Example 2

Protein category	Amino acid biosynthesis / Histidine family (multi complex)		
M.W.	40,052 22,381	Wet weight of *E. coli* cells	27 g 13 g
Theoretical pI	5.9 9.9	Purified protein	15 mg
ε_M ($M^{-1}cm^{-1}$)	21,900 10,200	Purification time	5 days

(Methods)

Cells expressing protein No.1 and cells expressing protein No.2 were combined.

cell suspension in 20 mM Tris-HCl, 50 mM NaCl,
5 mM β-mercaptoethanol, pH 8.0, 140 ml

↓

sonication (OUT PUT 8, DUTY 50 and 1 min × 10)

↓

heat-treatment at 70℃ for 10 min

↓

on ice for 12 min

↓

40,000 rpm × 1 h at 4℃

↓

column scouting RESOURCE ISO and RESOURCE PHE
(hydrophobic column)

↓

RESOURCE ISO (hydrophobic column)

↓ ← (desalting)

HiTrap Heparin (affinity column)

↓

Bio-Scale CHT-2 (hydroxyapatite column)

↓ ← (concentration)

HiLoad 16/60 Superdex 75 prep. grade (gel filtration column)

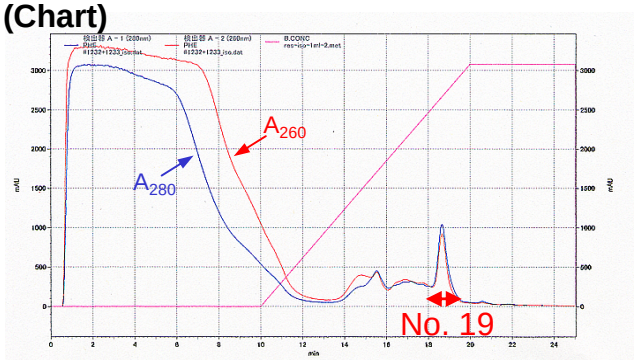
↓

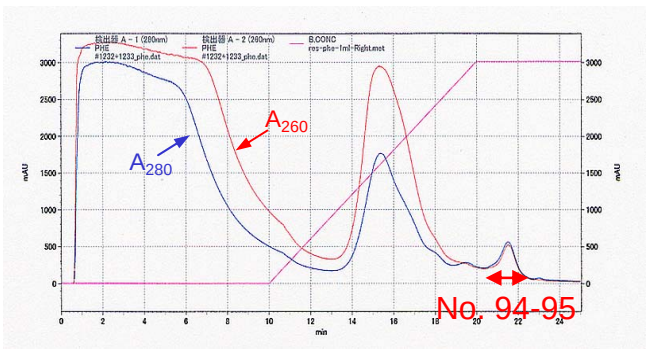
protein concentration determination

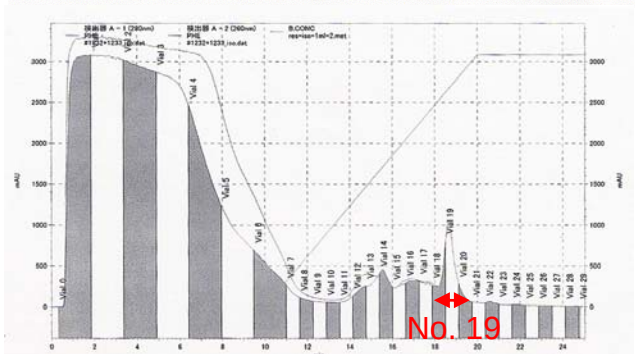
Step 1: Column Scouting

Flow rate	1 ml / min
Gradient (Volume)	1.35 → 0 M $(NH_4)_2SO_4$ (10 column volumes)
Buffer	A = 50 mM Na phosphate, 1.35 M $(NH_4)_2SO_4$, pH 7.0 B = 50 mM Na phosphate, pH 7.0
Eluted Conc.	RESOURCE ISO: 0.2 M $(NH_4)_2SO_4$ RESOURCE PHE: 0 M $(NH_4)_2SO_4$

