Protocol 105

Immobilization of Proteins or Antibodies on NHS beads

Materials

1) Beads and Protein or Antibody

NHS beads (Product No.: TAS8848N1141): 1 mg
(Functional groups: 200–300 nmol/mg)
NHS beads are stored in IPA (isopropyl alcohol)
Protein or antibody: 50 μg (= 1 nmol / 50 kDa of protein)

*** Please keep in mind that the shelf life of NHS beads are <1 mo. Prolonged storage will result in loss of NHS esters impacting loading capacity.**

2) Reagents

2-Morpholinoethanesulfonic acid (MES)
2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)
Sodium hydroxide (NaOH)
Potassium chloride (KCl)
Ethylenediaminetetraacetic acid (EDTA)
Glycerol
Methanol
2-Aminoethanol (Ethanolamine) (M.W. 61.08)

3) Apparatus

Micro centrifuge (HITACHI CF15RX2)
Microtube Mixer (TOMY MT-360)
Ultrasonic homogenizer (TAITEC VP-15 with cup horn)

Method

1) Preparation of Buffers

- **Immobilization buffer**

  25 mM MES-NaOH (pH 6.0) : for antibody

  25mM HEPES-NaOH (pH 7.0) : for protein

- **Washing and storage buffer**

  10 mM HEPES-NaOH (pH 7.9)

  50 mM KCl

  1 mM EDTA

  10 % glycerol

- **Masking solution**

  1 M Ethanolamine (pH 8.0)

2) Immobilization of Protein or Antibody on NHS beads

Prepare 50 μg / 50 μl of protein (or antibody) solution in immobilization buffer

*** Please remove Tris and BSA from the protein solution before immobilization because they inhibit protein immobilization on NHS beads.**

Start with 1 mg of NHS beads

(NHS beads are suspended in IPA)

↓

Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant

↓

Wash beads with 50 μl of methanol

(Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant)

↓

Add 50 μl of immobilization buffer and resuspend beads by using homogenizer

↓

Add 50 μl of protein solution (total 100 μl)

↓

Mix for 30 min at 4 °C by using Microtube Mixer

↓

Centrifuge at 15,000 rpm for 5 min at 4 °C

(Transfer the supernatant to another tube for quantification of protein)

↓

Add 250 µl of masking solution and resuspend beads by the Manual Agitation method, or by using homogenizer under chilled condition

↓

Mix for 16-20 h (over night) at 4 °C by using Microtube Mixer

↓

Wash beads with 200 µl of washing buffer, 3 times

(Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant)

↓

Resuspend in 200µl of storage buffer, and store at 4 °C