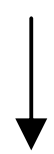
Example 5

Protein category	Other categories / Transposon-related functions		
M.W.	35,204	Wet weight of *E. coli* cells	24 g
Theoretical pI	10.4	Purified protein	9.0 mg
ε_M ($M^{-1}cm^{-1}$)	39,300	Purification time	8 days

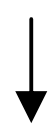
(Methods)

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)



without heat-treatment

40,000 rpm × 1 h at 4ºC

sup. ppt.

40,000 rpm × 1 h at 4ºC

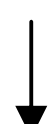
ammonium sulfate precipitation

(desalting)

HiTrap Heparin (affinity column)

(concentration)

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



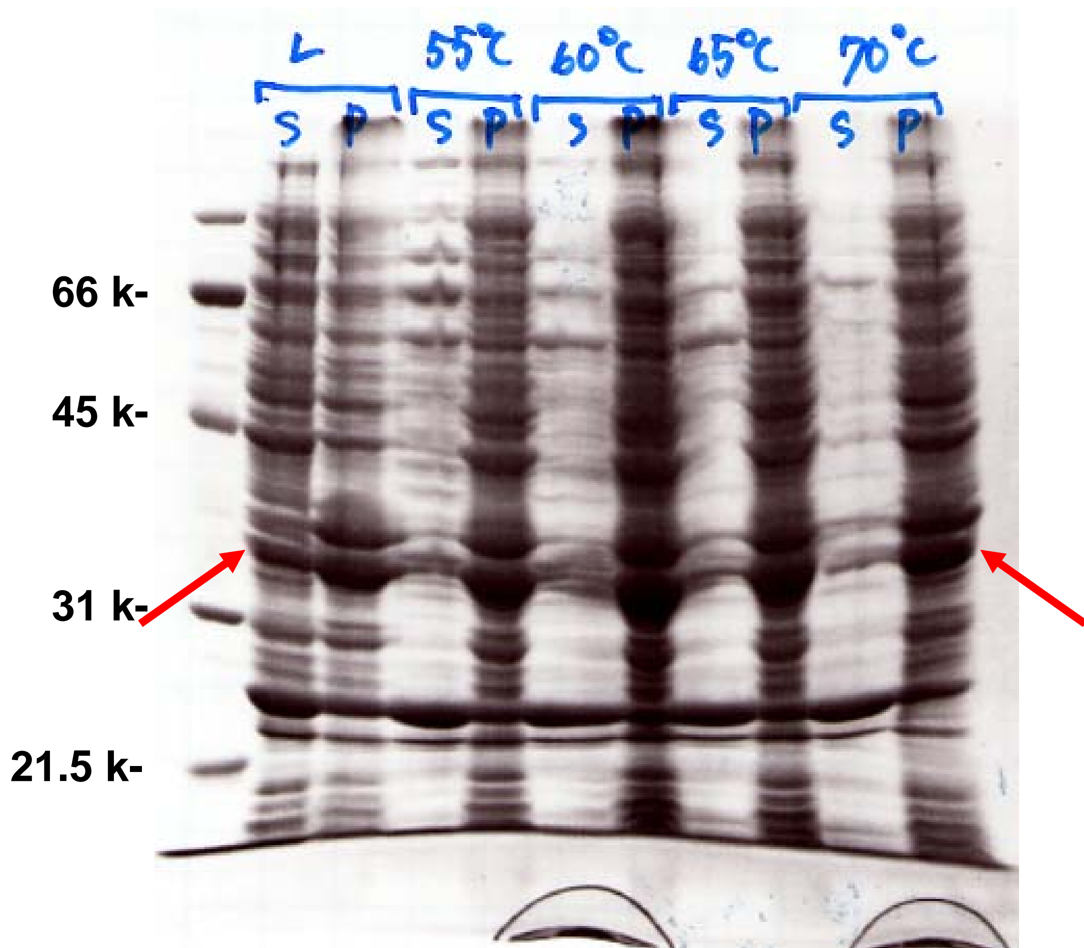
HiPrep 26/10 Desalting (desalting column)

(concentration)

protein concentration determination

Step 1: Checking the Heat-Treatment Condition

(SDS-PAGE)

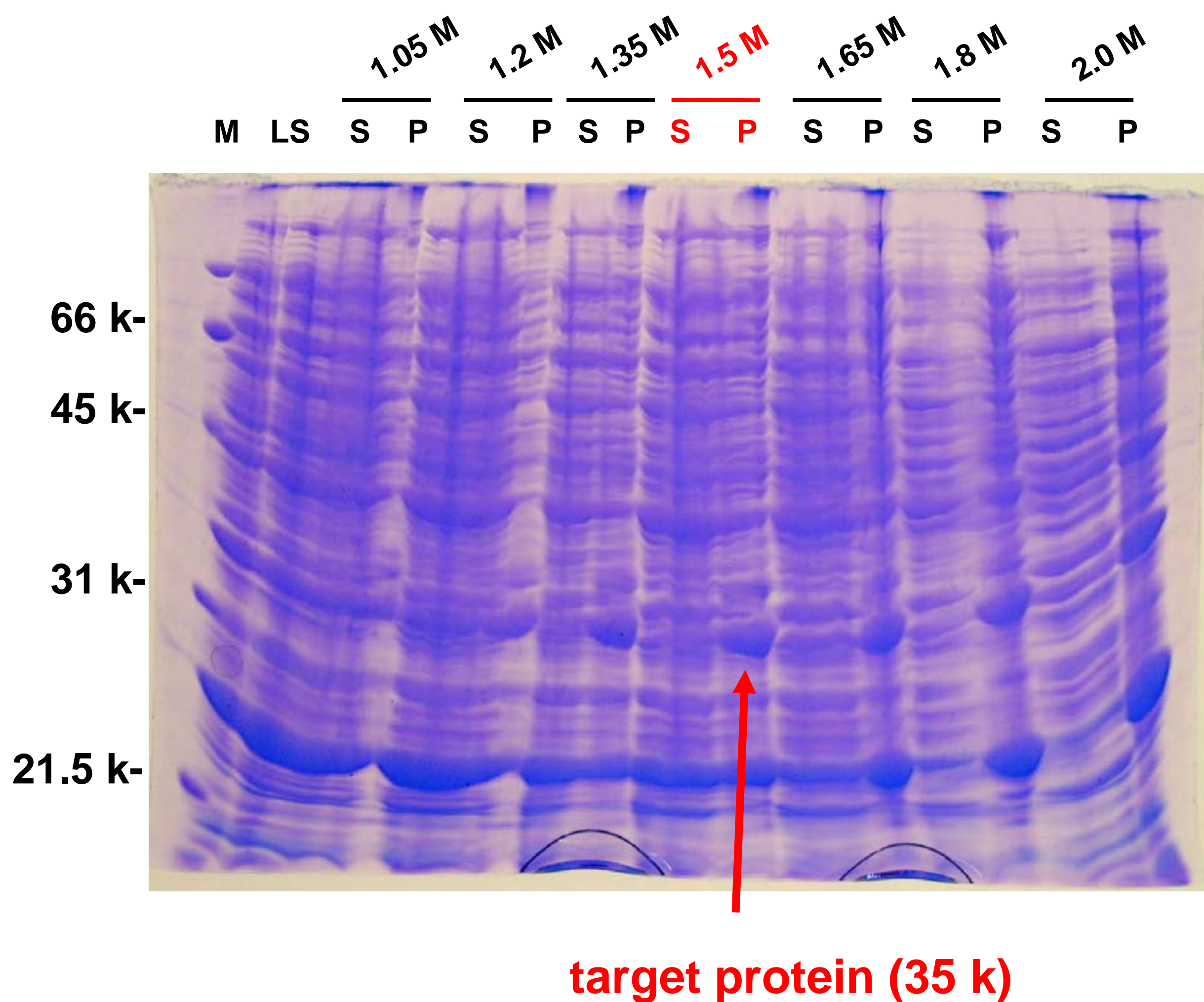


target protein (35 k)

