1ml His GraviTrap Kit

实验操作方法

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 8.0:** 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

Protocol

1. Remove the top cap, pour off excess liquid and place the column in the Workmate column stand.
2. Equilibrate the column with 10 ml 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 ml binding buffer.
5. Apply 3 ml elution buffer and collect the eluate. Under denaturing conditions, elute with 2 x 3 ml elution buffer.

1ml His GraviTrap Kit
实验操作方法（续）

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 10 ml 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 ml wash buffer(NPI-20).
5. Apply 3 ml elution buffer(NPI-500) and collect the eluate. Under denaturing conditions, elute with 2 x 3 ml elution buffer.