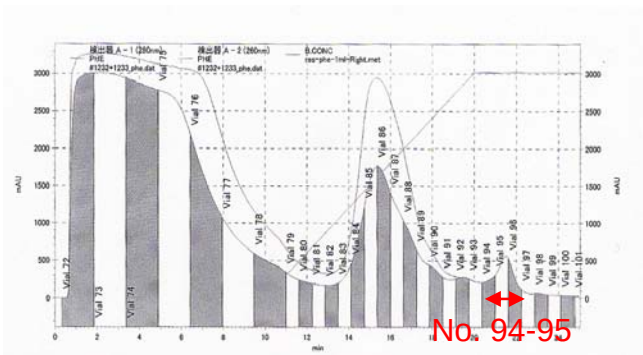
(Chart)







RESOURCE ISO



RESOURCE PHE

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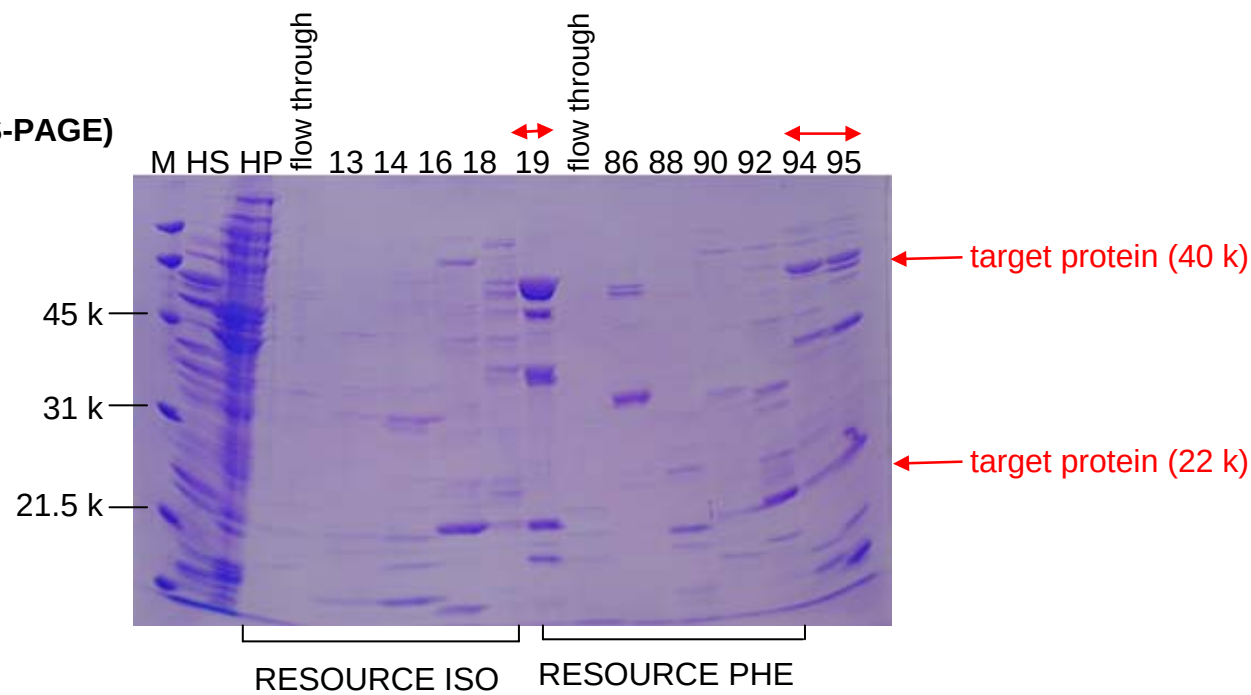
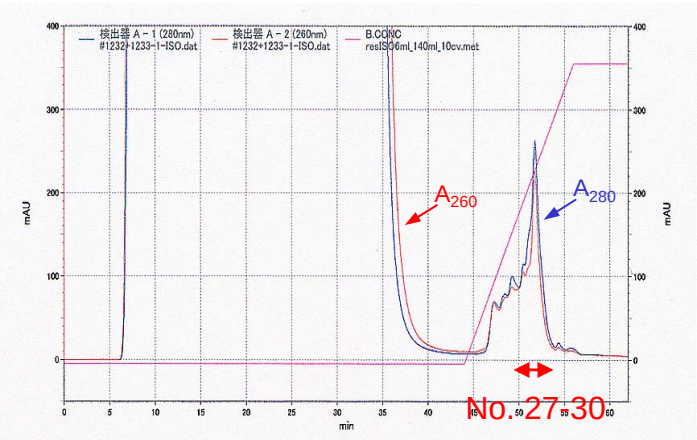


