Denaturing purification of Insoluble Proteins

**Buffer A**

<table>
<thead>
<tr>
<th>Component</th>
<th>500 ml</th>
<th>1 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 M Guanidine hydrochloride</td>
<td>286.59 g</td>
<td>573.18 g</td>
</tr>
<tr>
<td>0.1 M NaH$_2$PO$_4$</td>
<td>6.9 g</td>
<td>13.8 g</td>
</tr>
<tr>
<td>0.01 M Tris</td>
<td>5 ml</td>
<td>10 ml (of 1 M Tris HCl pH 8.0)</td>
</tr>
<tr>
<td>adjust pH to 8.0 with NaOH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Buffer B**

<table>
<thead>
<tr>
<th>Component</th>
<th>240.24 g</th>
<th>480.48 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 M Urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 M NaH$_2$PO$_4$</td>
<td>6.9 g</td>
<td>13.8 g</td>
</tr>
<tr>
<td>0.01 M Tris</td>
<td>5 ml</td>
<td>10 ml (of 1 M Tris HCl pH 8.0)</td>
</tr>
<tr>
<td>adjust pH to 8.0 with NaOH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Buffer C**

<table>
<thead>
<tr>
<th>Component</th>
<th>240.24 g</th>
<th>480.48 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 M Urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 M NaH$_2$PO$_4$</td>
<td>6.9 g</td>
<td>13.8 g</td>
</tr>
<tr>
<td>0.01 M Tris</td>
<td>5 ml</td>
<td>10 ml (of 1 M Tris HCl pH 8.0)</td>
</tr>
<tr>
<td>adjust pH to 6.3 with HCl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Buffer E**

<table>
<thead>
<tr>
<th>Component</th>
<th>240.24 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 M Urea</td>
<td></td>
</tr>
<tr>
<td>0.1 M NaH$_2$PO$_4$</td>
<td>6.9 g</td>
</tr>
<tr>
<td>0.01 M Tris</td>
<td>5 ml (of 1 M Tris HCl pH 8.0)</td>
</tr>
<tr>
<td>adjust pH to 4.5 with HCl</td>
<td></td>
</tr>
</tbody>
</table>

1. Resuspend the cells in Buffer A: 5 ml per gram weight.
2. Stir cells for 2 hours at room temperature.
3. Centrifuge lysate at 10,000 rpm for 30 minutes at 4°C, collect supernatant.
4. Take 1 ml of a 50% slurry of Ni-NTA resin, wash 3 times in Buffer A (50 ml), equilibrate in Buffer A (v/v).
5. Add 50% Ni-NTA resin to supernatant from step 3 and stir at room temperature for 1-2 hours.
6. Centrifuge at 1600 rpm for 5 minutes. Discard supernatant.
7. Wash beads 3 times with 10-50 ml of Buffer A (centrifuge at 1600 rpm for 5 minutes).
8. Wash beads 3 times with 10-50 ml of Buffer B.
9. Wash beads 3 times with 10-50 ml of Buffer C.
10. Elute protein with 500 ml of Buffer E (7 times).
11. Centrifuge the eluates for 20 seconds at 10,000 rpm to remove rest of beads.
12. Analyze samples on SDS-PAGE: 20 μl sample + 10 μl 3x Lämmli buffer.
**Protein gel electrophoresis (Lämmli)**

- **solutions:**
  - **Tris gel buffer:**
    - 375 mM Tris pH 8.8
    - 0.1% SDS
  - **stacking gel (4%):**
    - 26.67 ml acrylamide-stock (30%)
    - 0.2 g SDS (0.1%)
    - 3 g Tris base
    - adjust pH to 6.8
    - add H$_2$O to 200 ml
    - store at 4°C
  - **10x running buffer:**
    - 250 mM Tris
    - 30.3 g/l Tris base
    - 1920 mM glycine
    - 144.2 g/l 1% SDS
    - 10 g/l pH should be ~8.3 (do not adjust pH!!!)
  - **3x Lämmli:**
    - 187.5 mM Tris pH 6.8
    - 6% SDS
    - 30% glycerol
    - 15% -----mercaptoethanol
    - 0.003% bromphenolblue
    - make 1 ml aliquots
    - heat 5 min at 100°C in heating block

- **sample preparation:**
  - x [l protein extract
  - 5 [l 3x Lämmli
  - H$_2$O to 15 [l
  - denature 5 min at 95-100°C (heating block)
  - samples can be stored at –20°C (heat again before loading)

- **gel preparation:**
  - for Hoefer Minigels (Mighty Small II SE250)
  - clean 1 glass plate, 1 white plate and 2 spacers (0.75 mm) per gel
  - assemble for pouring (white plate in the back, do not tighten screws too much, make sure spacers and plates are level at the bottom)
  - prepare 10% APS (ammonium persulfate) in H$_2$O
  - make a mark on glass plate to indicate the volume of the gel (~1 cm below the comb)
  - acrylamide-solution: e.g. for two 10% gels:
    - 3.3 ml acrylamide-stock (30%)
    - 6.7 ml Tris gel buffer
    - 200 [l 10% APS
    - 6 [l TEMED
  - mix well and pour gel up to mark
  - add 200 [l n-butanol on top
  - polymerize for ~1 h
-remove gel from pouring unit, remove n-butanol with H₂O
-remove drops between plates using a whatman paper
-stacking gel: for 2 gels:
  4 ml stacking gel (4%)
  200 μl 10% APS
  4 μl TEMED
-mix well and fill gel up to the very top
-immediately add combs (stacking gel polymerize very fast)
-polymerize for 15 min

-gel run:
-prepare 250 ml 1x running buffer
-carefully remove combs and load samples
-add protein standard
-run gels for 50-60 min at 25-30 mA per gel (use water cooling and mix buffer) until bromphenolblue reaches bottom of gel and runs out
-the protein gels can be stained directly or used in a western blot procedure

Staining of protein gels with Coomassie

- solutions:  
  -Coomassie-solution 1: 0.025% Coomassie Brilliant Blue R250
    25% isopropanol
    10% HAc
  
  -Coomassie-solution 2: 0.0025% Coomassie Brilliant Blue R250
    10% isopropanol
    10% HAc

  -destaining solution: 10% isopropanol
    10% HAc

-shake protein gel 20-30 min in Coomassie-solution 1
-partially destain in Coomassie-solution 2 (gels can be left in this solution almost indefinitely)
-destain 2-3 h in destaining solution (add a rolled kim wipe to absorb the coomassie)
-fix for ~1 h in 10% HAc
-sandwich gel between wet cellophane foil, put sandwich on a whatman filter and cover with saran wrap
-dry for 1 h on gel drier at 80°C
-make sure gels do not curl