

Experiment Protocol 102

- 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°C, 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°C by using a micro tube mixer.
- 11) Centrifuge at 15,000 rpm for five minutes at 4°C, 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°C 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°C, 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°C, 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°C.
(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/af-notes.html> for moving pictures.)



- The amount of immobilized His-Tag proteins can be calculated from protein quantification (Bradford 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 amount of His-Tag proteins immobilized on beads, increase the amount of the proteins to be added.

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