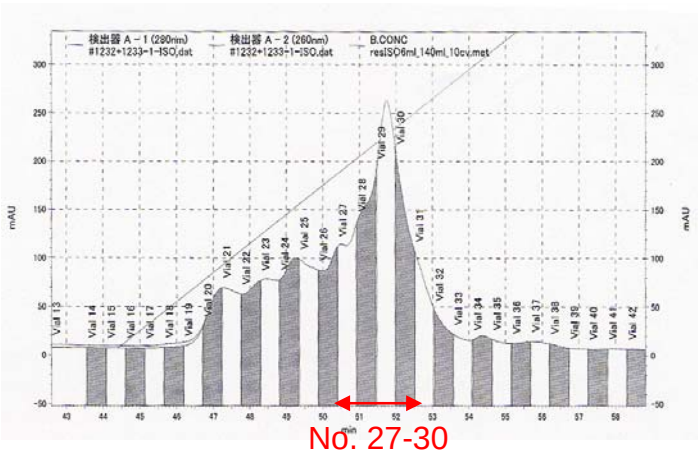
RESOURCE ISO column was selected.

Step 2: RESOURCE ISO (6 ml)

Flow rate	5 ml / min
Gradient (Volume)	1.35 → 0 M $(NH_4)_2SO_4$ (10 column volumes)
Buffer	A = 50 mM Na phosphate, 1.35 M $(NH_4)_2SO_4$, pH 7.0 B = 50 mM Na phosphate, pH 7.0
Eluted Conc.	0.5 M $(NH_4)_2SO_4$

(Chart)





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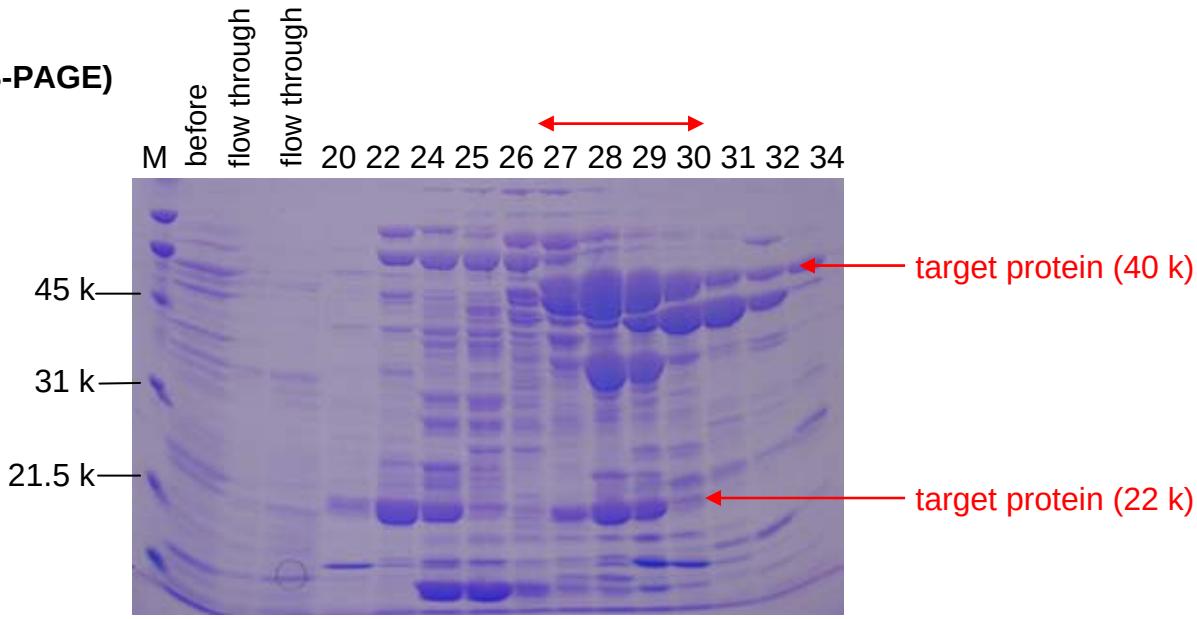
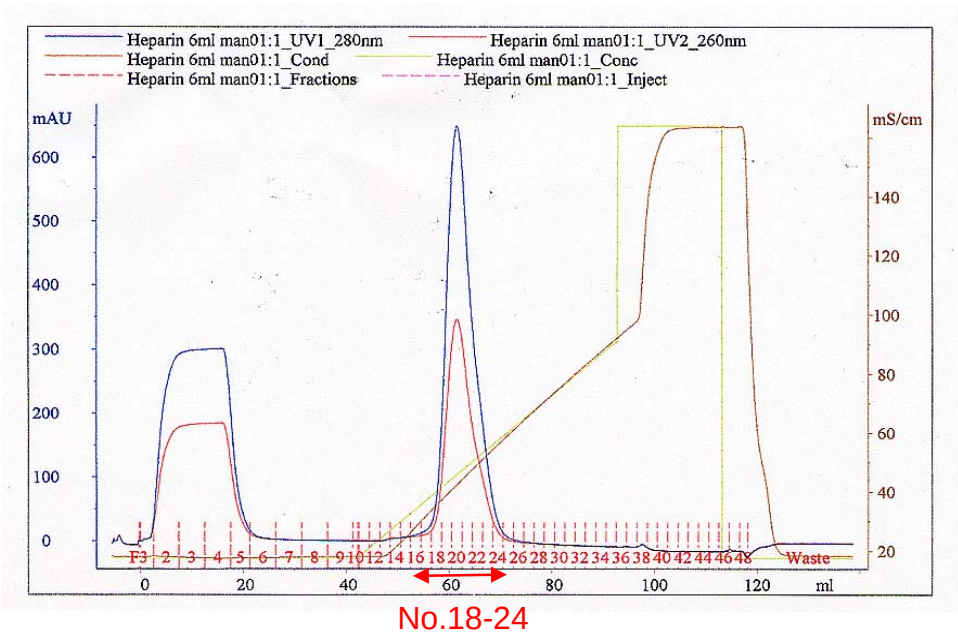


