Experiment Protocol 105

Immobilization of antibodies or proteins on NHS beads

1. Materials

1.1 Beads and Ligands (Antibodies or Proteins)

・ NHS beads (TAS8848N1141):1 mg (Functional groups：Approx 200nmol/mg)
・ Antibodies or Proteins : Approx 50 µg

1.2 Reagents

・ 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid(HEPES)
・ Morpholinoethane sulfonic acid (MES) ・ Sodium hydroxide ・ Potassium chloride
・ Ethylenediamine tetra acetic acid (EDTA) ・ Glycerol ・ Methanol
・ Amino ethanol M.W. 61.08 ・ Hydrochloric acid

Composition of protein immobilization buffer

(1) Protein immobilization buffer

25mM MES-NaOH(pH6.0) ・・・ Antibodies
25mM HEPES -NaOH(pH7.9) ・・・ Other proteins

(2) Protein immobilized beads wash/storage buffer

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

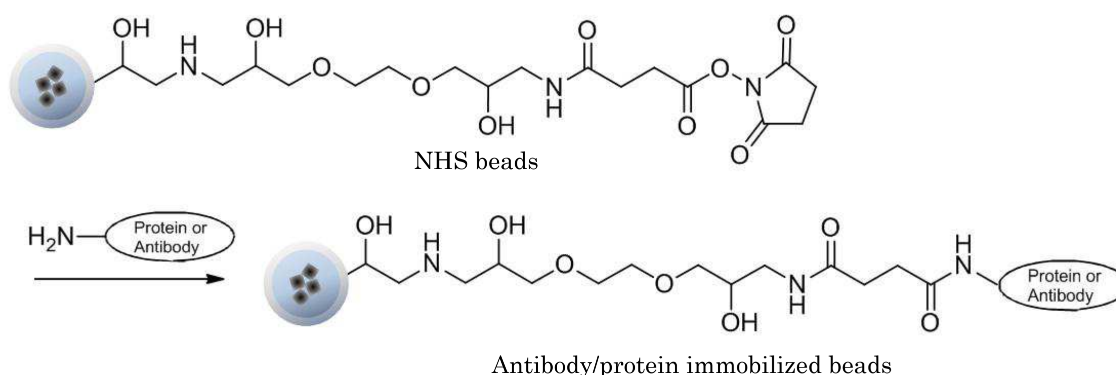
1.3 Apparatus

・ 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.



Antibody/protein immobilized beads

2.2 Procedures

1) Prepare protein immobilization buffer and protein immobilization beads washing/storing buffer.
2) Dilute antibodies or proteins with protein immobilization buffer, and prepare 50 µg/50 µL of antibody (or protein) solution.
3) Add 1 mg of NHS beads (TAS8848N1141) into a 1.5 mL micro-tube.
4) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
5) Add 50 µL of methanol, and disperse the NHS beads.
6) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
7) Add 50 µL of protein immobilization buffer, and disperse the NHS beads (an ultrasonic device may be used). Add 50 µL of antibody or protein solution.
8) React them for 30 minutes at 4℃ by using Micro-tube Mixer.
9) Centrifuge at 15,000rpm for five minutes at 4℃, and transfer the supernatant to a fresh micro-tube. (For protein quantification)
10) Add 250 µL of 1 M amino ethanol solution (pH8.0) to the remaining beads, and disperse the beads.
11) React them for 16) to 20) hours at 4℃ by using Micro-tube Mixer.
    (Masking of protein-non-binding carboxyl groups)
12) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
13) Add 200 µL of protein immobilization beads washing/storing buffer, and disperse the beads.
14) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
15) Repeat the above 13 to 14 two more times. (Wash the beads three times in total.)
16) Disperse the beads in 200 µL of protein immobilized beads wash/storage buffer, and store them at 4℃. (Concentration of protein immobilization beads: 0.1 mg/20 µL) (For protein quantification)

3. Supplements

・ Perfome the dispersion of the beads 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).
When you cannot disperse the beads easily, disperse them in a short time by using an ice-cold ultrasonic homogenizer or ultrasonic washer.
For more information, please visit FG beads web site and see the movie of the method.
(Please click: http://www.magneticnanoparticle.jp/en/htdocs/technique/affinity.html for moving pictures)

- When dispersing the beads after immobilizing antibodies or proteins, 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 antibodies and proteins 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 antibodies and proteins immobilized on beads, increase the volume of the proteins to be added.
- Because NHS (succinimide) is liberated quickly into water, perform the work as promptly as possible under a condition of 4℃.
- If the protein solution contains Tris or BSA, remove it before the reaction because it is immobilized on the beads.