Purification of His^6-tagged proteins produced in the BL21(DE3) *E. coli* strain.
(also check purification of GST-fusion proteins)

Production of His^6-tagged proteins

1. BL21(DE3) cells transformed with a pET28a derivative, use kanamycin (20 ug/ml).
    *No heat shock when transformed.
    *Do not leave colonies at 4˚C. Use fresh colonies.
2. Inoculate a single colony in 25 ml LB+kan, culture @25˚C O/N
3. Dilute in 750 ml (**s ml**) LB+kan
4. Cultivate at 30˚C until an OD660 of approx. 0.8 (you can start induction at lower OD, at least 0.4)
    *Take 1ml culture for an SDS-PAGE sample (**pre-induction sample**). (Screw top tube)
    *Spin down cells. Remove supernatant.
    *Add 1xSDS-PAGE loading buffer (OD x 300 ul) to the pellet.
    *Suspend cells by vortex and boil for 2min
    *Vortex again briefly and boil another 3min
    *Chill on ice, freeze at -20˚C
5. Add IPTG to the culture (final 0.67 mM IPTG) (0.1 ~ 1.0 mM depending on your protein)
6. Cultivate @30˚C for 2h (2 h ~5 h depending on you protein)
    *Measure OD, and prepare SDS-PAGE sample as described below (**post-induction sample**)
    *Take 1ml culture for SDS-PAGE sample. (Screw top tube)
    *Spin down cells. Remove supernatant.
    *Add 1xSDS-PAGE loading buffer (OD x 300 ul = **t**) to the pellet.
    *Suspend cells by vortex and boil for 2min
    *Vortex again briefly and boil another 3min
    *Chill on ice, freeze at -20˚C
7. Collect cells in 500 ml centrifuge tube (use two bottle, 375 ml each)
    (5,000 rpm, 15 min, 4˚C)
8. Suspend cells in 25ml of TBS, transfer the content to 50 ml tube
9. Collect cells (3,200 rpm, 15 min, 4˚C)
12. Store pellet @-80˚C

Purification of His^6-tagged protein

All the procedures must be performed at 4˚C or on ice unless otherwise specified

1. Add 10 ml (**w ml**) of ice-cold lysis buffer to cell pellet (4˚C)
   Add 10 mg of lysozyme, suspend cells, incubate on ice for 30 min.

2. Sonicate cell (4˚C)
    With Branson Digital Sonifier: amplitude 20%, processing time 30 sec, ON time 1 sec, OFF time 3 sec. Need to repeat 3 ~ 5 times.
    Keep the tube on ice while sonicating.
    *Cell suspension changes its color from white to brown
    *Avoid foaming. Put down the sonicator tip well below the buffer surface during sonication.

3. Transfer cell lysase to a 30ml centrifuge tube (4˚C)

4. Clarify lysate by centrifugation (JA-17, 15krpm, 30min, 4˚C) (**use adaptor if necessary**)

5. During centrifugation, pre-wash Ni-NTA beads
    a. Take 1 ml (50% slurry, 500 ul bed volume/sample) of beads in 15 ml tube
    b. Spin down to collect beads, 1000 rpm, 1sec, 4˚C (remove supernatant)
    c. Add 5 ml of lysis buffer, mix gently/thoroughly

d. repeat steps: b, c, b, c and b

6. After centrifugation, save (**w x 1/s) ml** of supernatant for SDS-PAGE sample (**sup sample, 4˚C**)

7. Transfer supernatant to the 15ml conical tube containing pre-washed Ni-NTA beads (50% slurry in lysis buffer) (4˚C). Do not vortex. Do not pipette up and down. Mix by inversion.

8. Rotate for 1h at 4˚C
9. During the step 8:
    *Suspend pellet in 10 ml (**w ml**) of lysis buffer. Save (**w x 1/s = u; 14 ul if the original culture is 750 ml) ml** for SDS-PAGE sample (**pellet sample, 4˚C**)
    *Prepare sup & pellet samples: sample must contains same concentration of proteins as pre- and post-induction sample
        *Add (**u x 0.5) ml** of 3XSDS-PAGE loading buffer.
        *Add (**t- 1.5u**) ul of 1XSDS-PAGE loading buffer (**total t ul**)
        *Suspend by vortex, and boil for 5min
        *on ice, then -20˚C.
10. Load the lysate-Ni-NTA mixture into a column with the bottom outlet capped (in the cold room). Remove the bottom cap and collect flow-through.
        *Save flow-through for SDS-PAGE analysis
        *Add (**u x 0.5) ml** of 3XSDS-PAGE loading buffer.
        *Add (**t- 1.5u**) ul of 1XSDS-PAGE loading buffer (**total t ul**)
        *Suspend by vortex, and boil for 5min
        *on ice, then -20˚C
11. Wash Ni-NTA with 5 ml of wash buffer; collect fractions for SDS-PAGE analysis. Do twice, but pool two washes in the same tube.
        *Save the wash for SDS-PAGE analysis
        *Add (**u x 0.5) ml** of 3XSDS-PAGE loading buffer.
        *Add (**t- 1.5u**) ul of 1XSDS-PAGE loading buffer (**total t ul**)
        *Suspend by vortex, and boil for 5min
        *on ice, then -20˚C
12. Elute the protein 4 times with 500 ul of elution buffer
        *Prepare SDS-PAGE sample with each fraction (5 fractions)
        *30 ul fraction + 15 ul 1x SDS-PAGE loading buffer
        *Suspend by vortex, and boil for 5min
        *on ice, then -20˚C
        *Store the rest of protein solution at 4˚C (O/N) or -80˚C (longer storage)
            (make 100 ul aliquots from each fraction)

SDS-PAGE analysis of purified proteins
Load 15 ul from each step/fraction

Reagents

Lysis buffer (prepare the amount you need)

	Stock soln			
50 mM NaH_2PO_4	1M			
300mM NaCl	5M			
10% Glycerol	60%			
0.25% Tween 20	20%			
10 mM Imidazole	1M			
10 mM beta-Mercaptoethanol**	14M			
1 mM PMSF**	1M			
Total				

****add before use**
##Adjust pH to 8.0 using NaOH

Wash buffer (prepare the amount you need)

	Stock soln			
50 mM NaH_2PO_4	1M			
300mM NaCl	5M			
10% Glycerol	60%			
0.25% Tween 20	20%			
20 mM Imidazole	1M			
10 mM beta-Mercaptoethanol**	14M			
1 mM PMSF**	1M			
Total				

****add before use**
##Adjust pH to 8.0 using NaOH

Elution buffer (prepare the amount you need)

	Stock soln			
50 mM NaH_2PO_4	1M			
300mM NaCl	5M			
10% Glycerol	60%			
0.25% Tween 20	20%			
250 mM Imidazole	1M			
10 mM beta-Mercaptoethanol**	14M			
1 mM PMSF**	1M			
Total				

****add before use**
##Adjust pH to 8.0 using NaOH

Lysozyme (powder)