Protein purification protocol

A. Note：

Before entering the laboratory: Put on facemasks and gloves to prevent enzymes dissolving the proteins.

B. Theory：

1. The experiment focuses on Immobilized Metal Affinity Chromatography (IMAC)
   - There are some special sequences in many proteins and they can help proteins to specifically bind some metal ions.
   - However, the specific binding character needs a kind of molecule called “supports”.
   - “Supports” can combine with groups that can covalently bond to metals, such as nitrilotriacetic acid, these groups would combine with metal ions, becoming affinity absorptions.
   - We usually modify our proteins and add a special sequence called “His-Tag”, which consists of 6 histidines. It appears in the end of the recombinant protein.
   - After recombinant protein attaches onto the supports with nickel, they can be washed out with imidazole.
2. Histidine tag (His-tag)
   - Histidine has high affinity towards metal ions.
   - His-tag could be added into N or C terminal of a recombinant protein.
   - It can be expressed using different protein expression systems: bacteria, yeast, insect cells, and mammalian cells etc.
   - There are several advantages：
     - ✓ Small molecular size (4 to 10 amino acids sequence)
     - ✓ High purification efficiency
     - ✓ It would not change the function and activity of protein (in most cases).
     - ✓ Broad applications

C. Materials and equipment：

- Column-purified
- Equivalent buffer, Wash buffer, Elution buffer
- Nickel Resin
- A few eppendorfs, 15 ml and 50 ml centrifuge tubes

D. Laboratory procedures：

1. Add NaCl and Imidazole into the supernatant of bacterial cell lysate and make the final concentration to the chart below and shake it for 30 mins at 4℃

NaCl	150 mM
Imidazole	10 mM

2. Nickel-agarose preparation：
   - Because nickel resin would form layers of clear supernatant liquid and precipitation, so it would be better to mix well.
   - Add 2 ml resin into the column
   - Open the valve of the column and discard the effusive liquid.
   - Wash the column with equivalent buffer buffer and discard the effusive liquid
   - Close the valve
3. Batch binding：
   - Add the cell lysate mixture into the column and mix it with resin well
   - Transfer all the liquid into a tube and shake it for 30 mins at 4℃
4. Flow Through：
   - After shaken, move the mixture back into the column
   - Open the valve and collect the liquid flowing out of the column.
   - Label the liquid as “Flow Through (FT)” and put it on ice.

   Note：All the samples should be put on ice from this step.
5. Collection-Wash buffer：
   - Wash the column with wash buffer
   - Use Eppendorfs to collect the liquid(1 ml for each Eppendorf)
   - Label the first 3 Eppendorfs as “W_1”, “W_2”, “W_3”
   - Label the last Eppendorf as “WF”
6. Collection-Elution buffer：
   - Elute the column with elution buffer
   - Use Eppendorfs to collect the liquid(1 ml for each Eppendorf)
   - Collect around 10 ml and label them as “E_1”, “E_2”, … “E_{10}”
7. Store all the sample at -20℃
8. Wash resin：
   - Wash the column with solution I (concentration：500 mM NaCl, 500 mM Imidazole)
   - Wash the column with ddH_2O
   - Wash the column with 30% alcohol
   - Close the valve
   - Mix the resin with 30% alcohol and store at 4℃.