We tested the condition for heat-treatment at 55ºC, 60ºC, 65ºC and 70ºC. Almost all target protein was precipitated by 55ºC heat-treatment.

Step 2: Ammonium Sulfate Precipitation

(SDS-PAGE)



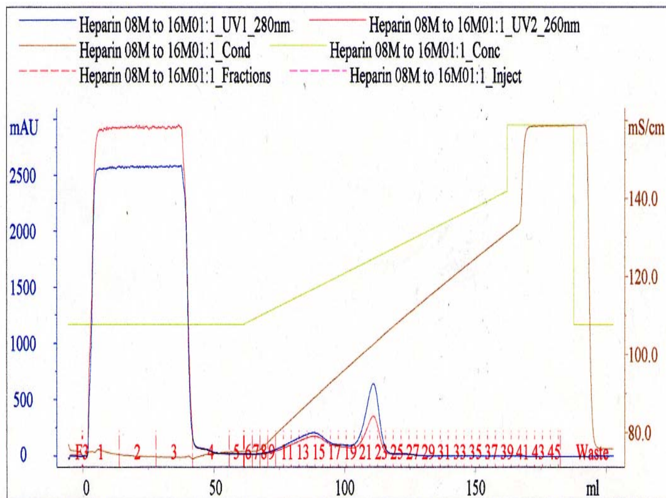
This protein was precipitated at 1.5 M $(NH_4)_2SO_4$. After the centrifugation, the precipitate was tried to dissolve in 10 mM Na phosphate, 1 M NaCl, pH 7.0. The target protein has tendency to aggregate, and about 1/3 of the target protein could not be solubilized. (See SDS-PAGE in Step 3).

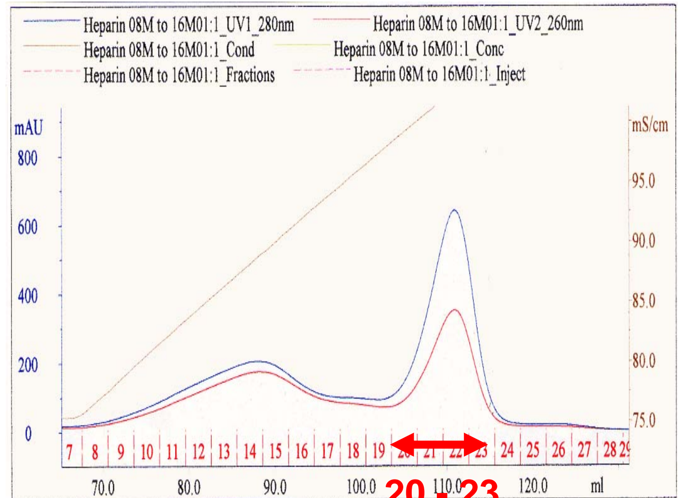
The solubilized protein was desalted on a desalting column equilibrated with 10 mM Na phosphate, 0.8 M NaCl, pH 7.0.

Step 3: HiTrap Heparin (5ml)

Flow rate	4 ml / min
Gradient (volume)	0.8 → 1.6 M NaCl (20 column volumes)
Buffer	A = 10 mM Na phosphate, pH 7.0 B = 10 mM Na phosphate, 2.0 M NaCl, pH 7.0
Eluted Conc.	1.2 M NaCl

(Chart)





(SDS-PAGE)

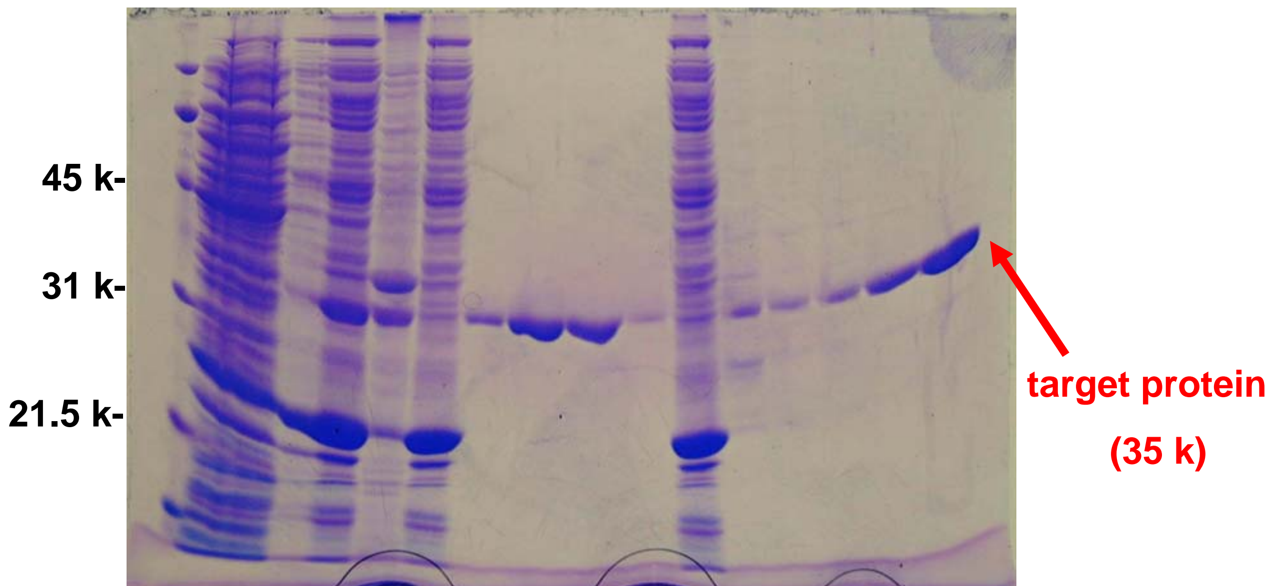
Ammonium sulfate precipitation

sup. ppt.

