PURIFICATION OF ACTIVE EUKARYOTIC PROTEINS FROM THE INCLUSION BODIES IN *E. Coli*

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The essence of this method is to purify the inclusion bodies followd by selective solubilization of the expressed protein.

This protocol was designed for a 1L culture. However, one can scale the volumes up or down, depending on the type of experimentation performed. Both 100ml cultures and 500 ml cultures were prepared for this protocol (the results section specifies further).

1. The cells from the 1L culture are transformed into two 500ml tubes and are pelleted by centrifugation at 5000rpm/min at 4 degrees C. To prevent contaminating the inclusion bodies by proteins from the outer membrane, the outer membrane is removed by resuspending the cell pellet from 1L culture in 50ml BUFFER A. (If 100ml culture was prepared, only 5 ml Buffer A would be required. This down -scaling of volumes is required whenever a volume is indicated).
2. Incubate on ice for 10 minutes. Pellet the cells by centrifugation at 6000rpm/5min at 4 degrees C. Resuspend in 50ml of ice cold water. Leave on ice for 10 min. Centrifuge at 8200 rpm/5 min at 4 degrees C.
3. Resuspend the pellet in 10ml Buffer P containing protease inhibitors.
4. Sonicate the resuspended mixture 3x (50W) each with a 30sec. pulse with a 30 sec. pause in between pulses. RNase T1 (1.3x103 U/10ml) + DNase I (400 ug/10ml) are added to the sonicated cell suspension and incubated at room temp for 10 min.
5. The suspension is further diluted by addition of 40 ml of Washing Buffer P and the crude inclusion bodies are pelleted by centrifugation at 11,000 rpm for 30 min at 4 degrees C. Keep aliquot and assay the supernatant for proteins.
6. The pellet (inclusion bodies) is suspended in 40 ml of Buffer W. Incubate on ice for 10 min and centrifuge at 15,000 rpm/5min at 4 degrees C. for 10 min. Keep aliquot of supernatant. Repeat this washing step and save the supernatant.
7. Resuspend the pellet in 10ml of Buffer D. A brief sonication with a 5 sec. pulse (50W) facilitates the solubilization of the aggregated proteins (NOTE: the pellet may also be resuspended in 4M or 8M urea in addition to BUFFER D).
8. The resultant suspension is incubated on ice for an additional 1 hour.
9. Centrifuge at 10,500 rpm for 30 min at 4 degrees C. Add the supernatant to 100ml of Renaturation Buffer R, and stir gently at 4 degrees C. over night to renature the proteins.
10. The next day, the supernatant is clarified by centrifugation at 12,000 rpm for 30 min at 4 degrees C. The supernatant contains highly purified expressed proteins.

SOME CONSIDERATIONS:

a) For plasmid #256 pGEX UTKMTB, step (7) of protocol:

Instead of suspending pellet in BUFFER D, 4M urea or 8M urea, inclusion bodies from #256 were resuspended in a BUFFER containing 0.5% Deoxycholate; 0.2M Na CO3 pH 11.5, 5mM EDTA.

b) After step (3), 1X PIN and freshly prepared 1mM PMSF are added at each following s step.

BUFFER A	BUFFER P	Buffer W
20mM Tris-HCl pH 7.5	PBS w/o Ca, Mg	PBS w/o Ca, Mg
20% Sucrose	5mM EDTA	25% Sucrose
1mM EDTA	1X PIN, PMSF,--added fresh	5mM EDTA
		1% Triton

BUFFER R	BUFFER D	BUFFER U1
50Mm Tris-HCl pH 8.0	50mM Tris-Hcl pH 8.0	50mM Tris-HCl pH 8.0
1mM DTT	5M Guanidium HCl	4M Urea
20% Glycerol	5mM EDTA	

BUFFER U2
50mM Tris HCl pH 8.0
8M Urea