-10 column with approximately 25 mL of start buffer.
3. Load 2.5 mL sample onto PD-10 column. Discard the eluent.
4. Wash the PD-10 column with start buffer to elute the proteins and collect the first 3.5 mL fraction.
5. Inject 50  $\mu$ L of exchanged sample onto the HPRP 2D Column to observe the detailed reversed phase elution profile for all the proteins in the sample prior to pl fractionation.

**Note: Depending on the complexity and amount of proteins expected to be in the sample, a very “spikey or porcupine” looking chromatogram should be obtained at this point. . Do not proceed to the CF fractionation step if you do not see a protein profile that makes sense for the sample you are analyzing!**

*Following please find an example of this type of spectra:*



6. The sample is now ready for injection on the HPCF 1D column. [See ProteoSep Protein Mapping Protocol.]

## **Lysis buffer Contents for membrane protein:**

5M urea  
2M thiourea  
10% glycerol  
2.5% SB3-10 (N-decyl-N,N-dimethyl-3-ammonio-1-propane sulfonate)  
50mM Tris  
2.0% n-octylglucoside  
5mM TCEP(Tris-(carboxyethyl)phosphine hydrochloride)  
1.0mM protease inhibitor

## **Membrane protein lysing procedure**

1. Suspend cell debris in 2.5 mL of above lysis buffer. Vortex rigorously.
2. Centrifuge the solution at >20,000g for 60 minutes.
3. Remove the supernatant and store at  $-20^{\circ}\text{C}$  before use.

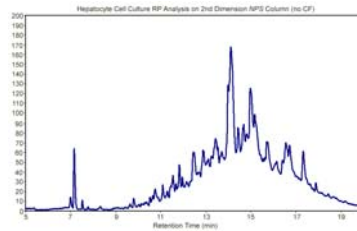
## **Exchanging the membrane protein solution with the start buffer**

1. Add start buffer to above supernatant for a final volume of 2.5 mL and vortex.

2. Equilibrate the PD-10 column with approximately 25 mL of start buffer.
3. Load 2.5 mL sample onto PD-10 column. Discard the eluent.
4. Wash the PD-10 column with start buffer to elute the proteins and collect the first 3.5 mL fraction.
5. Inject 50  $\mu$ L of exchanged sample onto the HPRP 2D Column to observe the detailed reversed phase elution profile for all the proteins in the sample prior to pl fractionation.

**Note: Depending on the complexity and amount of proteins expected to be in the sample, a very “spikey or porcupine” looking chromatogram should be obtained at this point. . Do not proceed to the CF fractionation step if you do not see a protein profile that makes sense for the sample you are analyzing!**

*Following please find an example of this type of spectra:*



6. The sample is now ready for injection on the HPCF 1D column. [See ProteoSep Protein Mapping Protocol.]

**Warning: Always store the cell lysate at  $-20^{\circ}\text{C}$  ~  $-80^{\circ}\text{C}$  to avoid protein degradation.**