# Goyama lab Protein expression and purification protocol

## (2022 version)

(All buffers using in this protocol are according to the targeting protein. In this protocol, buffers are suitable for CBFB. Different protein should confirm specific buffer condition previously. Ex. pH, salt or some agents.)

Preparation

Binding buffer

5mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

Wash buffer

50mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

20mM imidazwole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

10mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

Elute buffer

200mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

100mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

Cell lysis buffer

50mM imidazole 500mM Nacl 20mM Tris-Hcl 1mM PMSF (pH:8.0)

Protein store buffer

300mM Nacl 20mM HEPES 10mM EDTA 1mM DDT 1mM PMSF (pH:7.5)

Ps: If the protein first time to do Ni-NTA purification or protein size is large, please prepare 250mM imidazole 500mM Nacl 20mM Tris-Hcl (pH:8.0)

Day 1 Transformation

1. Taking BL21 with ice.
2. pET plasmid 500ng add in BL21 and keep it on ice 30 minutes.
3. Heat shock (42C° 45s), and then immediately put the tube back to the ice. Add SOC medium 100μl and incubate 30mins in 37C°.
4. Plating on the culture dish with antibiotics (which antibiotics used need to check on the websites previously). 37C° incubating overnight.

Day 2 Colony pick

1. Preparing LB medium which will be used next day. (Big flask is in -80C° room)

| | |
|---|---|
| MQ | 1000ml |
| NaCl | 10 g |
| Yeast Extract | 5 g |
| Bacto Trypton | 10 g |

2. Put LB medium (after autoclave) in the 4C° and take out in room temperature when tomorrow using before.
3. Pick up three single colony and put in the 15ml tube at the same time, and LB medium (2ml) with antibiotics (2μl). Put tube in the shaker (37C° 180rpm overnight).

