Example 7

Case of normal level expression protein

1

Protein category	Cellular processes / Other		
M.W	17802	Wet weight of E. coli cells	11 g
Theoretical pI	4.8	Purified protein	15 mg
ε M	13100	Purification time	3 days

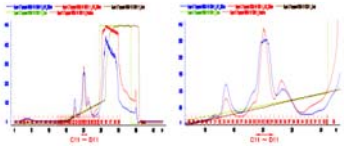
Method

Cell suspension in 20 mM Tris-HCl, 0.5 M NaCl, 1 mM PMSF, 5 mM β–mercaptoethanol, (pH 8.0) , Total vol. 20 ml
↓
Sonication (OUTPUT 6.5, Duty 50, 1 min × 10)
↓
Heat - treatment at 70°C for 11.5 min
↓
14,000 rpm × 30 min at 4°C
desalting
↓
Super Q TOYOPEARL 650M (30 ml) (anion exchange column)
desalting
↓
RESOURCE Q (6 ml) (anion exchange column)
desalting
↓
Bio-Scale CHT20-I (hydroxyapatite column)
↓
HiLoad 16/60 Superdex 75 pg (gel filtration column)
↓
Protein concentration determination
Wavelength scan, Native-PAGE analysis, DLS analysis,
N-terminal Amino acid sequencing

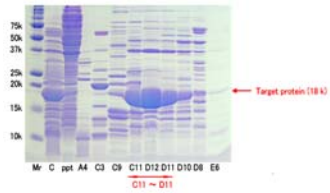
2 SuperQ TOYOPEARL 650M (30 ml)

Buffer	A : 20 mM Tris-HCl (pH 8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH 8.0)
Gradient (Volume)	0 → 0.4 M NaCl (4 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	0.21 M

Chart



SDS-PAGE

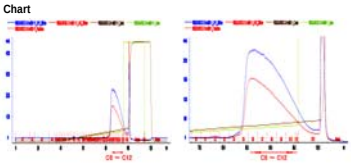


Fractions C11~D11 were pooled and desalted using a HiPrep 26/10 desalting column with 20 mM Tris-HCl (pH 8.0).

3 Resource Q (6 ml)

Buffer	A : 20 mM Tris-HCl (pH 8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH 8.0)
Gradient (Volume)	0 → 0.2 M NaCl (10 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	0.16 M

Chart



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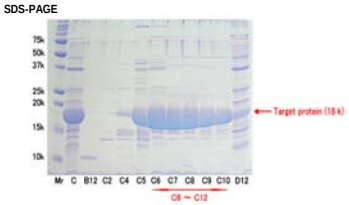
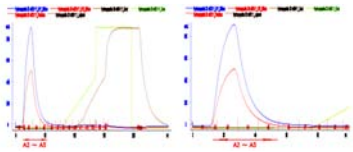


Fractions C6~C12 were pooled and desalted using a HiPrep 26/10 desalting column with 10 mM Na phosphate (pH7.0).

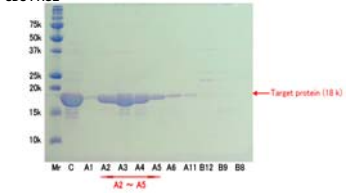
4 CHT20-I (20 ml)

Buffer	A : 10 mM Na phosphate (pH 7.0) B : 200 mM Na phosphate (pH 7.0)
Gradient (Volume)	0.01 → 0.1 M Na phosphate (3 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	10 mM

Chart



SDS-PAGE

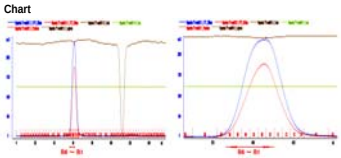


Fractions A2~A5 were pooled and concentrated using ultrafiltration (VIVASPIN 5 k cut).

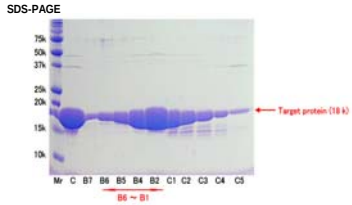
5 HiLoad 16/60 Superdex 75 (120 ml)

Buffer	A : 20 mM Tris-HCl, 200 mM NaCl (pH 8.0)
Flow rate	0.5 ml/min

Chart



SDS-PAGE

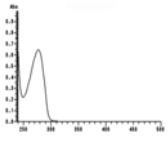


Fractions B6~B1 were pooled and concentrated using ultrafiltration (VIVASPIN 5 k cut).

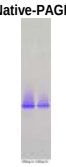
6 Protein concentration

	Abs.	dilution	Conc. (mg/ml)	Vol. (ml)	Total Protein (mg)
At 280 mm	0.62	20	17	0.91	15
Bio-Rad Protein Assay	0.48	20	17	0.91	15

UV spectrum

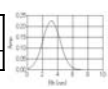


Native-PAGE



Dyna-Pro

Radius (nm)	Est. M.W (kDa)	Polydispersity (%)	Base line	SOS Noise
3.12	48.3	24	1	20.6



N-terminal amino acid sequencing

Predicted sequence	MVEPSL
Detected sequence	MVEPSL
Quality	>95%

Expression level of this protein sample was very good. We cultured *E. coli* in 2.5 L medium and purified the protein from 11 g cells following the normal method in our laboratory.

