

PURIFICATION OF PROTEINS FROM INCLUSION BODIES

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Recipes for all solutions highlighted **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 6 min.
 - c. Centrifuge cell lysate for approximately 13 min at 8000 rpm, 4°C. Discard the supernatant.
 - d. Re-suspend the pellets in 5 ml **TNMFx-2M Urea buffer** before transferring to a 10 ml centrifuge tube.
 - e. Sonicate the solution in an ice-bath at 200 W for 1 min.
 - f. Add an additional 5 ml **TNMFx-2M Urea** to the tube. Rotate for 30 min at 4°C.
 - g. Centrifuge for 20 min at 4000 rpm, 4°C. Discard the supernatant.
 - h. Repeat steps d-g.
 - i. Re-suspend the pellets in 5 ml of **TNMFx-0.1% Triton-X100**.
 - j. Sonicate the solution in an ice-bath at 200 W for 1 min.
 - k. Add an additional 5 ml **TNMFx-0.1% Triton-X100** to the tube. Rotate for 30 min at 4°C.
 - l. Centrifuge for 20 min at 4000 rpm, 4°C. Discard the supernatant.
 - m. Repeat steps i-l.
 - n. Vortex and wash the pellets with 2x volumes of dH₂O. Centrifuge at 1000 rpm for 2 min.
 - o. Repeat washing until the supernatant becomes clear. Collect the pellets.
 - p. Dissolve the proteins depending on intended application:
 - For immunization, dissolve in 1.5x volumes of **8 M urea (pH 8)**
 - For antibody purification, incubate in 2x volumes of **PBS with 2% Sarkosyl** overnight at 4°C. Collect the supernatant by centrifugation at 1000 rpm for 7 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	8.24 g
17 mM NaH ₂ PO ₄	2.04 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	1.65 g
17 mM Na ₂ HPO ₄	0.41 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	