

PURIFICATION OF PROTEINS FROM INCLUSION BODIES

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Recipes for all solutions (highlighted) in **bold** are included at the end of the protocol.

1.
 - a. Suspend the cell pellet (from 1 L culture) in 30–35 ml of **PBST buffer**.
 - b. Sonicate cells in an ice-bath at 200 W for 8 min.
 - c. Centrifuge cell lysate for approximately 15 min at 8000 rpm, 4°C. Discard the supernatant.
 - d. Re-suspend the pellets in 30 ml **TNMFx-2M Urea buffer** before transferring to a 50 ml centrifuge tube. Rotate for 30 min at 4°C.
 - e. Sonicate the solution in an ice-bath at 200 W for 1–4 min.
 - f. Rotate for 30 min at 4°C.
 - g. Centrifuge for 15 min at 8000 rpm, 4°C. Discard the supernatant.
 - h. Re-suspend the pellets in 30ml of **TNMFx-0.1% Triton-X100**. Rotate for 30 min. at 4°C.
 - i. Centrifuge for 15 min at 8000 rpm, 4°C. Discard the supernatant. Vortex and wash the pellets with 2x volumes of dH₂O before transferring to a 10 ml centrifuge tube. Centrifuge at 8000 rpm for 10 min.
 - j. Repeat washing until the supernatant becomes clear. Collect the pellets.
 - k. Repeat washing until the supernatant becomes clear. Collect the pellets.
 - l. Dissolve the proteins depending on intended application:
 - For immunization, dissolve in 1.5x volumes of **8 M urea** (pH 8.0)
 - For antibody purification, incubate in 2x volumes of **PBS with 2% Sarkosyl** overnight at 4°C. Collect the supernatant by centrifugation at 12000 rpm for 15 min.

Solutions

TNMFx-2M Urea	For 1000 ml
50 mM Tris-base	6.06 g
150 mM NaCl	8.77 g
1 mM EDTA	0.37 g
2 M Urea	120.20 g
Adjust to pH 8.0	
Add ddH ₂ O to 1000 ml	

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Solutions

PBST buffer	For 1000 ml
58 mM Na ₂ HPO ₄ • 12H ₂ O	20.77 g
17 mM NaH ₂ PO ₄ • 2H ₂ O	2.65 g
68 mM NaCl	3.98 g
1% Triton-X100	10 ml
Adjust to pH 7.4	
Add ddH ₂ O to 1000 ml	

TNMFx-0.1% Triton X100	For 1000 ml
50 mM Tris	6.06 g
150 mM NaCl	8.8 g
1 mM EDTA	0.4 g
0.1% Triton-X100	1 ml
Adjust to pH 8.0	
Add ddH ₂ O to 1000 ml	

PBS with 2% Sarkosyl	For 200 ml
58 mM Na ₂ HPO ₄ • 12H ₂ O	20.77 g
17 mM Na ₂ HPO ₄ • 2H ₂ O	2.65 g
68 mM NaCl	0.80 g
2% Sarkosyl	4 .00 g
Adjust to pH 8.0	
Add ddH ₂ O to 200 ml	

8 M Urea	For 200 ml
Urea	96.08 g
Adjust to pH 8.0	
Add ddH ₂ O to 200 ml	