Example 8

Case of very low level expression protein

7

Protein category	Transport and binding proteins/ Anions		
M.W	40934	Wet weight of E. coli cells	75 g
Theoretical pI	6.0	Purified protein	1.6 mg
ε M	43500	Purification time	4 days

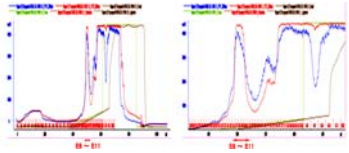
Method

Cell suspension in 20 mM Tris-HCl, 0.5 M NaCl, 5 mM β–mercaptoethanol, (pH 8.0) , 1 mM PMSF, Total vol. 150 ml
↓
Microfluidization (100 Mpa, 2 times)
↓
Heat - treatment at 70°C for 13 min
↓
15,000 rpm × 30 min at 4°C
desalting
↓
Super Q TOYOPEARL 650M (80 ml) (anion exchange column)
desalting
↓
RESOURCE Q (6 ml) (anion exchange column)
desalting
↓
Bio-Scale CHT10-I (hydroxyapatite column)
↓
HiLoad 16/60 Superdex 200 pg (gel filtration column)
↓
Protein concentration determination
Wavelength scan, Native-PAGE analysis, DLS analysis,
N-terminal Amino acid sequencing

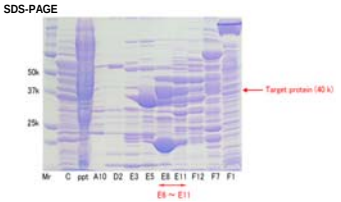
8 SuperQ TOYOPEARL 650M (80 ml)

Buffer	A : 20 mM Tris-HCl (pH 8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH 8.0)
Gradient (Volume)	0 → 0.3 M NaCl (3 Column Vol.)
Flow rate	8 ml/min
Elution Conc.	0.11 M

Chart



SDS-PAGE

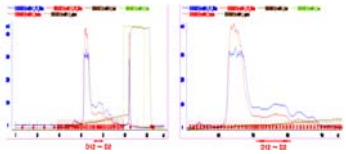


Fractions E6~E11 were pooled and desalted using a HiPrep 26/10 desalting column with 20 mM Tris-HCl (pH 8.0).

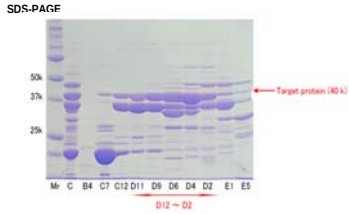
9 Resource Q (6 ml)

Buffer	A : 20 mM Tris-HCl (pH.8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH.8.0)
Gradient (Volume)	0 → 0.2 M NaCl (10 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	0.17 M

Chart



SDS-PAGE

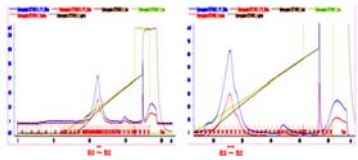


Fractions D12~D2 were pooled and desalted using a HiPrep 26/10 desalting column with 10 mM Na phosphate (pH7.0).

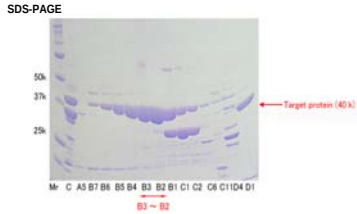
10 CHT10-I (10 ml)

Buffer	A : 10 mM Na phosphate (pH 7.0) B : 200 mM Na phosphate (pH 7.0)
Gradient (Volume)	0.01 → 0.1 M Na phosphate (10 Column Vol.)
Flow rate	3 ml/min
Elution Conc.	45 mM

Chart



SDS-PAGE

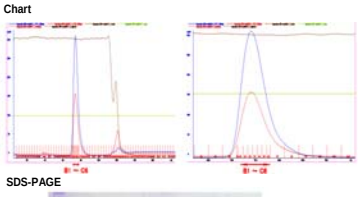


Fractions B3~B2 were pooled and concentrated using ultrafiltration (VIVASPIN 30 k cut).

11 HiLoad 16/60 Superdex 200 (120 ml)

Buffer	A : 20 mM Tris-HCl, 200 mM NaCl (pH 8.0)
Flow rate	0.5 ml/min

Chart



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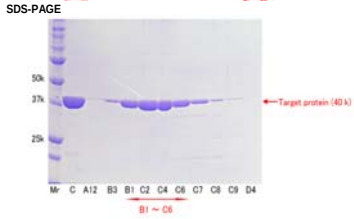
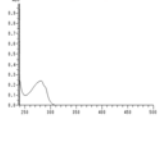


Fractions B1~C6 were pooled. This sample was not concentrated because of precipitation.

12 Protein concentration

	Abs.	dilution	Proteion Conc. (mg/ml)	Vol. (ml)	Total Protein (mg)
At 280 mm	0.22	1	0.23	7.0	1.6
Bio-Rad Protein Assay	0.24	1	0.36	7.0	2.5

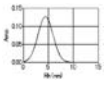
UV spectrum



Native-PAGE

Dyna-Pro

Radius (nm)	Est. M.W (kDa)	Polydispersity (%)	Base line	SOS Noise
5.12	155.5	51.2	1	52.5



N-terminal amino acid sequencing

Predicted sequence	MWLSG
Detected sequence	MWLSG
Quality	>95%

This protein sample was difficult to obtain sufficient amount of protein from small scale culture. We cultured *E. coli* in 18.0 L medium and obtained 75 g cells. In this case, microfluidizer was used to disrupt the cells completely.