Heparin 1ml scouting

Heparin 5ml

M S P S P FT 14 15 16 17 FT 13 17 18 19 20

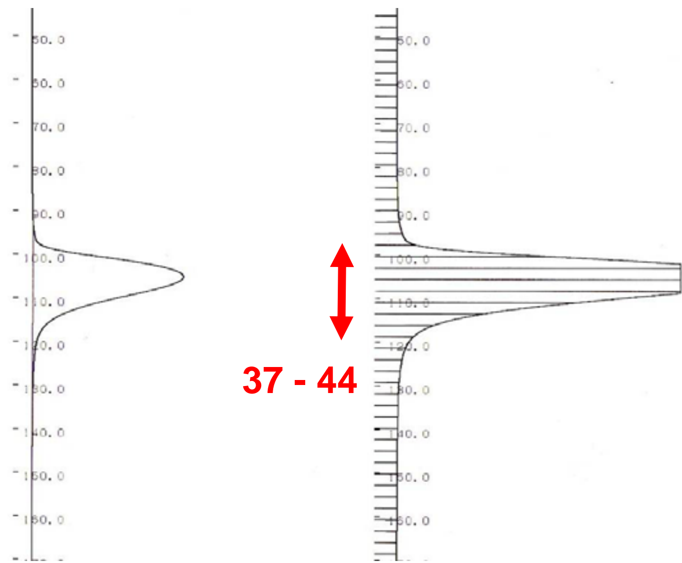


Fractions 20 to 23 were pooled.

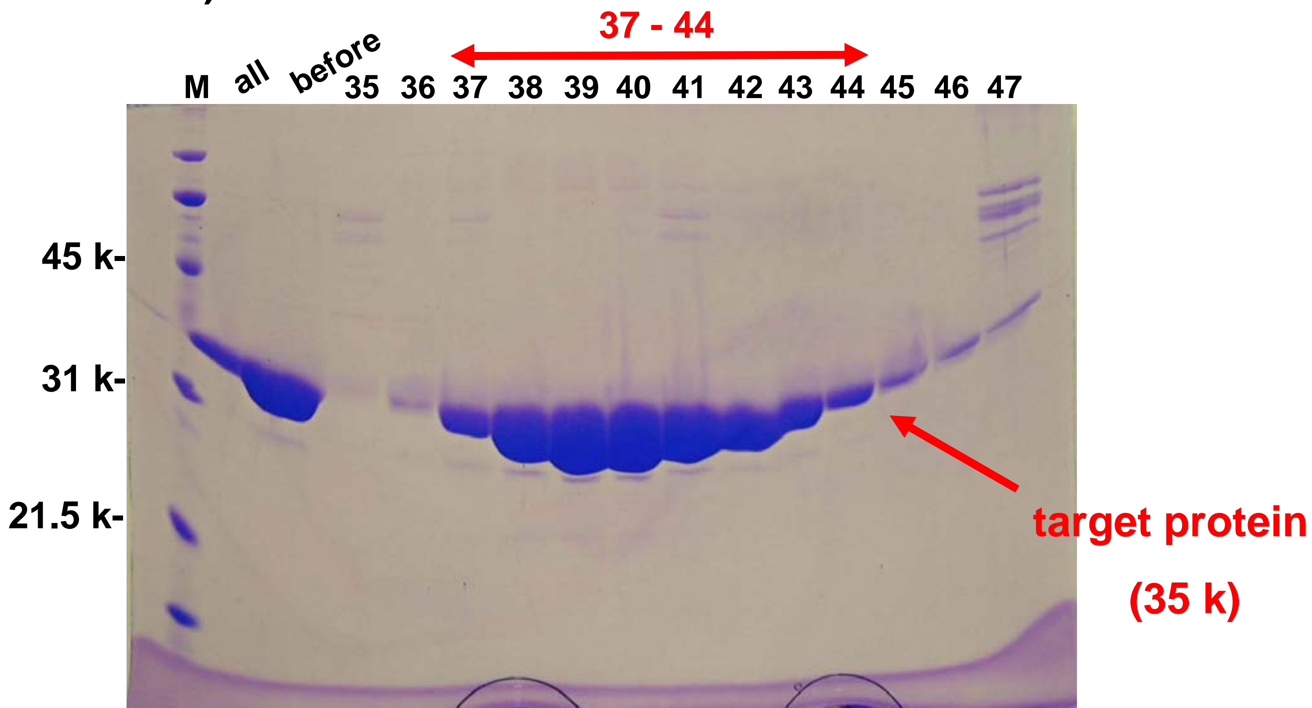
Step 4: Superdex 75 (120 ml)

Flow rate	0.5 ml / min
Buffer	20 mM Tris-HCl, 1 M NaCl, pH 8.0
Elution volume	52 ml

(Chart)



(SDS-PAGE)

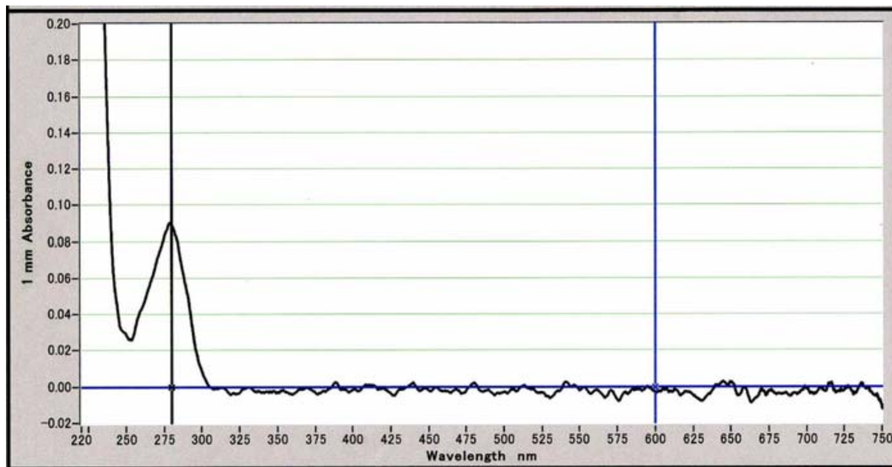


Fractions 37 to 44 were pooled, and desalted on a desalting column equilibrated with 20 mM Tris-HCl, 0.5 M NaCl, pH 8.0. After desalting the fractions were concentrated from 28 ml to 1.1 ml using VIVA SPIN 10,000 MWCO.

