ENDEXT® Technology

Purification of His-tagged protein

CellFree Sciences Co., LTD

Leading Venture Plaza 201, 75-1, Ono-cho, Tsurumi-ku,
Yokohama-city, Kanagawa, 230-0046, JAPAN
TEL +81-45-500-2119
FAX +81 45-500-2117

Ver.1.0 Mar 8, 2006

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.
- Abbreviations: GFP, Green Fluorescence Protein; GUS, β-D-glucuronidase; Akt1, v-akt murine thymoma viral oncogene homolog 1; ERK2, Extracellular signal Regulated Kinase 2; JNK2, Jun N-terminal kinase 2

1 Materials

Item	Concentration
Ni Sepharose High Performance (GE Healthcare Bio-Sciences, Code No.17-5268-01)	50 % slurry
Column	
Equilibration buffer Wash buffer	20 mM Phosphate buffer, 0.3 M NaCl, 20 mM Imidazole, pH 7.5
Elution buffer	20 mM Phosphate buffer, 0.3 M NaCl, 500 mM Imidazole, pH7.5

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

Note: The Gly, Gln, Arg, and His left in the solution unused for protein synthesis would little affect the adsorption of the His-tagged protein by the resin.

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

Note: Washing with wash buffer containing a higher concentration of imidazole can increase the purity of a recombinant His-tagged protein, although it decreases the yield.

In general, washing with wash buffer containing 20 mM imidazole is recommended. (See Fig. 3 on page 6.)

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

3 Practical examples of His-tagged protein purification

3.1 Examples of His-tagged GFP and His-tagged GUS purification.

N-terminal $(His)_6$-tagged GFP and N-terminal $(His)_6$-tagged GUS were 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®), buffers, and resin used for the test are shown in the table below:

WEPRO®	**WEPRO1240** or **WEPRO1240H**	250 µl
Synthesized Protein	N-terminal $(His)_6$-tagged GFP and N-terminal $(His)_6$-tagged GUS	6 ml each
Resin	Ni sepharose High Performance (GE Healthcare Bio-Sciences, Code No. 17-5268-01)	100 µl (50 % slurry)
Equilibration buffer	20 mM Phosphate buffer, 0.3 M NaCl, **10 mM Imidazole**, pH 7.5 or 20 mM Phosphate buffer, 0.3 M NaCl, **50 mM Imidazole**, pH 7.5	5 ml
Wash buffer	Same as Equilibration buffer	5 ml
Elution buffer	20 mM Phosphate buffer, 0.3 M NaCl, 500 mM Imidazole, pH 7.5	500 µl

Note: WEPRO1240H is specialized for higher degree of purification of His-tagged protein, while WEPRO1240 is a standard type of wheat germ extract.

3.1.1 Result of $(His)_6$-tagged GFP purification

When WEPRO1240H and wash buffer containing 10 mM imidazole were used, recovery rate* and purification rate were both about 70 % and about 0.25 mg of purified His-GFP yielded from 0.25 ml of WEPRO1240H. (Fig.1)