Fractions 27 to 30 were pooled and desalted on a desalting column equilibrated with 10 mM Na phosphate, 0.15 M NaCl, pH 7.0.

Step 3: HiTrap Heparin (5 ml)

Flow rate	5 ml / min
Gradient (Volume)	0 → 0.5 M NaCl (20 column volumes)
Buffer	A = 10 mM Na phosphate, 0.15 M NaCl, pH 7.0 B = 10 mM Na phosphate, 2.0 M NaCl, pH 7.0
Eluted Conc.	0.4 M NaCl

(Chart)



No.18-24

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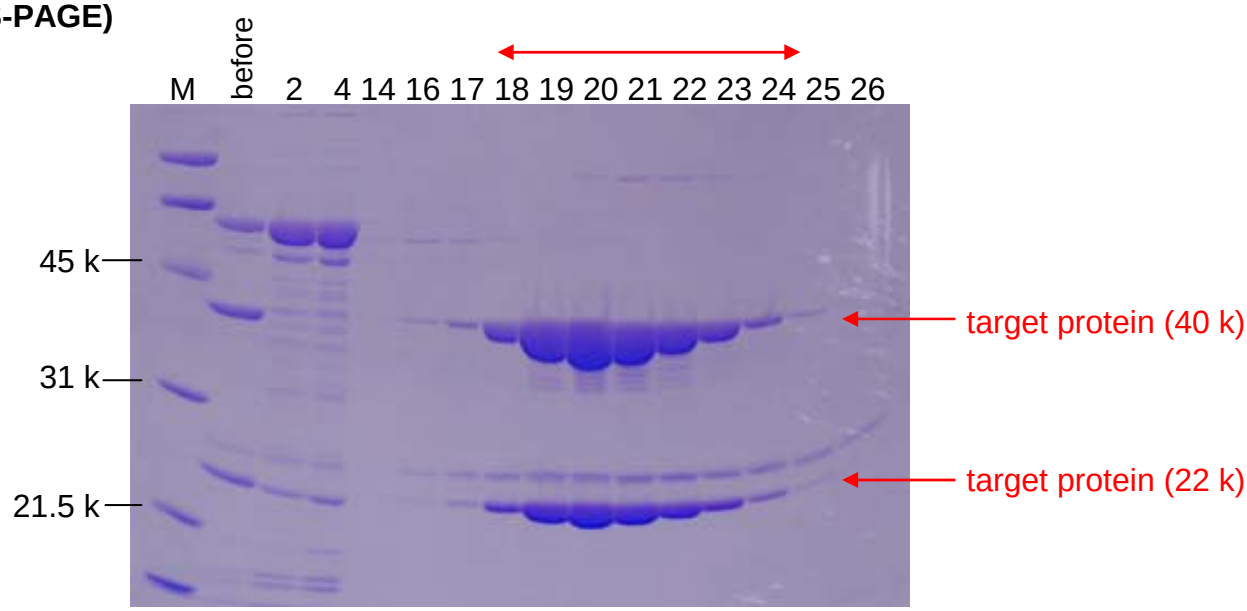
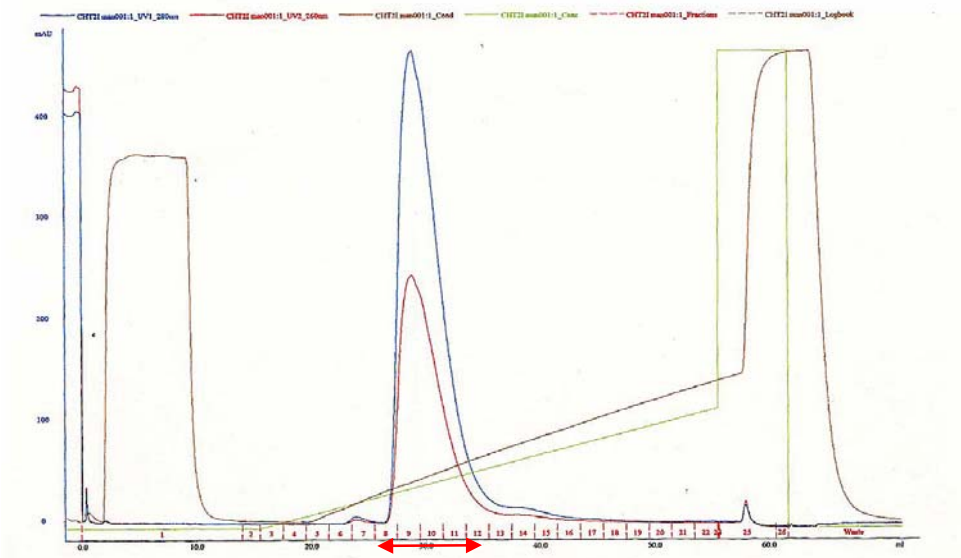


Fractions 18 to 24 were pooled for hydroxyapatite.

Step 4: Hydroxyapatite (2 ml)

Flow rate	2 ml / min
Gradient (Volume)	10 → 250 mM Na phosphate (20 column volumes)
Buffer	A = 10 mM Na phosphate, 0.15 M NaCl, pH 7.0 B = 500 mM Na phosphate, 0.15 M NaCl, pH 7.0
Eluted Conc.	40 mM Na phosphate

(Chart)