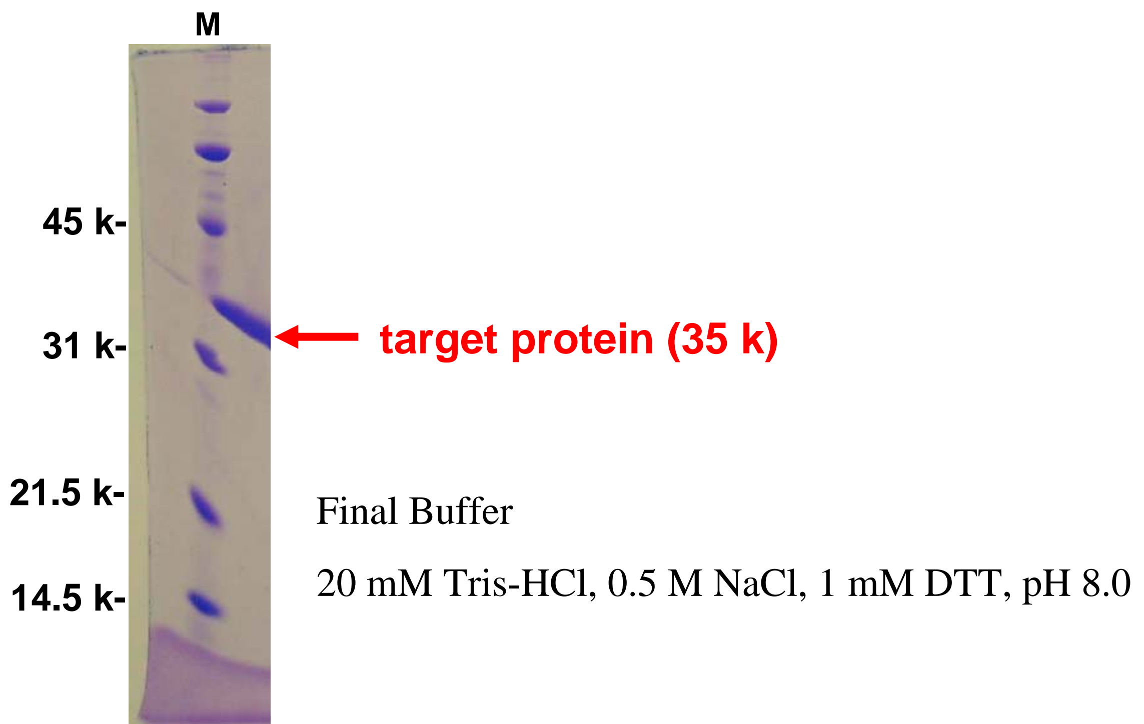
Step 5: Protein Concentration

ε_M	Abs. (280 nm)	Dilution rate	Mol. conc. (M)	M.W.	Protein conc. (mg/ml)	Vol. (ml)	Total protein (mg)
39,300	0.09	100	2.3×10^4	35,204	8.2	1.1	9.0

(UV spectrum)



(SDS-PAGE)

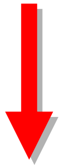


Final Buffer

20 mM Tris-HCl, 0.5 M NaCl, 1 mM DTT, pH 8.0

Point

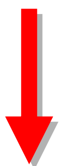
Without heat-treatment,



Step 1: Ammonium sulfate precipitation

Step 2: Affinity column

Step 3: Gel filtration column



this protein was purified by a small number of purification steps !

Example 6

Protein category	Regulatory functions / Other , p		
M.W.	16,555	Wet weight of *E. coli* cells	15 g
Theoretical pI	9.5	Purified protein	24 mg
ε_M ($M^{-1}cm^{-1}$)	10,200	Purification time	6 days

(Methods)

cell suspension in 20 mM Tris-HCl, 50 mM NaCl, 5 mM β-mercaptoethanol, pH 8.0, 70 ml

↓

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

↓

heat-treatment at 70ºC for 10 min

↓

on ice for 12 min

↓

40,000 rpm × 1 h at 4ºC

↓

column scouting RESOURCE ISO and RESOURCE PHE (hydrophobic column)

↓

RESOURCE ISO (hydrophobic column)

↓

RESOURCE PHE (hydrophobic column)

↓ ←(desalting)

RESOURCE Q (anion exchange column)

↓ ←(desalting)

RESOURCE S (cation exchange column)

↓ ←(concentration)

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

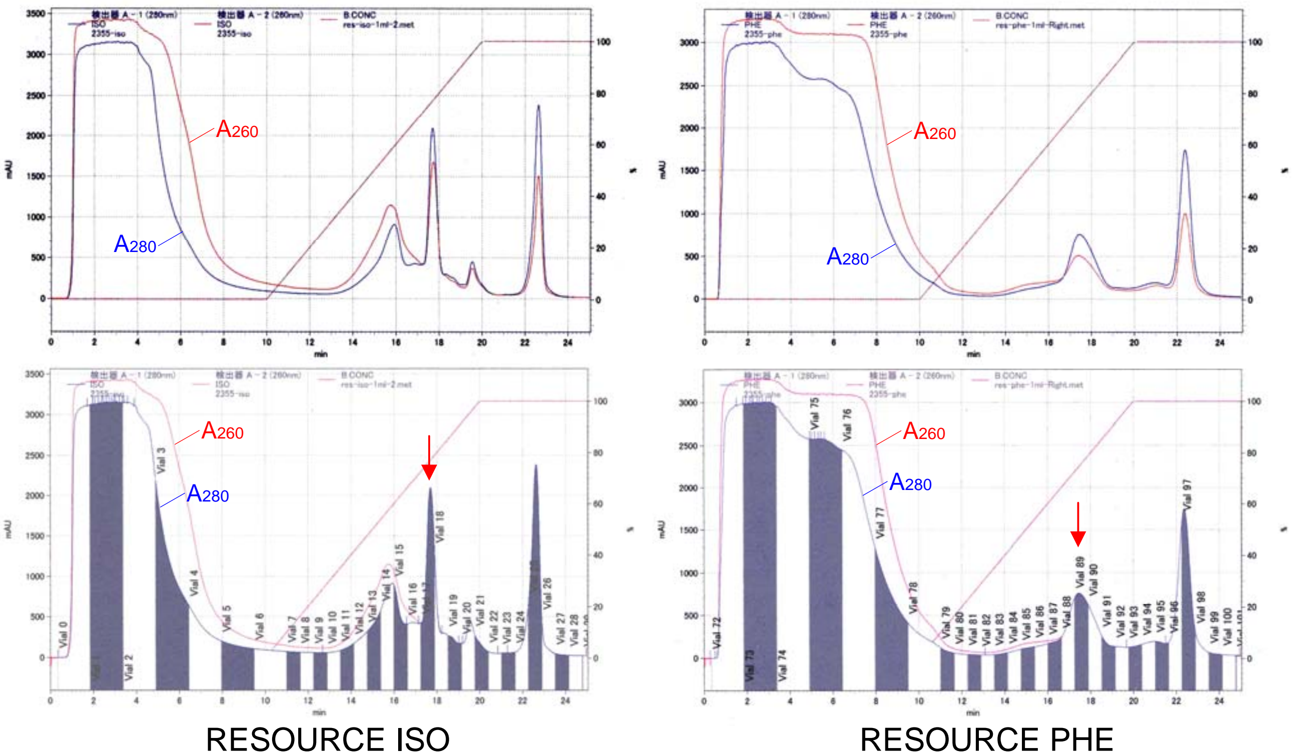
↓ ←(desalting, concentration)

