Protein Purification Protocol

Material:

Buffer			Total volume
Balancing Buffer	20mM Tris+ 150mM NaCl	18.6ml ddH2O+ 0.4ml Tris(1M)+ 1ml NaCl(3M)	20ml
Wash Buffer	20mM Tris+ 300mM NaCl+ 20mM Imidazole	17.52ml ddH2O+ 0.4ml Tris(1M)+ 2ml NaCl(3M)+ 0.08ml imidazole(5M)	20ml
Elution Buffer	20mM Tris+ 300mM NaCl + 250mM imidazole	8.3ml ddH2O+ 0.2ml Tris(1M)+ 1ml NaCl(3M)+ 0.5ml imidazole(5M)	10ml

Steps:

1. Unfreeze the cell lysate, and reserve 100ul as control group (L).
2. Make the others to the final concentration 150mM NaCl+10mM imidazole, And shake it on the rotary mixer for 10mins under 4℃.
3. **Nickel-agarose preparation**:

   First Time:

   (1) Add 2 ml **resin** into the purification column.
   (2) Open the valve of the column and discard the effusive liquid.
   (3) Wash the column with balancing buffer(20ml) and discard the effusive liquid.
   (4) Close the valve

   Second Time:

   (1) Add all the recycled resin(about 10ml) into the column.
   (2) Open the valve of the column and discard the effusive liquid.
   (3) Wash the column with balancing buffer and discard the effusive liquid.
   (4) Close the valve.
4. **Binding**:

   (1) Add the cell lysate mixture into the column and mix it with resin well by drawing in and out with a plastic dropper.
   (2) Transfer all the liquid into an eppendorf and shake it on the rotary mixer for 30mins at 4℃.

   Tip: Prepare the eppendorfs for the following steps while waiting.
5. **Flow through**

   (1) After shaking, move the mixture back into the column via plastic dropper.

(2) Open the valve to the largest flowing rate and collect the liquid flowing out of the column.
(3) Move the liquid back to the column again, but open the valve to a lower flowing rate and collect the liquid in a clean 15ml centrifuge tube.
(4) Label the liquid as FT(Flow Through) and put it on ice.
Note: All the samples should be on ice from this step.

6. Washing

(1) Wash the column with washing buffer(2~3ml/each time)
Note: The valve should be closed when adding buffer
(2) Open the valve to a lower flowing rate for the first 10ml, and a higher flowing rate for the rest 10ml.
(3) Use Eppendorfs to collect the first 1ml and last 1ml liquid and mark them as W1 and WF.

7. **Elution**

(1) Wash the column with elution buffer(1ml/each time)
(2) Open the valve to a lower flowing rate.
(3) Use Eppendorfs to collect the liquid(1 ml for each Eppendorf)
Note: Collect a little more for E1~E5(approximately 1.2ml)
(4) Label them as E1, E2, E3……to E10.

8. **Column Cleanin**g

Add the cleaning buffer in order to the column(2~3ml/each time)
(1) 0.5M imidazole 20ml
(2) DdH2O 20ml
(3) 0.5M NaCl 20ml
(4) DdH2O 20ml
(5) 20% EtOH 20ml
(6) Mix the resin with 10ml 20% EtOH, then transfer to a clean 15ml centrifuge tube and keep in 4℃
(7) Rinse the column with water.

9. Sample confirmation:
Collect 100ul from each eppendorfs (L, FT, W1, WF, E1~E10), and add 100ul 2XSB(sampling buffer).
Store the samples at -20℃
10. Heat at 100℃ for 10 mins, and run SDS page or Western page.