Example 9

Case of AKTAxpress

1

Protein category	Hypothetical / Conserved		
M.W	36814	Wet weight of E. coli cells	9.0 g
Theoretical pI	5.5	Purified protein	5.5 mg
ε M	58000	Purification time	2 days

Method

Cell suspension in 20 mM Tris-HCl, 0.5 M NaCl, 5 mM β-mercaptoethanol, (pH 8.0) , Total vol. 17 ml

↓

Sonication (OUTPUT 6.5, Duty 50, 1 min × 10)

↓

Heat - treatment at 70°C for 13 min

↓

15,000 rpm × 30 min at 4°C

↓

HiTrap Chelating HP5 (affinity column)

↓

HiLoad 16/60 Superdex 200 pg (gel filtration column)

↓ desalting

RESOURCE Q (6 ml) (anion exchange column)

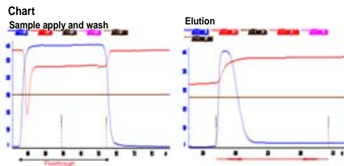
↓

Protein concentration determination
Wavelength scan, Native-PAGE analysis, DLS analysis,
N-terminal Amino acid sequencing

2 HiTrap Chelating HP5 (5 ml)

Buffer	A : 50 mM Tris-HCl, 50 mM Imidazole, 500 mM NaCl (pH 8.0) B : 50 mM Tris-HCl, 500 mM Imidazole, 500 mM NaCl (pH 8.0)
Gradient (Volume)	0.05 → 0.5 M Imidazole (stepwise.)
Flow rate	5 ml/min

Chart

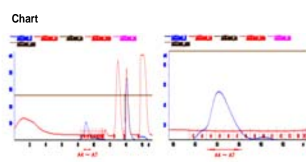


Fraction of peak was pooled at loop (6 ml) and applied to a HiLoad 16/60 Superdex 200 column automatically.

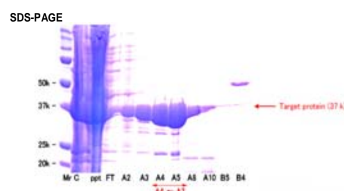
3 HiLoad 16/60 Superdex 200 (120 ml)

Buffer	A : 20 mM Tris-HCl, 200 mM NaCl (pH 8.0)
Flow rate	1.5 ml/min

Chart



SDS-PAGE

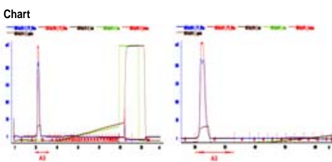


Fractions A4~A7 were pooled and desalted using a HiPrep 26/10 desalting column with 20 mM Tris-HCl (pH 8.0).

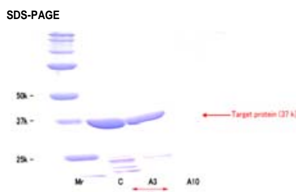
4 Resource Q (6 ml)

Buffer	A : 20 mM Tris-HCl (pH 8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH 8.0)
Gradient (Volume)	0 → 0.3 M NaCl (10 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	0 M

Chart



SDS-PAGE

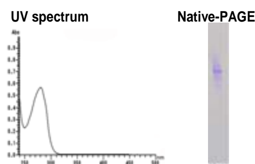


Fraction A3 was pooled and concentrated using ultrafiltration (VIVASPIN 10 k cut).

5 Protein concentration

	Abs.	dilution	Proteion Conc. (mg/ml)	Vol. (ml)	Total Protein (mg)
At 280 mm	0.57	31	11	0.5	5.5
Bio-Rad Protein Assay	0.22	31	13	0.5	6.5

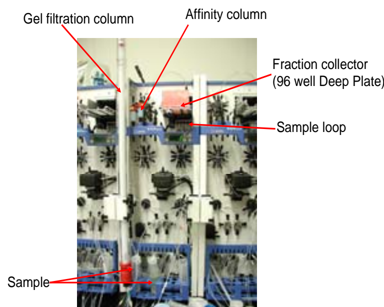
UV spectrum



Native-PAGE

N-terminal amino acid sequencing

Predicted sequence	MSDKIILH
Detected sequence	SDKIILH
Quality	85~95%



This protein sample was His-tag at N-terminal. In our laboratory, we use the AKTAxpress system for purification of protein having His-tag. The column chromatography was automatically performed after disruption of the cells and centrifugation. Sample was applied to chelating column and eluted. The eluted protein was collected in loops, applied to gel filtration column and colleted to the fraction collector (deep-well plate). Because the protein quality was not good, we manually purified it by additional chromatography.

