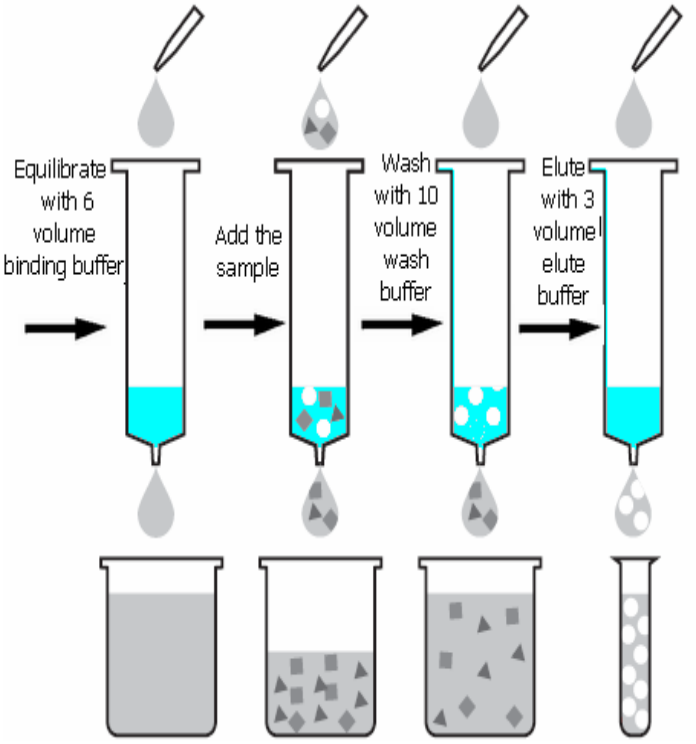
Ni-NTA Agarose

实验操作方法

蛋白纯化流程如下：



Sample preparation

1.**Dilute the cell paste:** Add 5-10 ml of binding buffer for each gram of cell paste.

2.**Enzymatic lysis:** 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM MgCl2, 1 mM PMSF (final concentrations). Stir for 30 minutes at room temperature or +4℃ depending on the sensitivity of the protein.

3.**Mechanical lysis:** Sonication, homogenization, repeated freeze/thaw or similar techniques.

4.**Adjust the pH of the lysate to pH 7.4:** Do not use strong bases or acids for pH-adjustment (precipitation risk).

5.**Centrifuge the lysate:** Transfer to tubes and centrifuge at 12 000 g for 20 minutes at room temperature or +4℃depending on the sensitivity of the protein.

6. **Filtration:** Filtrate the supernatant with 0.45um filter

Protein Purification under Denaturing Conditions from E. coli Lysates（变性条件下纯化蛋白）

试剂准备

Buffer A （**Lysis/Binding Buffer**）: 6 M GuHCl; 0.1 M NaH2PO4; pH 8.0

Buffer B （**Lysis/Binding Buffer**）: 7 M urea: 7 M urea; 0.1 M NaH2PO4; pH 8.0

Buffer C （**Wash Buffer**）: 8 M urea; 0.1 M NaH2PO4; pH 6.3

Buffer D （**Elute Buffer**）: 8 M urea; 0.1 M NaH2PO4;·HCl; pH 5.9

Buffer E（**Elute Buffer**） : 8 M urea; 0.1 M NaH2PO4; pH 4.5

Ni-NTA Agarose
实验操作方法
（续）

Protocol

1. Remove the top cap, pour off excess liquid and place the column in the Workmate column stand.
2. Equilibrate the column with 6 volume binding buffer. The frits protect the column from running dry during the run.
3. Add the sample (see Sample preparation). A volume of 0.5-35 ml is recommended. The protein binding capacity of the column is high, approx. 40 mg histidine-tagged protein/column (protein-dependent).
4. Wash with 10 volume wash buffer.
5. Apply 3 volume elution buffer and collect the eluate. Under denaturing conditions, elute with 2 x 3 ml elution buffer.

Protocol: Protein Purification under Native Conditions from E. coli Lysates（天然条件下纯化蛋白）

试剂准备

Lysis/binding Buffer (NPI-10): 50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole, pH 8.0
Wash Buffer (NPI-20): 50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole, pH 8.0
Elution Buffer (NPI-500): 50 mM NaH2PO4, 300 mM NaCl, 500 mM imidazole, pH 8.0

Protocol

1. Remove the top cap, pour off excess liquid and place the column in the Workmate column stand.
2. Equilibrate the column with 6 volume binding buffer(NPI-10). The frits protect the column from running dry during the run.
3. Add the sample (see Sample preparation). A volume of 0.5-35 ml is recommended. The protein binding capacity of the column is high, approx. 40 mg histidine-tagged protein/column (protein-dependent).
4. Wash with 10 volume wash buffer(NPI-20).
5. Apply 3 volume l elution buffer(NPI-500) and collect the eluate. Under denaturing conditions, elute with 2 x 3 ml elution buffer.

Small scale purification – batch method

Ni-NTA Agarose can also be used in a batch format for small scale purification. Settled bed volumes of 50–200 µl can be handled in a 1.5 ml microcentrifuge tube. In the following protocol, one volume is equivalent to the settled bed volume (e.g. 100 µl of Ni-NTA Agarose yields 50µl of resin for a settled bed volume of 50 µl).

1. Transfer 100–400 µl Ni-NTA Agarose to a 1.5 ml microcentrifuge tube, centrifuge at 400–1000 × g and remove the supernatant.
2. Wash the Agarose with 4 times with 2 volumes of 1X Binding Buffer. For each wash step, invert the tube several times to mix, and spin for 1 min at 400–1000 × g.
3. Add the cell extract to the microcentrifuge tube containing the prepared resin. Mix gently by inversion several times and incubate for 5 minutes. Centrifuge 400–1000 × g and discard the supernatant.
4. Wash the resin with 3 times with 3 volumes 1X Binding Buffer.
5. Wash the resin with 2 times with 3 volumes 1X Wash Buffer.
6. Elute the bound protein 2 times with 3 volumes 1X Elute Buffer. Alternatively, 1X Strip Buffer may also be used to elute the protein by stripping the Ni2+ from the Agarose.