protein concentration determination

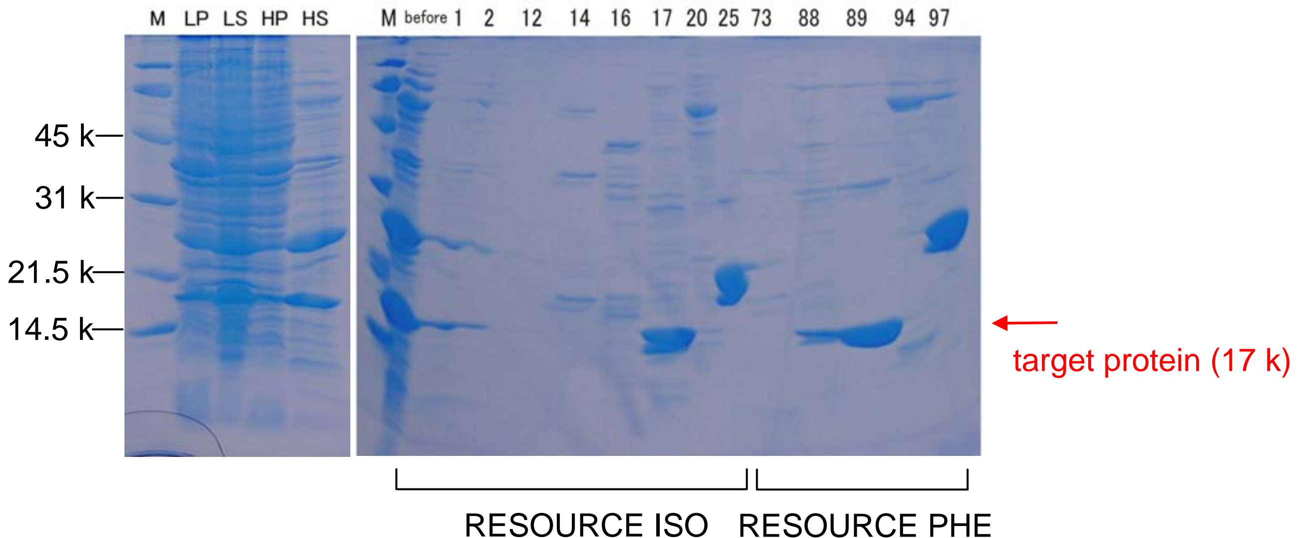
Step 1: Column Scouting

Flow rate	1 ml / min
Gradient (Volume)	1.5 → 0 M $(NH_4)_2SO_4$ (10 column volumes)
Buffer	A = 50 mM Na phosphate, 1.5 M $(NH_4)_2SO_4$, pH 7.0 B = 50 mM Na phosphate, pH 7.0
Eluted Conc.	RESOUREC ISO : 0.3 M $(NH_4)_2SO_4$ RESOURCE PHE: 0.4 M $(NH_4)_2SO_4$

(Chart)



(SDS-PAGE)

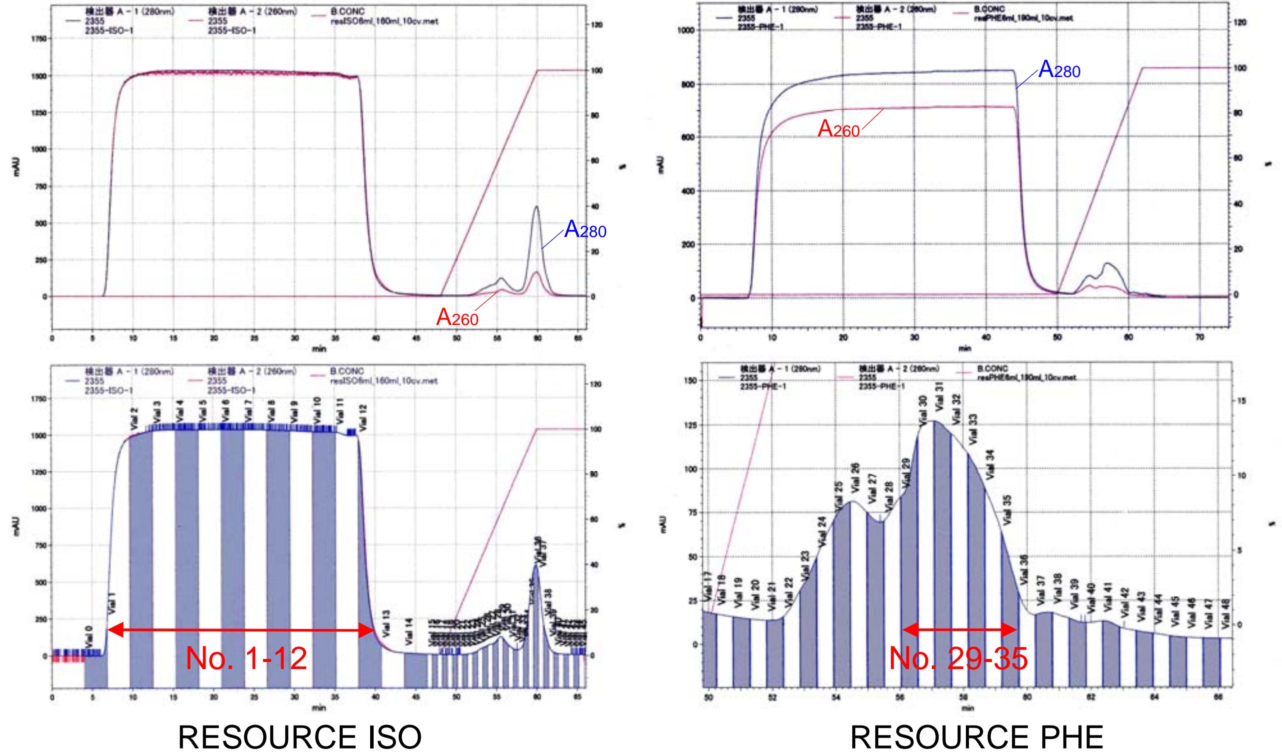


RESOURCE ISO (6 ml) column was selected.

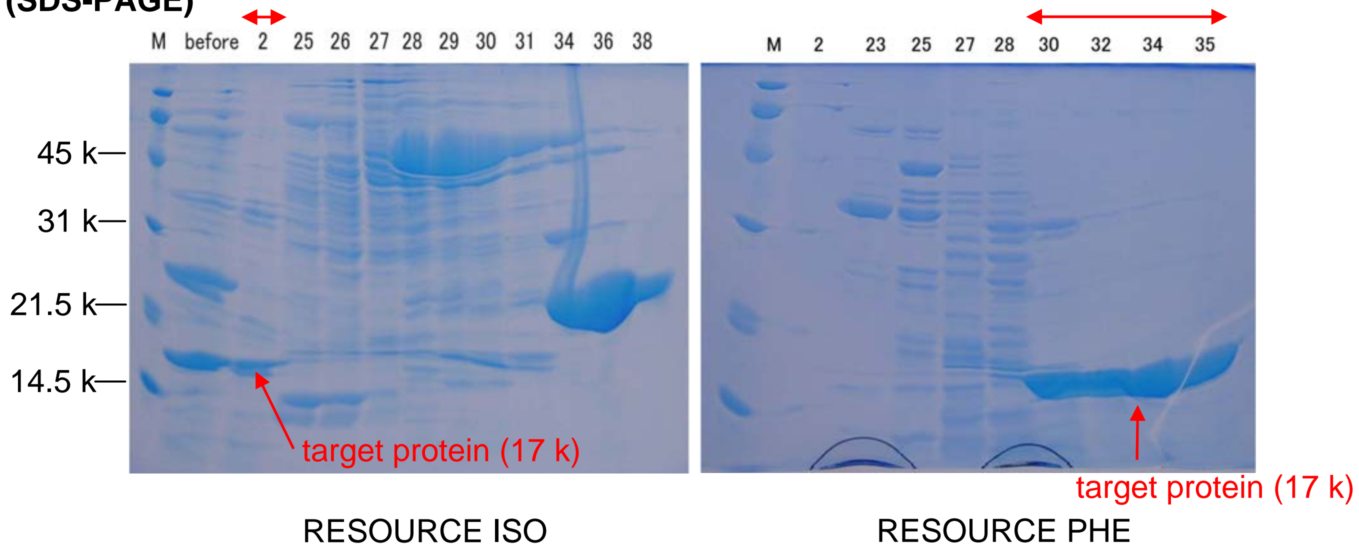
Step 2: RESOURCE PHE (6 ml)

