Thermus thermophilus HB8のシステム生物学へ向けて：タンパク質精製

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Protein Purification

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Abstract

We utilized heat treatment as an effective purification for *T. thermophilus* proteins overproduced in *E. coli*. After heat treatment, the target proteins were purified at room temperature by combination of hydrophobic interaction, ion exchange, hydroxyapatite and gel filtration column chromatographies. If the expression level of a protein was insufficient, We try to express the N-terminal His-tagged protein. Fusion of the His-tag often improves the protein expression level and enables the efficient purification with affinity column chromatography. Out of about 2,200 proteins from *T. thermophilus* HB8, we have purified 951 proteins.

① Purification — Current status

Total ORF	2238
Total protein purified	951 (42%)

② Tag-free protein — Purification procedure

Method 1 (using Shimadzu system and AKTA 10S system)

Cell suspension : 20 mM Tris-HCl, 50 mM NaCl, 5 mM β-mercaptoethanol (pH 8.0) (Lysis buffer)

↓

Sonication

↓

Heat treatment (70°C, 10 min in water bath)

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Ultracentrifugation : 40,000 rpm, 1 hour, 4°C （to remove the cell debris and insoluble proteins）

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Ammonium sulfate scouting : Check an occurrence of precipitation and determine a salt concentration for hydrophobic column scouting in the range of 1.05 M to 1.5 M ammonium sulfate.

↓

Hydrophobic column scouting : RESOURCE ISO, RESOURCE PHE (GE Healthcare)

Analytical HPLC system (Shimadzu)

The following purification steps

1. **Hydrophobic column chromatography on RESOURCE ISO, PHE (GE Healthcare)**
   (Otherwise) Ion-exchange column chromatography on SuperQ-TOYOPEARL 650 (TOSOH) or ammonium sulfate precipitation

Preparative HPLC system (Shimadzu)

2. **Ion-exchange column chromatography : RESOURCE Q, Mono Q, RESOURCE S, Mono S (GE Healthcare)**
3. **Hydroxyapatite column chromatography : Bio-scale CHT (Bio-Rad)**
   or Affinity column chromatography : HiTrap Heparin, HiTrap Blue
   or Hydrophobic column chromatography : RESOURCE ETH, RESOURCE ISO, RESOURCE PHE, HiTrap Butyl, HiTrap Octyl (GE Healthcare)

AKTA explorer 10S (GE Healthcare)

4. **Gel filtration: Superdex 75 pg, Superdex 200 pg, Sephacryl-S200 (GE Healthcare)**

↓

Protein concentration determination
Measurement of UV absorption at 280 nm

Gel filtration system (Shimadzu)

Method 2 (Using AKTA 10S system)

Cell suspension : 20 mM Tris-HCl, 500 mM NaCl, 5 mM β-mercaptoethanol (pH 8.0) (Lysis buffer)

↓

Sonication

↓

Heat treatment (70°C, 10 min in water bath)

↓

Centrifugation : 15,000 rpm, 30 min, 4°C （to remove the cell debris and insoluble proteins）

↓

Desalting

↓

The following purification steps

1. **Ion-exchange column chromatography : SuperQ-TOYOPEARL 650**
2. **Ion-exchange column chromatography : RESOURCE Q, RESOURCE S**
3. **Hydroxyapatite column chromatography : Bio-scale CHT**
   or Affinity column chromatography : HiTrap Heparin, HiTrap Blue
   or Hydrophobic column chromatography : RESOURCE ETH, RESOURCE ISO, RESOURCE PHE
4. **Gel filtration : Superdex 75 pg, Superdex 200 pg**

↓

Protein concentration determination
Measurement of UV absorption at 280 nm

AKTA explorer 10S (GE Healthcare)

③ His-tagged protein — Purification procedure

Method 3 (Automatic purification of protein using AKTA Xpress and AKTA Crystal system)

Cell suspension : 20 mM Tris-HCl, 500 mM NaCl, 5 mM β-mercaptoethanol (pH 8.0) (Lysis buffer)

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Sonication

↓

Heat treatment (70°C, 10 min in water bath)

↓

Centrifugation : 14,000 rpm, 30 min, 4°C (to remove the cell debris and insoluble proteins)

Automatic Purification System

HiTrap Chelating HP (affinity column)

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

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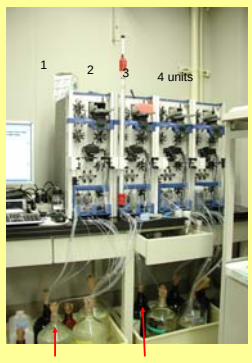
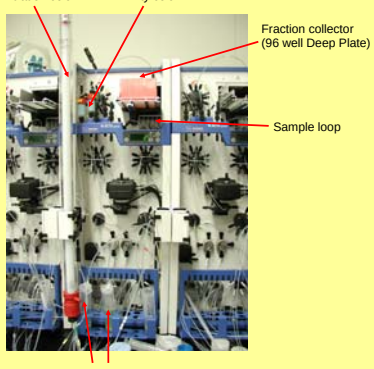
tobacco etch virus (TEV) protease cleavage reaction (Recognition site : E-N-L-Y-F-Q ↓ G)

(succeed)	(fail)
HiTrap Chelating HP (affinity column)	**HiTrap Chelating HP (affinity column)**
↓	↓
HiPrep 26/10 Desalting (desalting column)	**HiPrep 26/10 Desalting (desalting column)**
	↓
	RESOURCE Q (anion exchange column)
↓	↓
Protein concentration determination	**Concentration and buffer exchange**
Measurement of UV absorption at 280 nm	↓
	Protein concentration determination
	Measurement of UV absorption at 280 nm

AKTA Xpress (GE Healthcare)





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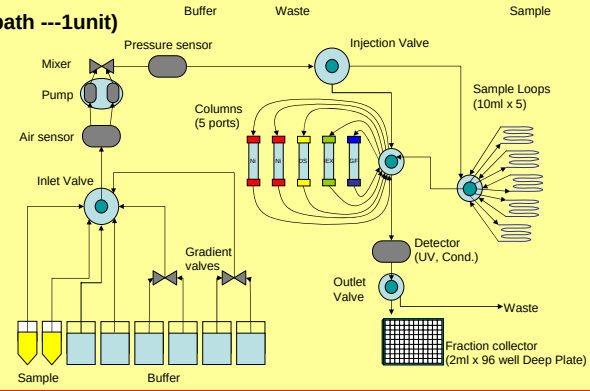

◆ Run time
・Affinity →Gel filtration: **2.7 hrs**
・Affinity →Desalting →Ion exchange →Gel filtration: **5.2 hrs**
(per 1sample)

◆ Maximum number of samples
・Affinity →Gel filtration: **4 samples/unit**
・Affinity →Desalting →Ion exchange →Gel filtration: **2 samples/unit**

AKTA Crystal (GE Healthcare)

◆ Run time (per 1sample)
・Affinity →Desalting→ reequilibration : **1 hrs**

◆ Maximum number of samples
・Affinity →Desalting→ reequilibration : **4 samples**

(Flow path)