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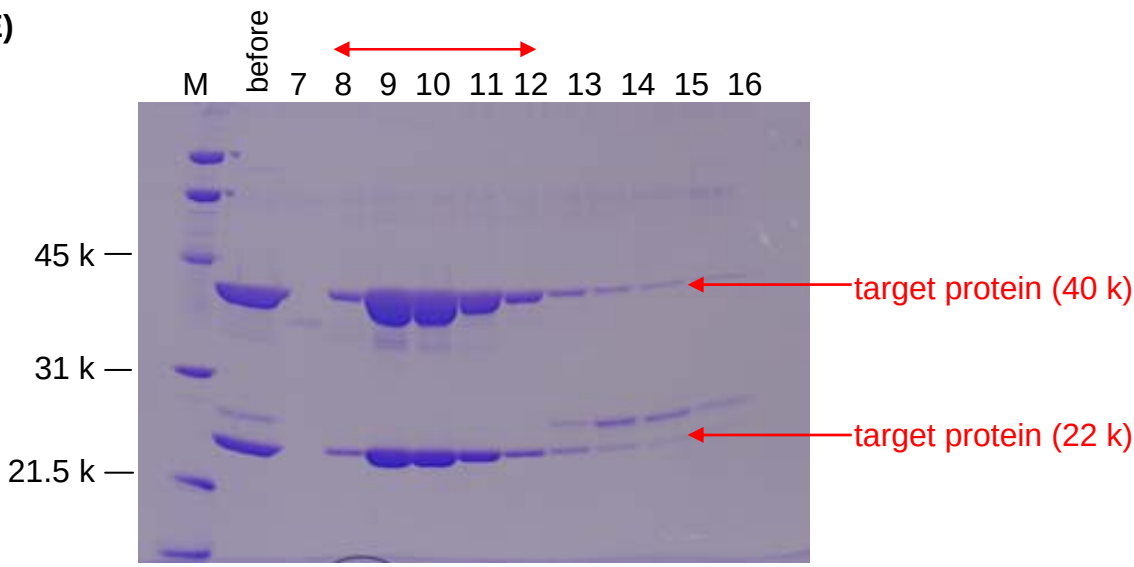
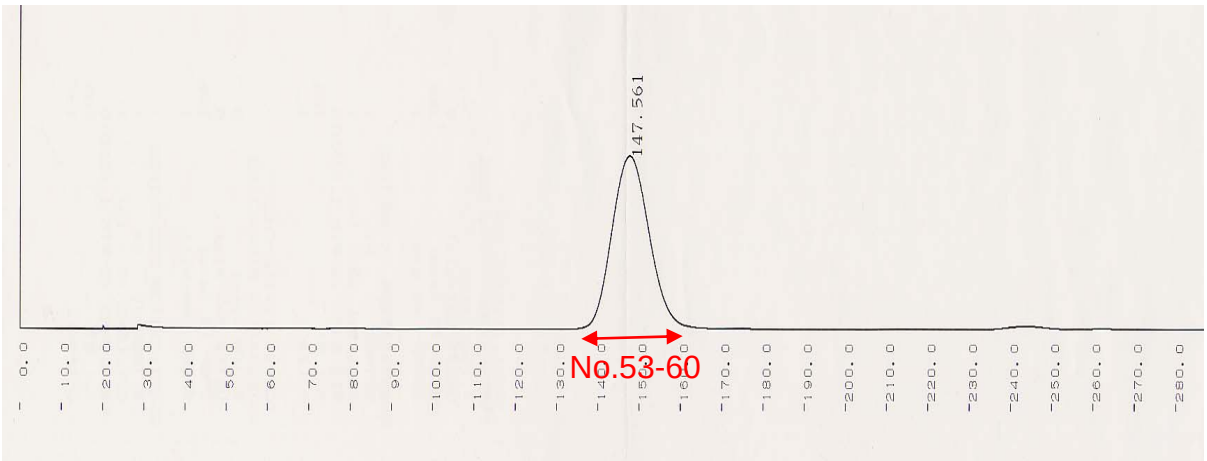


Fractions 8 to 12 were pooled and concentrated from 20 ml to 4 ml using VIVA SPIN 30,000 MWCO.

Step 5: Superdex 200 (120 ml)

Flow rate	0.5 ml / min
Buffer	20 mM Tris-HCl, 0.15 M NaCl, pH 8.0
Elution volume	74 ml

(Chart)