Flow rate	5 ml / min
Gradient (Volume)	1.5 → 0 M $(NH_4)_2SO_4$ (10 column volumes)
Buffer	A = 50 mM Na phosphate, 1.5 M $(NH_4)_2SO_4$, pH 7.0 B = 50 mM Na phosphate, pH 7.0
Eluted Conc.	0.6 M $(NH_4)_2SO_4$

(Chart)



(SDS-PAGE)

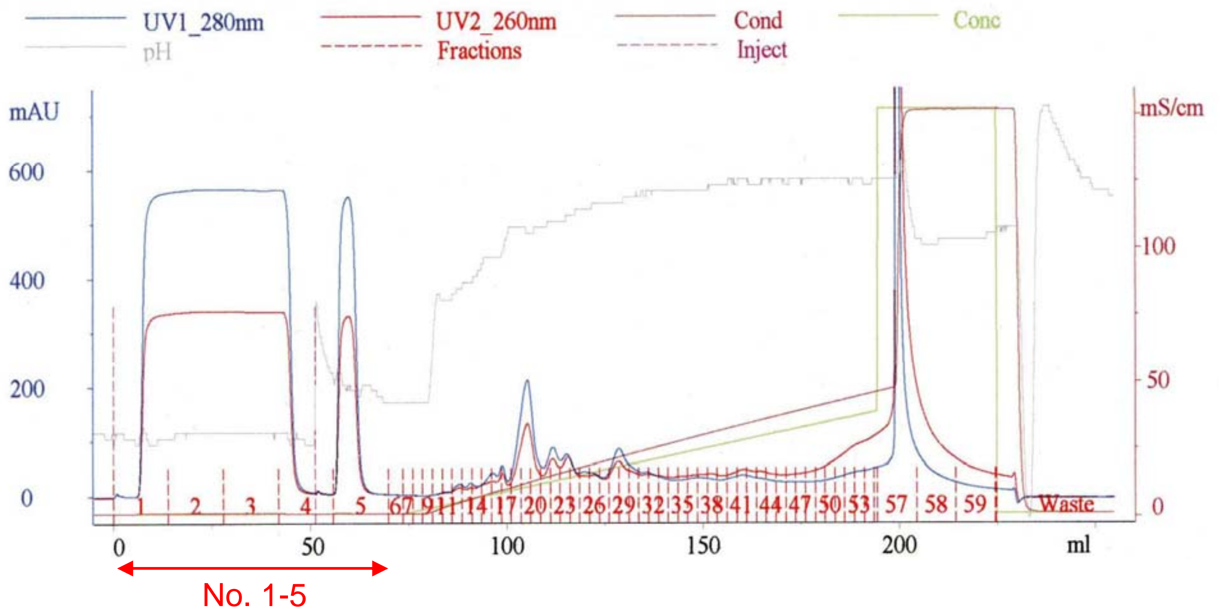


Fractions 1 to 12 (168 ml) of RESOURCE ISO were pooled, and applied to RESOURCE PHE. Fractions 29 to 35 (17.5 ml) of RESOURCE PHE were pooled, and desalted on a desalting column equilibrated with 20 mM Tris-HCl, pH 8.0.

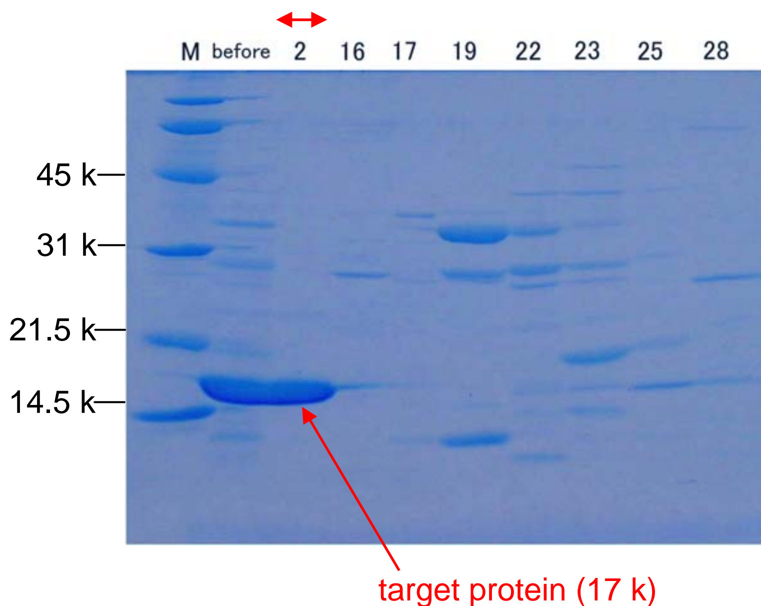
Step 3: RESOURCE Q (6 ml)

Flow rate	5 ml / min
Gradient (Volume)	0 → 0.5 M NaCl (20 column volumes)
Buffer	A = 20 mM Tris-HCl, pH 8.0 B = 20 mM Tris-HCl, 2.0 M NaCl, pH 8.0
Eluted Conc.	flow through

(Chart)



(SDS-PAGE)

