ENDEXT® Technology

Purification of GST-tagged protein

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General remarks

This protocol is based on the purification procedure used with the automatic protein synthesizer Protemist® DT, but it can be used for manual purification as well.

1 Materials

Item	Concentration
Glutathione Sepharose 4B (GE Healthcare Bio-Sciences, Code No.17-0756-01)	50 % slurry
Column	
Equilibration buffer (PBS) Wash buffer (PBS)	8.1 mM Disodium hydrogen phosphate, 1.47mM Potassium dihydrogen phosphate, 2.68mM Potassium chloride, 137mM Sodium chloride, pH 7.4
Elution buffer	50mM Tris-HCl, 10mM Glutathione (Reduced form), pH 8.0

Note: Use the resin specified in the above list.
Protein solution expressed by ENDEXT® Technology contains 4mM dithiothreitol.

2 Protocol

2.1 Decant the liquid portion of the 50% slurry and replace it with distilled water.

2.2 Stir the mix to resuspend the resin and pour the resultant slurry into the column.

Note: Follow the supplier's instruction to determine the optimum amount of the resin. Too much resin may cause unspecific adsorption, while too little resin may not be able to adsorb all of the GST-tagged target protein.

2.3 Equilibrate the column with the equilibration buffer.

2.4 Apply the protein solution first and then the flow-through solution to the column. Repeat this 10 times or more.

2.5 Wash the column with 10 times as much volume of the wash buffer as the column volume.

2.6 Elute with 2 to 10 times as much volume of the elution buffer as the column volume.

3 Example of GST-tagged protein purification

N-terminal GST-tagged GUS was used as models. For their synthesis, large scale bi-layer method (using a well in a 6 multi-well plate) was used (see protocol of “Bi-layer”). The wheat germ extract (WEPRO®), buffer, and resin used for the test are shown in the table below:

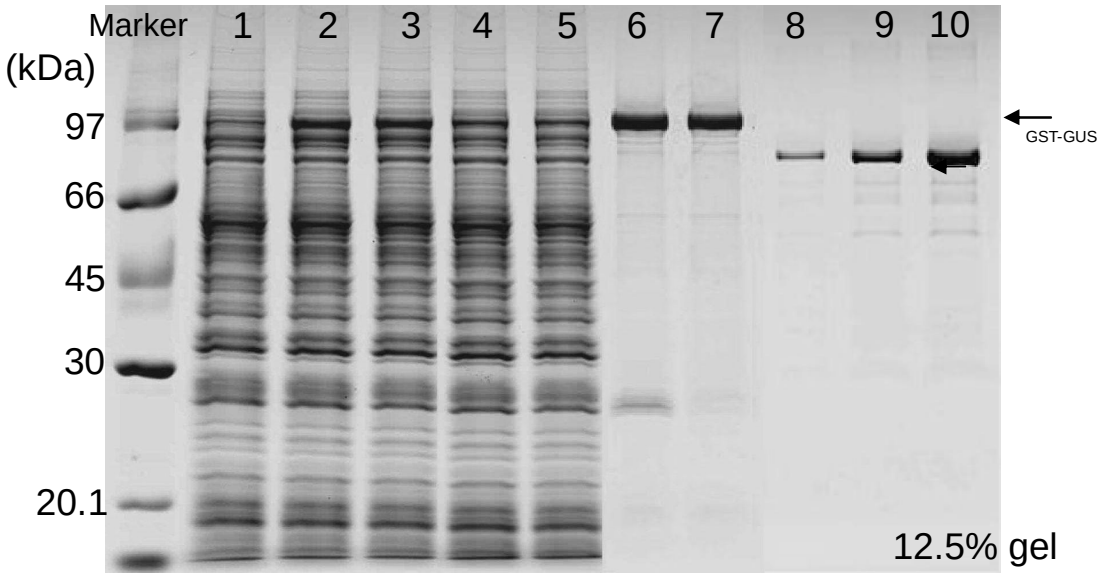
WEPRO®	**WEPRO®1240** or **WEPRO®1240G** (*1)	250 µl
Protein	N-terminal GST-tagged GUS	6 ml
Resin	Glutathione Sepharose 4B (GE Healthcare Bio-Sciences, Code No.17-0756-01)	400 µl (50% slurry)
Equilibration buffer	PBS, pH 7.4	5 ml
Wash buffer	Same as Equilibration buffer	5 ml
Elution buffer	50mM Tris-HCl, 10mM Glutathione (Reduced form), pH 8.0	500 µl

*1: WEPRO1240G is specialized for higher degree of purification of GST-tagged protein, while WEPRO1240 is a standard type of wheat germ extract.

3.1 Result of GST-tagged GUS purification

When WEPRO®1240G was used, recovery rate(*1) was about 35% and purification rate was about 70 %. About 150μg of purified GST-GUS yielded from 0.25 ml of WEPRO®1240G (Fig.1).

*1: recovery rate(%) = (amount of target protein in eluate / amount of target protein in crude fraction) x 100



No.	WEPRO®	Fraction	apply (μl / lane)
1	1240	Negative ctrl (crude)	3
2	1240	Crude	3
3	1240G	Crude	3
4	1240	Flow through	3
5	1240G	Flow through	3
6	1240	Elute	2
7	1240G	Elute	2
8	-	BSA 100ng	
9	-	BSA 250ng	
10	-	BSA 500ng	

Fig. 1 Result of SDS-PAGE (coomassie stained)