

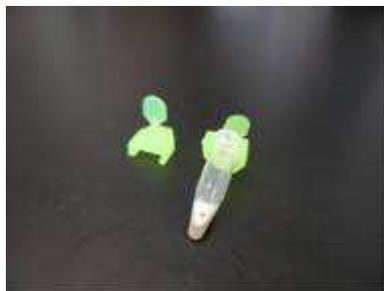


## Experiment Protocol 106

- 7) Repeat the above 5) to 6) two more times. (Wash the beads three times in total.)
- 8) Add 50  $\mu$ L of protein immobilization buffer, and disperse the beads with an ultrasonic device.
- 9) Add 50  $\mu$ L of antibody solution or protein solution.
- 10) React for 16 to 20 hours (overnight) at 37°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 tube. (For protein quantification)
- 12) Add 50  $\mu$ L of protein immobilized beads wash/storage buffer, and disperse the beads by the manual agitation.
- 13) Centrifuge at 15,000 rpm for five minutes at 4°C, and discard the supernatant.
- 14) Repeat the above 12) to 13) two more times. (Wash the beads three times in total.)
- 15) Resuspend in 200  $\mu$ L of protein immobilization beads washing/storing buffer, and store at 4°C. (Concentration of antibody immobilized beads: 0.1 mg/20  $\mu$ L)

### 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.html> for moving pictures.)



- When dispersing the beads after immobilizing antibodies, 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 proteins and antibodies immobilized on beads 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 volume of proteins immobilized on beads, increase the volume of the proteins to be added.