Experiment Protocol 107

Direct Quantification of Immobilized Proteins (Antibodies)

When screening target proteins, you may need to measure the amount of proteins or antibodies immobilized on beads. You can quantify it by using BCA Assay.

1. Materials

1.1 Beads

- Protein (Antibody) immobilized beads (5mg/mL)

1.2 Reagents

- BCA Protein Assay Reagent A (Pierce 23221, etc.)
- BCA Protein Assay Reagent B (Pierce 23224, etc.)
- Bovine Serum Albumin (BSA) Standard (Pierce 23209, etc.)
- Bovine Gamma Globulin (BGG) Standard (Pierce 23212, etc.)

1.3 Apparatus

- Micro tube Mixer (TOMY MT-360, etc.)
- Incubator
- Micro-tube filter : Ultrafree-MC (Pore size : 0.2μm) (MILLIPORE UFC30LG00, etc.)
- Micro centrifuge
- Microplate reader
- 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 Preparation of solutions

1) Prepare Working Reagent (WR) by mixing 50 parts of BCA Protein Assay Reagent A with 1 part of BCA Protein Assay Reagent B (50:1, Reagent A: B)
   The total volume of WR required: 200μL of WR is required for each sample and standard.
2) When quantifying proteins, dilute BSA Standards, and when quantifying antibodies, dilute BGG Standards, at 500, 250, 125, 62.5, 31.3, 15.6, 0 μg/mL respectively to obtain solutions for standard curves.

2.2 Measurement

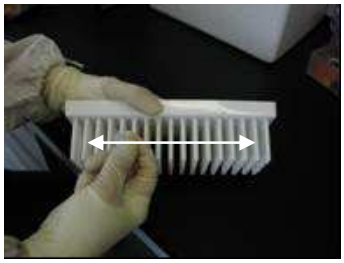
1) Mix 200μL of WR with 40μL (0.2mg of beads) of a sample (standard curves, beads, and blanks) (Mix the beads in suspension.)
2) Mix for 30 minutes in a 37℃ incubator by using a micro tube mixer.
3) Take the tube out of the incubator, and allow the solution to cool to room temperature.
4) Centrifuge at 15,000 rpm for five minutes at room temperature, and transfer the supernatant to another tube.
5) Filter the supernatant (by using a micro-tube filter, and centrifuging at 5000×g for one minute) to remove the beads thoroughly.
6) Measure the absorbance at or near 562nm on a microplate reader.

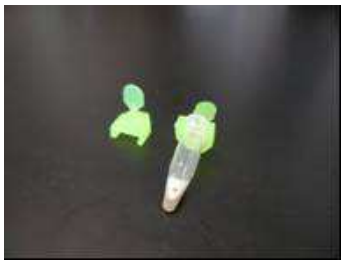
2.3 Data assay

1) Subtract the measurement of a blank sample from the measurement of a standard curve sample and a beads sample to correct the measurement.
2) Prepare a standard curve by plotting the average Blank-corrected measurement for each standard vs. its concentration in μg/mL. Use the standard curve to determine the protein (antibody) concentration of Blank-corrected beads sample.
3) Use the following formula to calculate the amount of immobilized proteins (antibodies).
   The amount of immobilization (μg/mg) = Protein concentration (μg/mL) / Bead concentration (mg/mL)

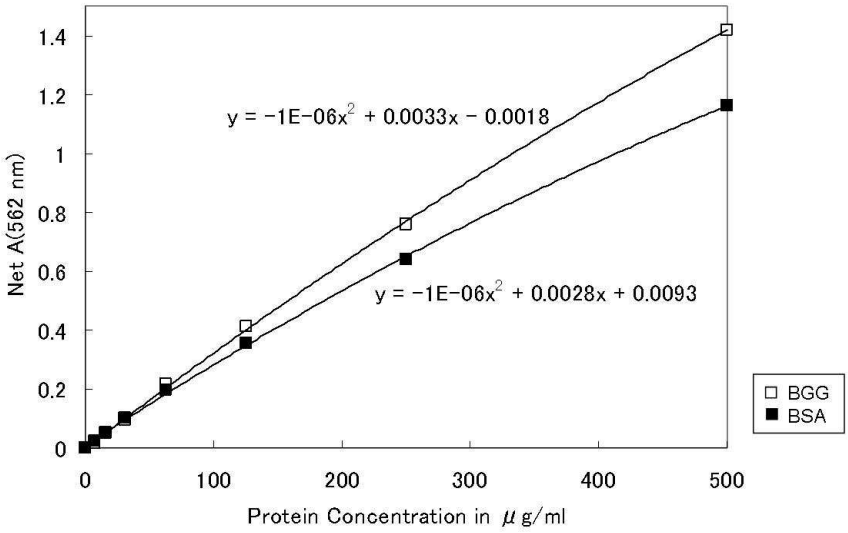
3. Supplements

・ 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)





・ An example of typical standard curves



4. Note

・If beads remain in a tube at the time of measurement, they may cause an error. Be sure to remove them thoroughly.