Experiment Protocol 106

Immobilization of Antibodies or Proteins on Epoxy Beads

1. Materials

1.1 Beads and Ligands (Antibodies)

- Epoxy beads (TAS8848N1110): 1 mg (Functional groups：Approx. 200 nmol/mg)
- Antibodies or Proteins: approximately 50 µg

1.2 Reagents

- 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)
- Sodium hydroxide
- Potassium chloride
- Ethylenediaminetetraacetic acid (EDTA)
- Glycerol

Composition of protein immobilization buffer

10mM HEPES-NaOH (pH7.9)

Composition of protein immobilization bead washing/storing buffer

10mM HEPES-NaOH (pH7.9)
50mM KCl
1mM EDTA
10% glycerol

1.3 Apparatus

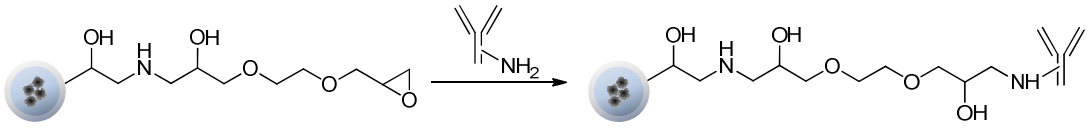
- Micro high-speed cold centrifuge
- Micro tube Mixer (TOMY MT-360, etc.)
- Ultrasonic dispersing device

  We have performed operation checks with an ultrasonic homogenizer:VP-15S with a cup horn (TAITEC), and an ultrasonic dispersing device:TA4905 (Tamagawa Seiki).

2. Method

2.1 Outline

The following is a schematic view of ligand immobilization. Refer to the next section 2.2 “Procedures” for details.



Epoxy beads　　Antibody (protein) immobilized beads

2.2 Procedures

1) Prepare protein immobilization buffer and washing/storing buffer
2) Dilute antibodies with protein immobilization buffer, and prepare 50µg/50µL antibody solution by 50µL or more. (Prepare the solution slightly more than 50µL so that you can fully secure 50µL.)
3) Add 1 mg of epoxy beads (TAS8848N1110) into a 1.5 mL micro-tube.
4) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
5) Add 50µL of protein immobilization buffer, and disperse the epoxy beads with an ultrasonic device.
6) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.

7) Repeat the above 5) to 6) two more times. (Wash the beads three times in total.)
8) Add 50µL of protein immobilization buffer, and disperse the beads with an ultrasonic device.
9) Add 50µL of antibody solution or protein solution.
10) React for 16 to 20 hours (overnight) at 37℃ by using a micro tube mixer.
11) Centrifuge at 15,000 rpm for five minutes at 4℃, and transfer the supernatant to a flesh tube. (For protein quantification)
12) Add 50µL of protein immobilization buffer, and disperse the beads by the manual agitation.
13) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
14) Repeat the above 12) to 13) two more times. (Wash the beads three times in total.)
15) Resuspend in 200 µL of washing buffer, and store at 4℃. (Concentration of antibody immobilized beads：0.1 mg/20 µL)

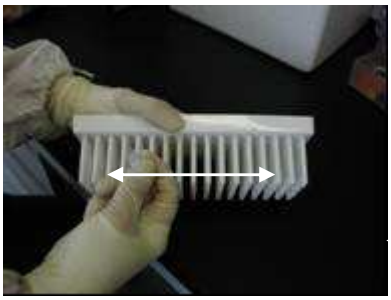
3. Supplements

・ Beads Beads are easily dispersed by using an ultrasonic dispersing device. But if you do not have such a device, they are dispersed by using an ultrasonic washer, or by the manual agitation. In the manual dispersion method, the bottom of a micro-tube is glided over an uneven surface (side of plastic test tube rack in this case) creating turbulence through the collisions. (see left side picture below)

Please make sure to use well-constructed tubes with the caps tightly secured in order to prevent leakage/breakage. Use of cap lock is recommended in order to prevent leakage. (see right side picture below).

For more information, please visit FG beads web site and see the movie of the method.

(Please click：http://www.magneticnanoparticle.jp/en/htdocs/af-notes.html for moving pictures.)



・ When dispersing the beads after immobilizing antibodies, disperse them by the manual agitation. When you cannot disperse the beads easily, disperse them in a short time by using an ice-cold ultrasonic homogenizer or ultrasonic washer.・ Recover the beads not by magnetic separation but by centrifugation.

・ The amount of proteins and antibodies immobilized on beads can be calculated from protein quantification (Bladford method or SDS-PAGE) of the transferred supernatant. It can also be calculated directly from protein immobilized beads by BCA method.

・ When you want to increase the volume of proteins immobilized on beads, increase the volume of the proteins to be added.