Experiment Protocol 108

Immobilization of biotinylated molecule on Streptavidin beads and NeutrAvidin beads

1. Materials

1.1 Streptavidin beads (or NeutrAvidin beads), Biotinylated molecule solution (compound, antibod, protein, DNA, RNA, other biotinylated molecules)

- Streptavidin beads (or NeutrAvidin beads): 2.0 mg
  (1.0 mg of the 2.0 mg is used as (-) beads that no biotinylated molecule is immobilized.)
- Concentration of the biotinylated molecule solution
  1) Biotinylated protein: 0.4 mg/ml (Prepare 120 µL for 1 mg of beads.)
  2) Biotinylated compound: 4 to 12 mM
  (Prepare 10µl for 1 mg of beads. Dissolve the compound in DMSO when it does not dissolve in PBS.)
  3) Biotinylated DNA: 0.4 mg/ml (Prepare 120µL for 1 mg of beads.)

1.2 Reagents

- 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)
- Sodium hydroxide (NaOH) ・ Potassium chloride (KCl)
- Ethylenediamine tetra acetic acid (EDTA) ・ Glycerol (Glycerin)
- Nonidet P-40 (NP-40) ・ Sodium chloride (NaCl) ・ Disodium hydrogen phosphate
- Potassium dihydrogen phosphate ・ Tris (hydroxymethyl) amino methane (Tris)
- Hydrochloric acid (HCl) ・ Dimethylsulfoxide (DMSO)

Buffer composition

1) Binding buffer for biotinylated protein and biotinylated compound
PBS (-)

2) Binding buffer for biotinylated DNA
5 mM Tris-HCl (pH 7.5)
0.5 mM EDTA
1 M NaCl

3) Washing/preservative buffer
10 mM HEPES-NaOH (pH 7.9)
50mM KCl
1 mM EDTA
10% glycerol

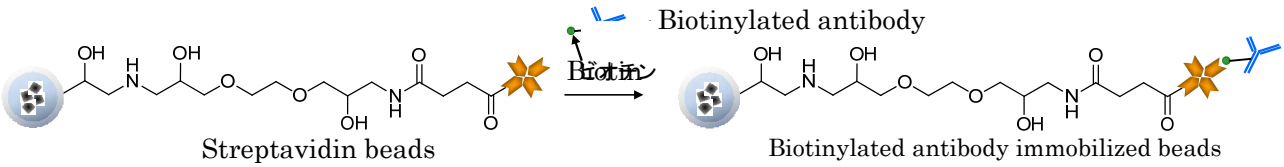
1.3 Apparatus

- Desktop centrifuge (for spin down) ・Magnetic separation stand
- Micro tube mixer (TOMY MT-360, etc.)

2. Method

2.1 Outline

The following is a schematic view of biotinylated molecule immobilization (Ex.: Biotinylated antibody immobilization).
Refer to the next section 2.2 “Procedures” for details.



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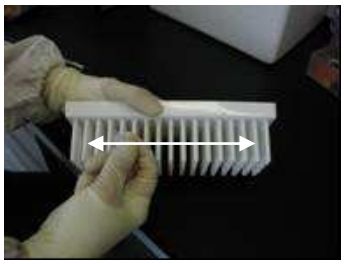
2.2 Procedures

1) Place the binding buffer on ice, and cool it.
2) Adjust the concentration of the biotinylated molecule to a target concentration (Refer to 1-1) with the binding buffer.
3) Prepare 2×1.5 mL micro-tubes, and add 1 mg of streptavidin beads (i.e. add 50µL of 20 mg/mL beads) to each of the micro-tubes. When investigating additive concentrations of the biotinylated molecule, prepare the appropriate number of the micro-tubes. (Secure one of them for control beads, to which the biotinylated molecule is not added.)
4) Add 200 µL of the binding buffer, and disperse the beads (by the manual agitation – refer to the section 3 "Supplements")
5) After spin down, separate magnetically, and discard the supernatant.
6) Repeat the above 4) to 5) two more times.(Wash the beads with buffer three times in total.)
7) Add the binding buffer to the supernatant discarded micro-tube, and disperse the beads by the manual agitation. Then, add biotinylated molecule solution to the micro-tube as follows.
   a) Biotinylated protein and biotinylated DNA: After adding 100 µL of binding buffer to the micro-tube, disperse the beads by manual agitation, and add 100 µL of biotinylated protein or DNA solution to it to obtain 200 µL of mixture.
   b) Biotinylated compound: After adding 195µL of binding buffer, disperse the beads by manual agitation, and add 5µL of biotinylated compound to it to obtain 200 µL of mixture.
8) React the mixture with a micro tube mixer for one hour at 4℃ (on a scale of 6).
9) After spin down, separate magnetically, and transfer the supernatant to a clean micro-tube. (When quantifying the concentration of the biotinylated molecule in the supernatant, store it.)
10) Add 500 µL of washing/preservative buffer, and disperse the beads by the manual agitation.
11) After spin down, separate magnetically, and discard the supernatant.
12) Repeat the above 10) to 11) two more times. (Wash the beads with buffer three times in total.)
13) Add 200 µL of washing/preservative buffer, disperse the beads by the manual agitation, and store the biotinylated molecule immobilized Streptavidin or NeutrAvidin beads at 4℃. (Concentration of the biotinylated molecule immobilized beads:0.1 mg/20 µL)

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)



- The amount of immobilized biotinylated protein can be calculated from protein quantification (Bladford method or SDS-PAGE) of the transferred supernatant.
- The amount of immobilized biotinylated DNA can be calculated from nucleic acid quantification (absorbance measurement at wavelength of 260 nm and 280 nm) of the transferred supernatant.
- When you want to increase the amount of biotinylated molecule immobilized on beads, increase the amount of the biotinylated molecule solution to be added or the concentrations of it.