Experiment Protocol 110

Immobilization of antibodies on Protein A beads and Protein G beads

1. Materials

1.1 Beads and ligands (antibodies)

・ Protein A beads or Protein G beads：2.0 mg
(1.0 mg of the 2.0 mg is used as (-) beads that no antibody is immobilized.)
・ Antibody solution 0.5 mg/mL (Prepare 120 µL of the solution for 1 mg of beads.)
(When increasing the amount of antibodies immobilized on beads, raise the concentration of the solution.)

1.2 Reagents

・ 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) ・ Glycerin (Glycerol)
・ Sodium hydroxide (NaOH) ・ Sodium chloride (NaCl) ・ Potassium chloride (KCl)
・ Disodium hydrogen phosphate ・ Potassium dihydrogen phosphate ・ Ethylenediamine tetra acetic acid (EDTA)

Buffer composition
1) Binding buffer
PBS (-)
2) Washing/preserving buffer
10 mM HEPES-NaOH (pH 7.9)
50 mM KCl
1 mM EDTA
10% glycerol

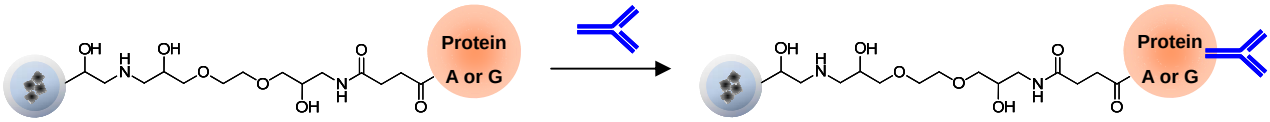
1.3 Apparatus

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

2. Method

2.1 Outline

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



Protein A beads/Protein G beads　　Antibody immobilizing beads

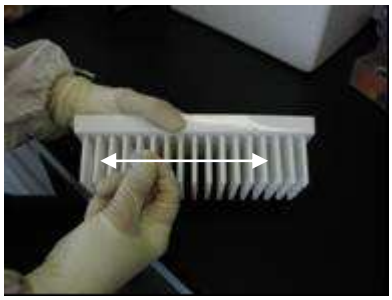
2.2 Procedures

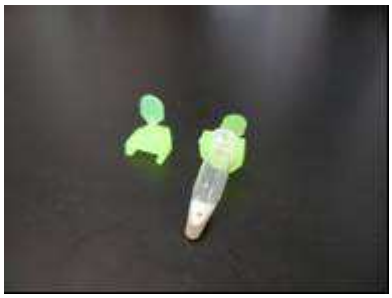
1) Place the antibody binding buffer (PBS) on ice, and cool it.
2) Adjust the concentration of your antibody to a target concentration (Refer to 1-1) with the antibody binding buffer.
3) Completely resuspend Protein A or Protein G beads with a vortex mixer, and add 1 mg of the beads (50µL of 20 mg/mL beads) to each 1.5 mL micro-tube. (When investigating additive concentrations, prepare the appropriate number of the micro-tubes.)

4) Add 200 µL of the antibody binding buffer to the micro-tube, 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) one more time.(Wash the beads with buffer twice in total.)
7) Add 100 µL of the antibody binding buffer to the supernatant discarded micro-tube, and disperse the beads by the manual agitation. Then, add 100 µL of the antibody solution.
8) Incubate with rotation for thirty minutes at room temperature.
9) After spin down, separate magnetically at room temperature, and discard the supernatant. (When quantifying the concentration of the antibody in the supernatant, store it.)
10) Add 500 µL of washing/preserving buffer to the maicrotube, 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 the buffer three times in total.)
13) Add 200 µL of washing/preserving buffer to the micro-tube, disperse the beads by the manual agitation, and store the antibody immobilized Protein A or Protein G beads at 4℃. (The concentration of the antibody 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 antibodies immobilized on beads can be calculated from protein quantitation (Bladford method or SDS-PAGE) of the transferred supernatant.
・ When you want to increase the amount of antibodies immobilized on beads, increase the volume of the antibody solution to be added, or the concentrations of it.