Experiment Protocol 102

Immobilization of His-Tag proteins on Ts beads

For screening, you need, first of all, to optimize the amount of immobilization of proteins on beads. You can change the amount of immobilization of proteins by changing the concentration of proteins. This experiment protocol shows a method to immobilize proteins at four various concentrations, i.e. 0μM(0nmol/mg), 2μM(0.4nmol/mg), 10μM(2nmol/mg), and 50μM (10nmol/mg) when immobilizing proteins on Ts beads

1. Materials

1.1 Beads and Ligands (His-Tag proteins)

- Ts beads (TAS8848N1150): 10mg(2.5mg/condition)
- His-tag proteins: Approx. 2 mg

1.2 Reagents

- 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid(HEPES)
- Sodium hydroxide ・ Potassium chloride ・ Ethylenediamine tetraacetic acid (EDTA) ・Glycerol
- Tris(hydroxymethyl)aminomethane M.W. 121.14 ・ Hydrochloric acid

Composition of protein immobilization buffer
10mM HEPES-NaOH(pH7.9)
50mM KCl
1mM EDTA
10% glycerol

1.3 Apparatus

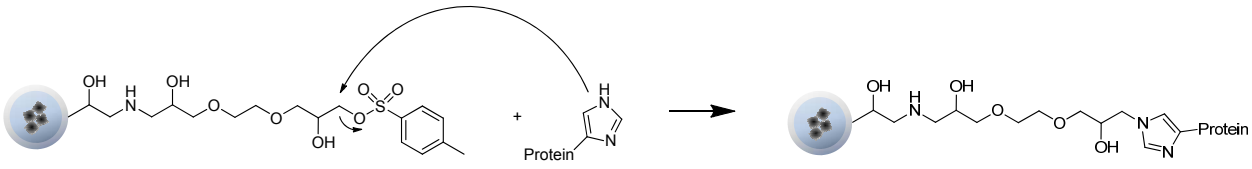
- Micro centrifuge ・Micro tube Mixer (TOMY MT-360, etc.)
- Ultrasonic dispersing device

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 for details.



Ts beads His-Tag proteins His-Tag protein immobilized beads

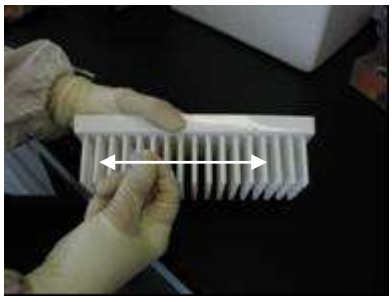
2.2 Procedures

1) Prepare protein immobilization buffer.
2) Dilute proteins with protein immobilization buffer, and prepare 500 μl of protein solution for each concentration, 0 μM, 2 μM, 10 μM, and 50 μM.
   ※We recommend dialyzing the protein immobilization buffer when the buffer composition of protein solution differs.
3) Dissolve tris(hydroxymethyl)aminomethane in ultrapure water, and prepare 3mL of 1M tris (hydroxymethyl)aminomethane (pH8.0). (Adjust the pH with hydrochloric acid.)
4) Add 2.5 mg of Ts beads (TAS8848N1150) into each of four 1.5 ml micro-tubes.

5) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
6) Add 500 µL of protein immobilization buffer, and disperse the Ts beads with an ultrasonic device.
7) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
8) Repeat the above 6) to 7) two more times. (Wash the beads three times in total.)
9) Add 500 µL of the prepared protein solution of each concentration, and disperse the beads with an ultrasonic dispersing device.
10) React for 16 to 20 hours (over night) at 4℃ by using a micro tube mixer.
11) Centrifuge at 15,000 rpm for five minutes at 4℃, and transfer the supernatant to a fresh micro-tube. (For protein quantification)
12) Add 500 µL of 1M tris hydroxymethyl amino methane (pH8.0) to the remaining beads, and disperse the beads.
13) React for 16 to 20 hours (over night) at 4℃ by agitating with a micro tube mixer. (Masking of protein-non-binding tosyl groups)
14) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
15) Add 500 µL of protein immobilization buffer, and disperse the Ts beads with an ultrasonic device.
16) Centrifuge at 15,000 rpm for five minutes at 4℃, and discard the supernatant.
17) Repeat the above 15) to 16) two more times. (Wash the beads three times in total.)
18) Disperse the beads in 500 µL of protein immobilization buffer, and store them at 4℃. (Concentration of His-Tag protein immobilized beads:0.5 mg/100 µL)

3. Supplements

・ 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/technique/affinity.htmlfor moving pictures.)



・ The amount of immobilized His-Tag proteins 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.

Experiment Protocol 102

- When you want to increase the amount of His-Tag proteins immobilized on beads, increase the amount of the proteins to be added.

- When beads and proteins attach to the wall of a micro-tube, disperse them as much as possible.