Example 10

Case of Co-expression protein

6

Protein category	Amino acid biosynthesis / Aromatic amino acid family (multisubunit proteins)		
M.W	28946 45339	Wet weight of E. coli cells	9 g
Theoretical pI	6.1	Purified protein	12 mg
ε M	51100	Purification time	3 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. 17.5 ml

↓

Sonication (OUTPUT 6.5, Duty 50, 1 min × 10)

↓

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 CHT20-I (hydroxyapatite column)

↓

HiLoad 16/60 Superdex 200 pg (gel filtration column)

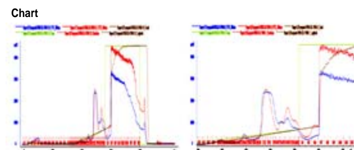
↓

Protein concentration determination
Wavelength scan, Native-PAGE analysis, DLS analysis,
N-terminal Amino acid sequencing

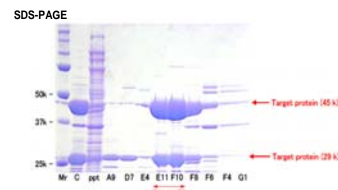
7 SuperQ TOYOPEARL 650M (80 ml)

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.	0.17 M

Chart



SDS-PAGE

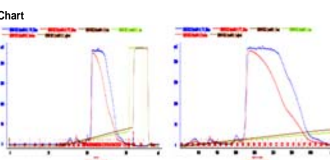


Fractions E11~F9 were pooled and desalted using a HiPrep 26/10 desalting column with 20 mM Tris-HCl (pH 8.0).

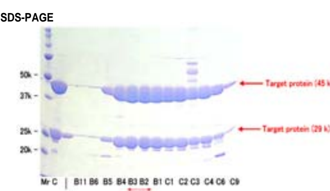
8 Resource Q (6 ml)

Buffer	A : 20 mM Tris-HCl (pH 8.0) B : 20 mM Tris-HCl, 2 M NaCl (pH 8.0)
Gradient (Volume)	0 → 0.3 M NaCl (15 Column Vol.)
Flow rate	4 ml/min
Elution Conc.	0.13 M

Chart



SDS-PAGE

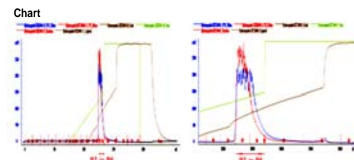


Fractions B3~B2 were pooled and desalted using a HiPrep 26/10 desalting column with 10 mM Na phosphate (pH 7.0).

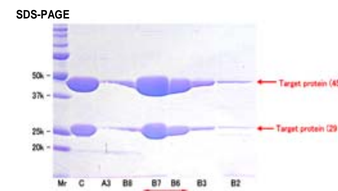
9 CHT20-I (20 ml)

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.	36 mM

Chart



SDS-PAGE

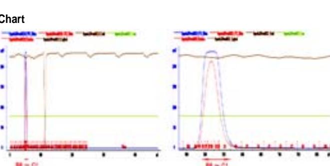


Fractions B7~B6 were pooled and concentrated using ultrafiltration (VIVASPIN 10 k cut).

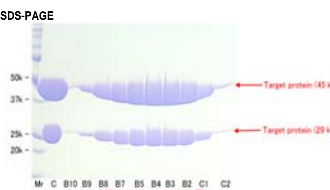
10 HiLoad 16/60 Superdex 200 (120 ml)

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

Chart



SDS-PAGE

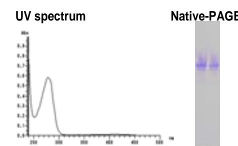


Fractions B8~C1were pooled and concentrated using ultrafiltration (VIVASPIN 10 k cut).

11 Protein concentration

	Abs.	dilution	Proteion Conc. (mg/ml)	Vol. (ml)	Total Protein (mg)
At 280 mm	0.58	80	67	0.18	12
Bio-Rad Protein Assay	0.42	80	60	0.18	11

UV spectrum



Native-PAGE

Dyna-Pro

Radius (nm)	Est. M.W (kDa)	Polydispersity (%)	Base line	SOS Noise
4.7	125.9	13.9	1	3.7

N-terminal amino acid sequencing

Predicted sequence	MTTLE + MLTLP
Detected sequence	MTTLE + MLTLP (1 : 1)
Quality	>95%

This sample is co-expression proteins. We coexpressed a pair of proteins using incompatible two plasmids (pET 9a (Kan^r), pET 11a (Amp^r)). We constructed different antibiotics resistance plasmid pair and these plasmids were transformed into *E. coli*. We cultured *E. coli* in medium containing two antibiotics and purified following the normal methods in our laboratory.