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

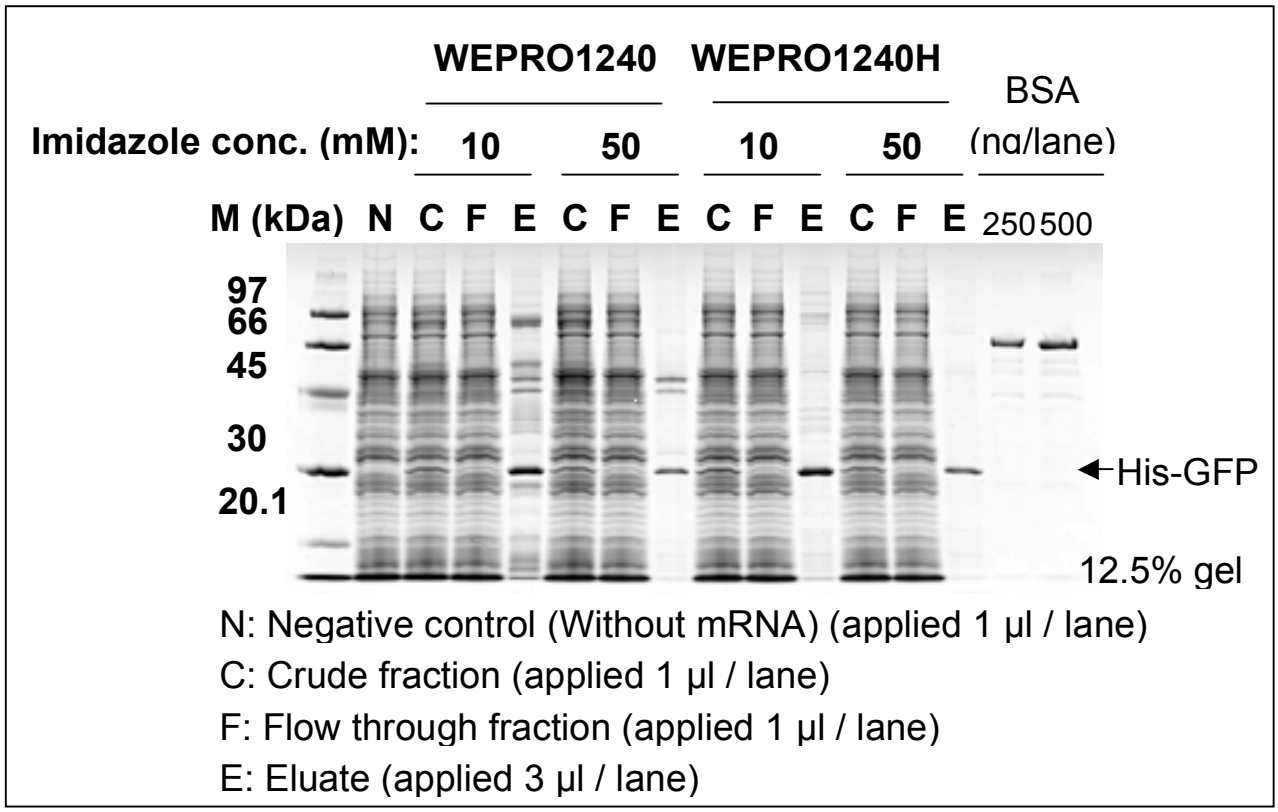

Fig. 1 Result of SDS-PAGE (coomassie stained)

3.1.2 Result of $(His)_6$-tagged GUS purification

When WEPRO1240H and wash buffer containing 10 mM imidazole were used, recovery rate* and purification rate were both about 70 % and about 0.25 mg of purified His-GFP yielded from 0.25 ml of WEPRO1240H. (Fig.2)