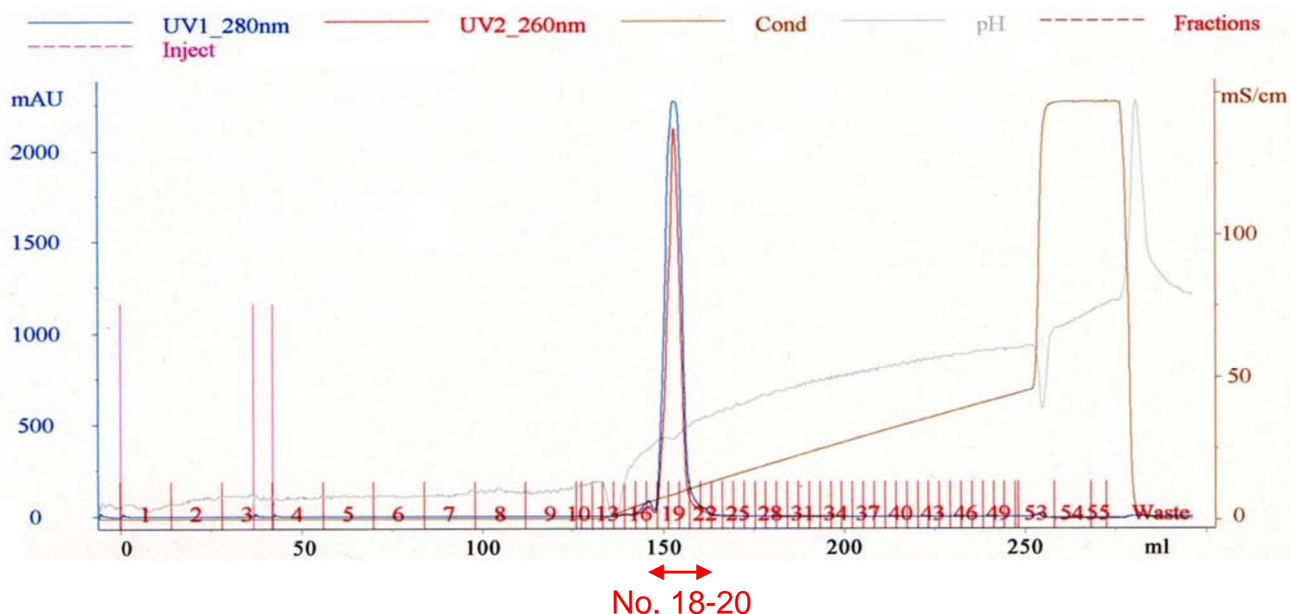


Fractions 1 to 5 (70 ml) were pooled and desalted on a desalting column equilibrated with 20 mM MES, pH 6.0.

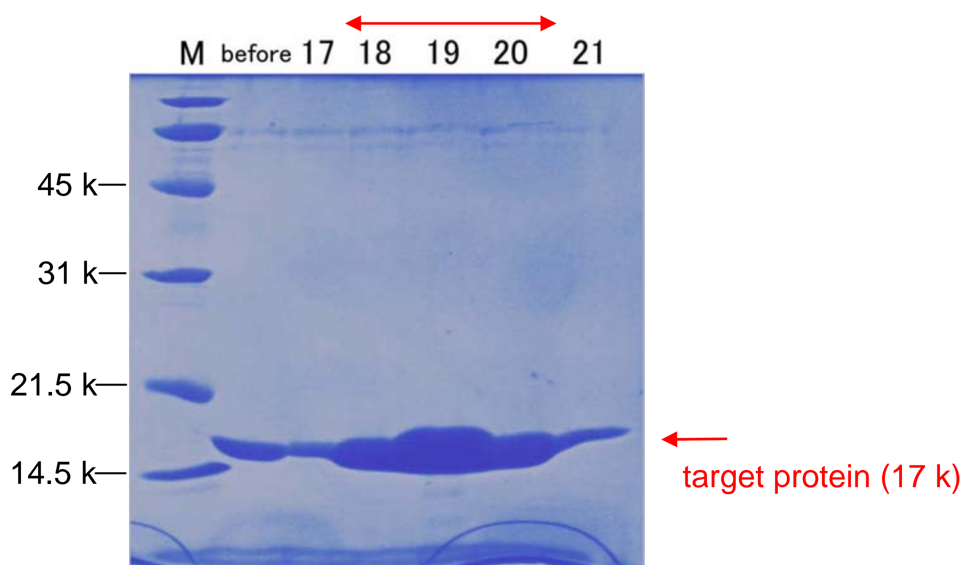
Step 4: RESOURCE S (6 ml)

Flow rate	5 ml / min
Gradient (Volume)	0 → 0.5 M NaCl (20 column volumes)
Buffer	A = 20 mM MES, pH 6.0 B = 20 mM MES, 2.0 M NaCl, pH 6.0
Eluted Conc.	0.9 M NaCl

(Chart)



(SDS-PAGE)

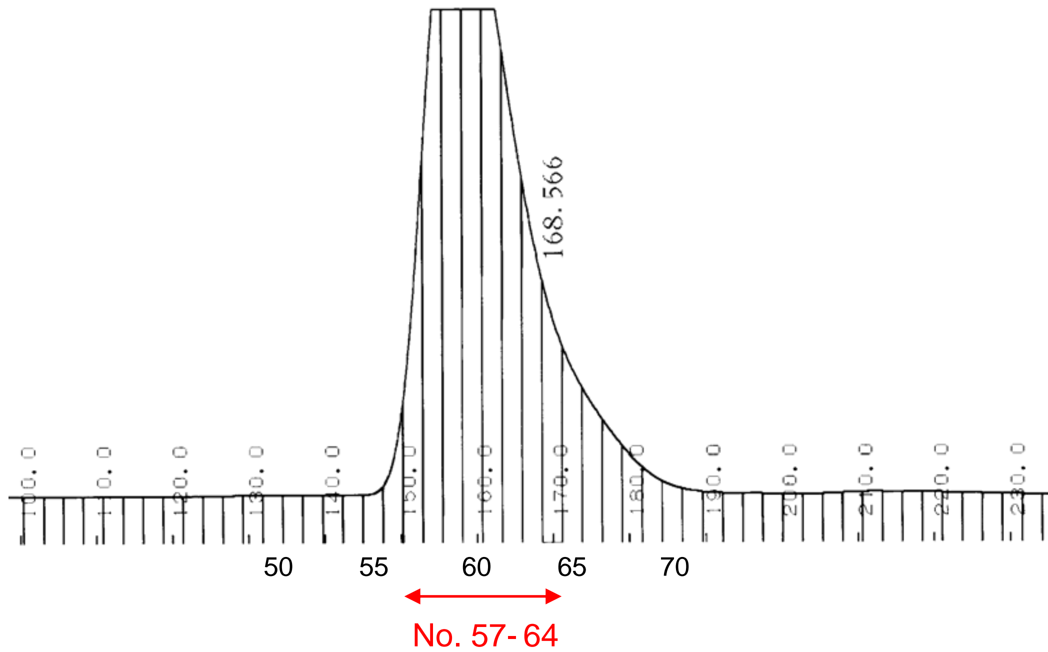


Fractions 18 to 20 (9 ml) were pooled, and concentrated from 9 ml to 5 ml by VIVA SPIN 5,000 MWCO.