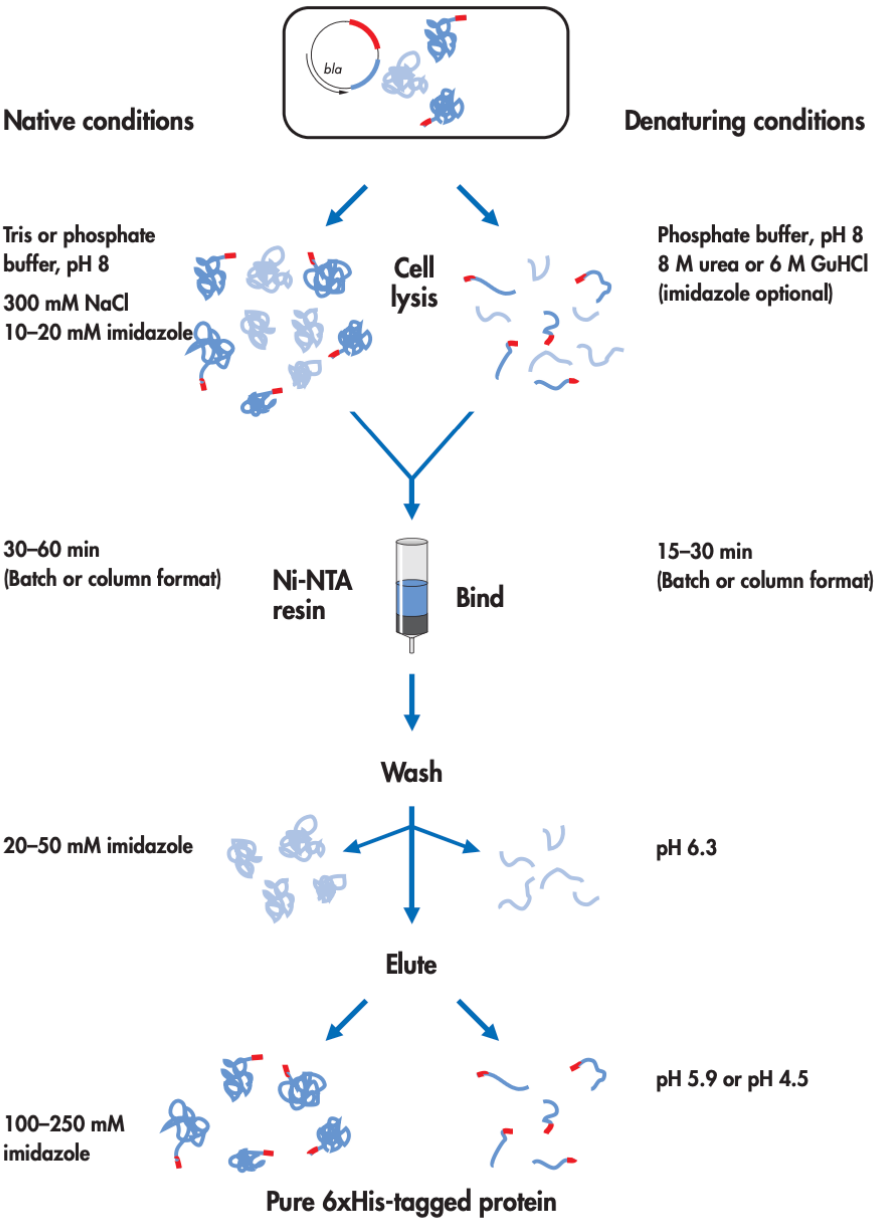
Day 3 Large scale incubate

1. Put 2ml overnight culture bacteria into 1L LB medium. Shaking 3 hours with 37C° and 180rpm.
2. Detecting the OD600 value, when OD600 is arrived at o.5, add 500μl IPTG. Continue to shake in 20C° and 180rpm overnight. (If OD600 value is not arrive at 0.5 continue to shake until it arrives)

Ps: This day also need prepare Nickle columns.

Add Ni-NTA Agarose 4ml in the big column, wash the Ni-NTA Agarose (when start adding liquid lightly). Using Binding Buffer wash the column (5mM imidazole 500mM Nacl 20mM Tris-Hcl pH:8.0). Store the column with 3ml binding buffer and close the lid. Keep the column in 4C° room overnight.

Details of Ni-NTA purification




Day 4 Protein extraction and purification

1. Take out bacteria from shaker, centrifuge (NA-16 mode, 7000G, 10mins, 4C°).
2. Collect all the bacteria into 50ml tube (safe to stock, stock method: put bacteria into nitrogen immediately, and store it in the -80C°) and add 20ml buffer (50mM imidazole 500mM Nacl 20mM Tris-Hcl 1mM PMSF, pH8.0)
3. Mix well and put the sample on the ice.
4. Sonicate bacteria on ice using sonicator. (OUT PUT/7, DUTY/50, 15min)
5. Transfer the protein liquid into tubes and centrifuge. (40000 g, 4C°, 30 min)

6. Put the supernatant into the new 50mL tube.
7. Do Ni-NTA purification.
   i. Pure all sample liquid into Ni column and waiting 20 min.
   ii. Open the lip below using one 50mL tube collect the flow through.
   iii. Use wash buffer 10mL wash one to two times and collect all liquid.
   iv. Use different concentration imidazole elution buffer and collect all liquid.
   v. Make small amount protein sample and run SDS-Page.

| Protein Size | Gel Percentage |
|---|---|
| 4-40 kDa | Up to 20% |
| 12-45 kDa | 15% |
| 10-70 kDa | 12.5% |
| 15-100 kDa | 10% |
| 50-200 kDa | 8% |
| >200 kDa | 4-6% |

Gel percentage chose rule

PS. After confirmed which concentration of imidazole elution buffer contains main part of target protein, transfer the protein from elution buffer to protein store buffer (no imidazole) as soon as possible.

8. If need store protein for long time, concentrated protein at less 1mg/mL. Immediately put protein into the nitrogen, and put into -20C° or -80C° for future using.