Protocol 101

Immobilization of Proteins on COOH Beads (EDC method)

Materials

1) Beads and Proteins

COOH beads (Product No.: TAS8848N1140): 1 mg
(Functional groups: 200–300 nmol/mg)
Proteins: 50 μg (= 1 nmol / 50 kDa of protein)

2) Reagents

2-Morpholinoethanesulfonic acid (MES)
2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)
Sodium hydroxide (NaOH)
Potassium chloride (KCl)
Ethylenediaminetetraacetic acid (EDTA)
Tween 20
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (M.W. 191.70)
2-Aminoethanol (Ethanolamine) (M.W. 61.08)

3) Apparatus

Micro centrifuge (HITACHI CF15RX2)
Microtube Mixer (TOMY MT-360)

Method

1) Preparation of buffers

- **Binding buffer**

  25 mM MES-NaOH (pH 6.0)

- **Washing buffer**

  10 mM HEPES-NaOH (pH 7.9)
  50 mM KCl
  1 mM EDTA
  0.1 % Tween 20

- **Masking solution**

  1 M Ethanolamine
  10 mM HEPES-NaOH (pH 7.9)
  50 mM KCl
  1 mM EDTA

2) Immobilization of Proteins on COOH Beads

Start with 1 mg of COOH beads
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Wash beads with H_2O, 3 times
(Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant)
↓
Add 200 µl of 20 mg/ml EDC·HCl solution in H_2O
↓
Mix for 30 min at 4 °C by using Microtube Mixer
↓
Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant
↓
Add 500 µl of cold H_2O and mix rapidly
↓
Centrifuge at 15,000 rpm for 5 min at 4 °C and remove the supernatant

↓

Add 500 µl of cold Binding buffer and Mix rapidly

↓

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

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Add 50 µg of proteins in 50 µl Binding buffer

↓

Mix for 2 h at 4 °C by using Microtube Mixer

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Add 5 µl of Masking solution

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

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Wash with 1 ml of Washing buffer, 5 times

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

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Resuspend in Washing buffer, and store at 4 °C