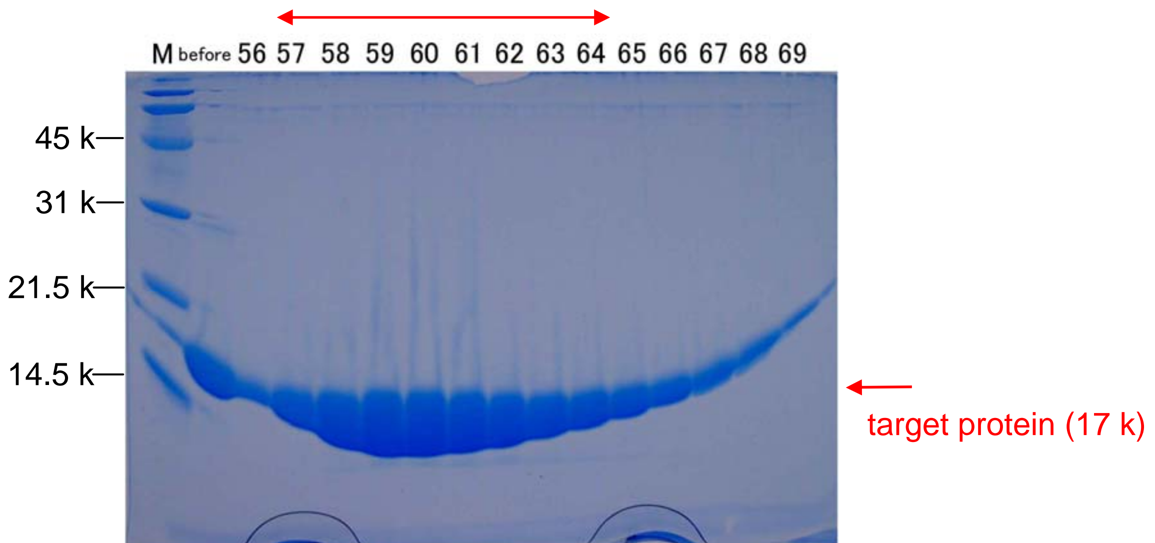
Step 5: Superdex 75 (120 ml)

Flow rate	0.5 ml / min
Buffer	20 mM Tris-HCl, 0.15 M NaCl, pH 8.0
Elution volume	79 ml

(Chart)



(SDS-PAGE)

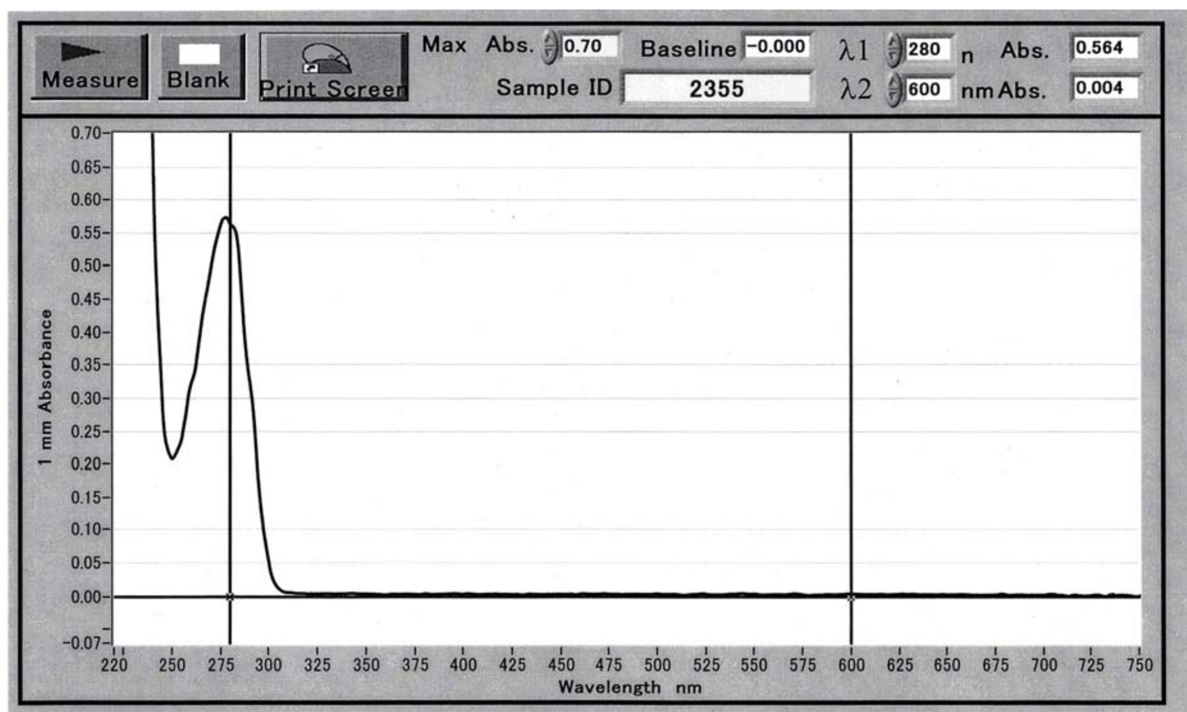


Fractions 57 to 64 (10.4 ml) were pooled and desalted on a desalting column equilibrated with 20 mM Tris-HCl, pH 8.0 and concentrated from 14 ml to 2.6 ml by VIVA SPIN 5,000 MWCO.

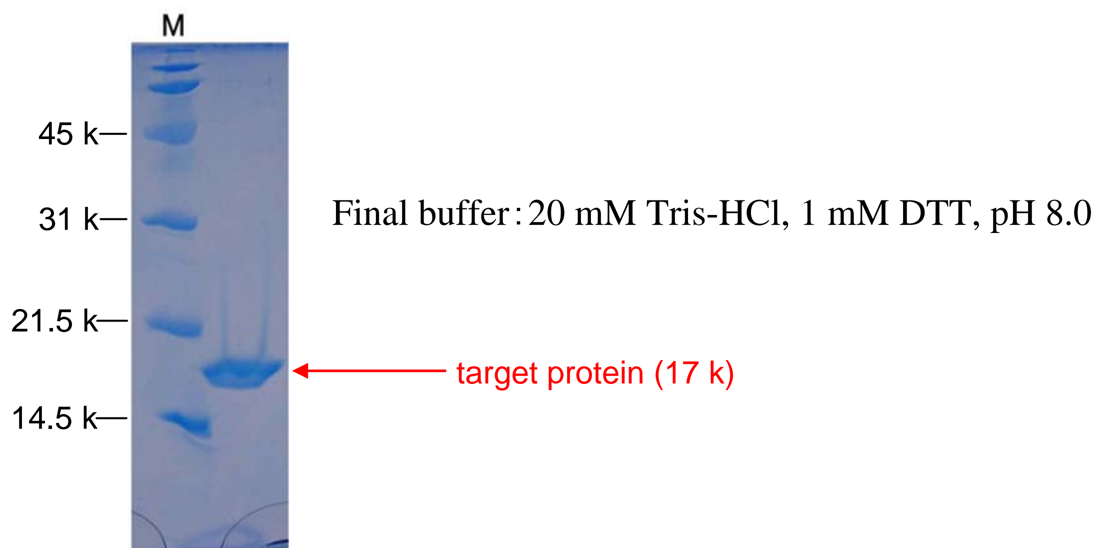
Step 6: Protein Concentration

ε_M ($M^{-1}cm^{-1}$)	Abs. (280 nm)	Dilution rate	Mol.conc. (M)	M.W.	Protein conc. (mg/ml)	Vol. (ml)	Total protein (mg)
10,200	0.56	10	5.5×10^{-4}	16,555	9.2	2.6	24

(UV spectrum)



(SDS-PAGE)



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

Point

The two types of ion exchange chromatography were effective!

- This protein behaved according to expectation from the theoretical pI.
- Purity improved as chromatography proceeded.

- Target protein was highly purified in these steps.