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

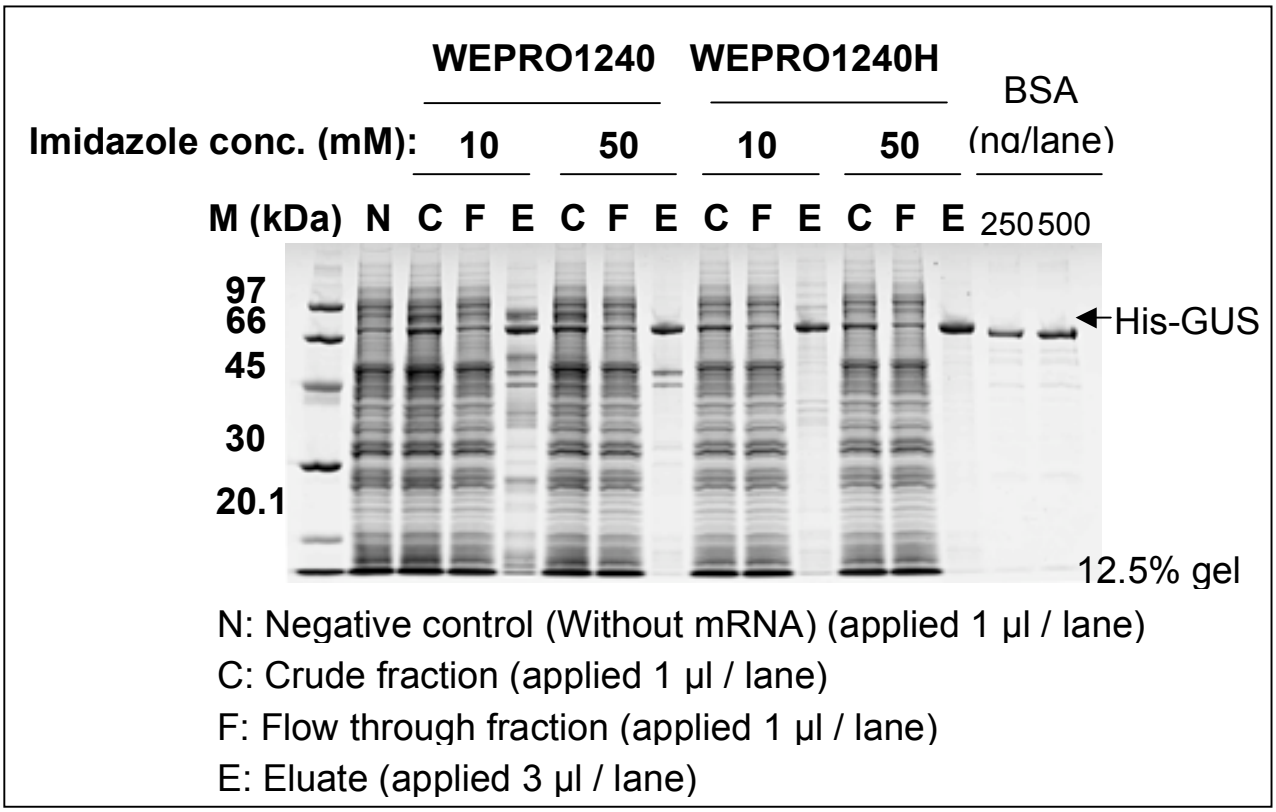

Fig. 2 Result of SDS-PAGE (coomassie stained)

3.2 Effect on elution of imidazole concentration in wash buffer.

N-terminal $(His)_6$-tagged GFP, DHFR, Akt1, ERK2, and JNK2 were compared. 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®), buffers, and resin used for the test are shown in the table below:

WEPRO®	**WEPRO1240H**	250 µl
Resin	Ni sepharose High Performance (GE Healthcare Bio-Sciences, Code No. 17-5268-01)	100 µl (50 % slurry)
Equilibration buffer	20 mM Phosphate buffer, 0.3 M NaCl, **X mM* Imidazole**, pH 7.5 ***X = 10 mM, 20 mM, or 50 mM**	5 ml
Wash buffer	Same as Equilibration buffer	5 ml
Elution buffer	20 mM Phosphate buffer, 0.3 M NaCl, 500 mM Imidazole, pH 7.5	500 µl

3.2.1 Test results

The concentration of imidazole, whether 10 mM or 20 mM, in the wash buffer little affect the yield of each His-tagged protein, but it affected its purity; a higher purity was obtained with 20 mM imidazole than with 10 mM imidazole. (Fig.3)

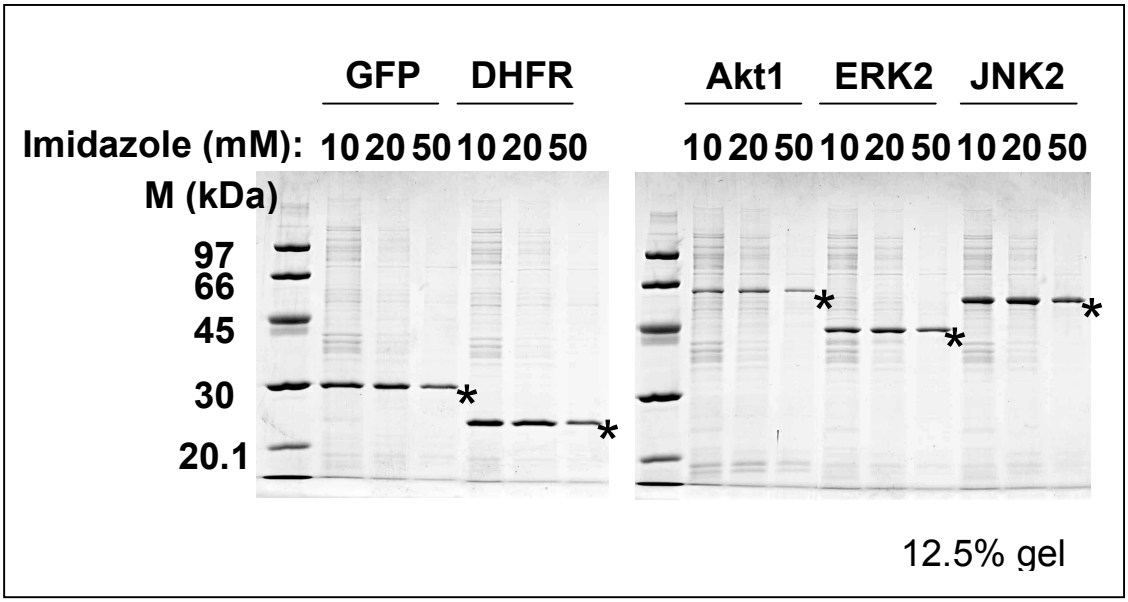

Fig. 3 SDS-PAGE of each eluate. (coomassie stained)

3.3 Discussion.

In general, washing with wash buffer containing a higher concentration of imidazole can increase the purity but at the expense of the yield. As exemplified by His-GUS, however, some His-tagged protein can be purified with 50 mM imidazole buffer at the same recovery rate as with 10 mM buffer (Fig. 2). For ordinary proteins, washing with wash buffer containing 20 mM imidazole is recommended (Fig. 3), but the imidazole concentration can be varied depending on your needs.