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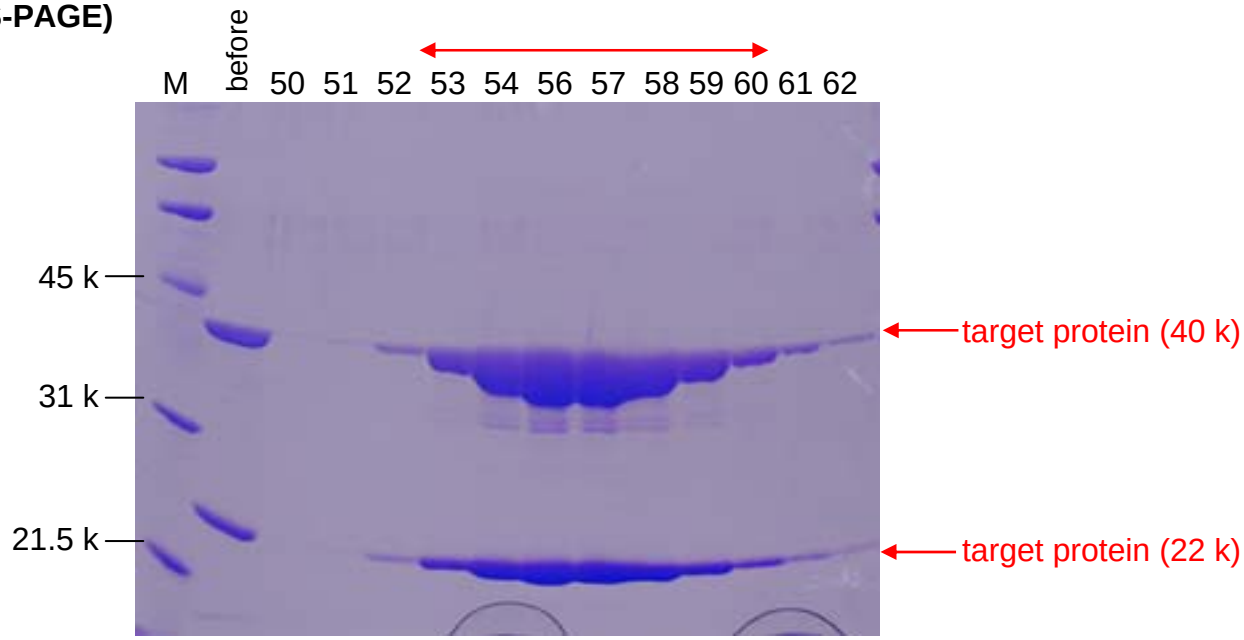

Fractions 53 to 60 were pooled.

Step 6: Protein Concentration

Bio-Rad Protein Assay (Bradford method)

Abs. (280 nm)	Dilution rate	Protein conc. (mg/ml)	Vol.(ml)	Total protein (mg)
0.176	5	1.4	10.4	15

Calibration curve: Y (mg/ml) = { X (Abs.) - 0.0119 } / 0.5744 (standard: BSA)

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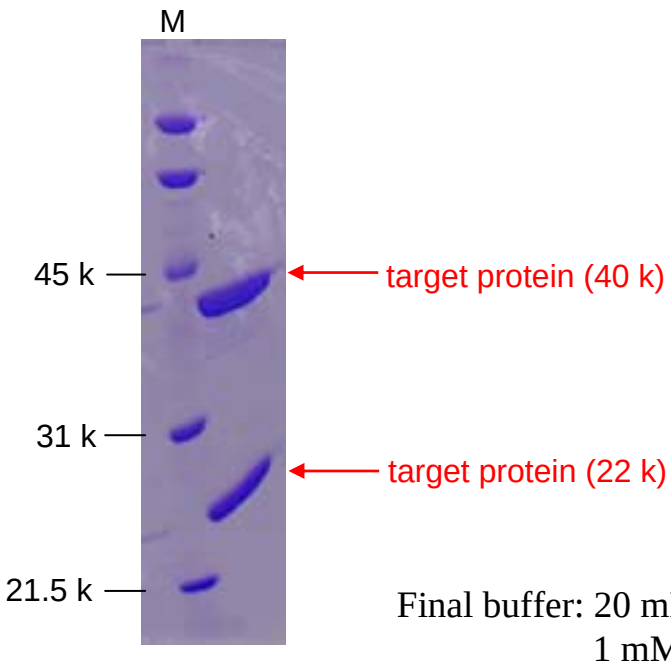
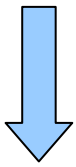


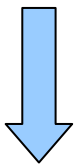
Final buffer: 20 mM Tris-HCl, 0.15 M NaCl, 1 mM DTT, pH 8.0

Point!

Expression of each subunit in *E. coli*



Combination of the cells in lysis buffer



Successful in co-purification of two subunits