TECH TIP #75

Measure protein bound to Pierce® NHS-Activated Magnetic Beads

TR0075.0

Introduction

The direct measurement of protein coupled to Pierce NHS-Activated Magnetic Beads (Product No. 88826, 88827) can be performed using the Micro BCA Protein Assay Kit (Product No. 23235). This kit uses bicinchoninic acid to detect Cu^{+}, which is formed when Cu^{2+} is reduced by protein in an alkaline environment. After the deep purple color develops from bicinchoninic acid coupling with Cu^{+}, the magnetic beads are separated from the solution by centrifugation and the color is measured using a spectrophotometer. The bound protein is calculated as micrograms of protein per milligram of magnetic beads.

Important Information

- This procedure is a modified version of the Micro BCA Protein Assay (Product No. 23235).
- Quenched NHS-activated magnetic beads are used as a control in the measurement. The procedure to prepare these quenched beads (Section A) requires 2 hours to perform and should be completed before setting up the assay.
- For best results, prepare standards using the same protein that is conjugated to the Pierce NHS-Activated Magnetic Beads. If the specific protein is not available, use either Bovine Serum Albumin Standard (Product No. 23209, which is also included with the Micro BCA Protein Assay Kit) or Bovine Gamma Globulin Standard (Product No. 23212).
- Results obtained by direct measurement of protein conjugated to the magnetic beads are similar to results obtained indirectly by subtracting the protein in the flow-through from protein loaded.

Materials Required

- 1mg/mL conjugate protein in buffer
- Protein-coupled Pierce NHS-Activated Magnetic Beads
- Non-coupled Pierce NHS-Activated Magnetic Beads (100µL of beads will be quenched for use as a control)
- Micro BCA Protein Assay Kit (Product No. 23235)
- Phosphate-buffered saline (PBS, Product No. 28372)
- Quenching Buffer: 3M ethanolamine, pH 9.0
- 2mL microcentrifuge tubes and microcentrifuge
- 96-well plate and plate reader
- 50mL conical tube
- 1.5mL microcentrifuge tubes
- Magnetic Stand (e.g., Thermo Scientific MagnaBind Magnet for 6 × 1.5mL Microcentrifuge Tubes; Product No. 21359)

Procedure

A. Prepare Quenched "Control" Beads

Note: Vortex magnetic beads until homogeneous; then place them on a rocking platform to continuously mix before use.

1. Add 100µL of non-coupled Pierce NHS-Activated Magnetic Beads to a 1.5mL microcentrifuge tube.
2. Place the tube on a magnetic stand, collect the beads and discard the storage solution.
3. Add 1mL of Quenching Buffer to the beads and incubate for 2 hours at room temperature on a rotating platform. During the first half hour of incubation, vortex the tube for 15 seconds every 5 minutes.
4. Place the tube on a magnetic stand, collect the beads and discard the supernatant.

5. Add 1mL of purified water to the beads and vortex to mix. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
6. Add 1mL of PBS to the beads and vortex to mix. Place the tube on a magnetic stand, collect the beads and discard the supernatant. Repeat this step twice.
7. Add 100µL of PBS to the quenched beads and save for use as a control in the assay below.

B. Prepare Standards and Diluted Bead Samples for Testing

Note: Heat a water bath to 55°C for use in Section D. In addition, vortex magnetic beads (both coupled and "quenched" bead samples) until homogeneous and then place the beads on a rocking platform to continuously mix before use.

1. Prepare standards using the same protein that is conjugated to the magnetic bead. Starting with a 1mg/mL protein solution (ideally in PBS), prepare Stock standards in PBS equal to 1000, 750, 500, 250, 125, 62.5, 0µg/mL.
2. Prepare Test standards (in duplicate) from the Stock standards by adding 475µL of purified water and 25µL of Stock standard to appropriately labeled 2mL microcentrifuge tubes. Vortex each tube to mix.

Stock standard concentration (µg/mL)	Volume of water added to 25µL of standard (µL)	Final Test standard concentration (µg/mL)
1000	475	50
750	475	37.5
500	475	25
250	475	12.5
125	475	6.25
62.5	475	3.125
0	475	0

3. For each bead-sample to be assayed, pipette 25µL of protein-coupled Pierce NHS-Activated Magnetic Beads and 475µL of water into labeled 2mL microcentrifuge tubes. *This is a 20-fold dilution of each bead sample (originally with beads at 10mg/mL), resulting in TEST beads at 0.5mg/mL.* Prepare in duplicate. Include the quenched beads (prepared in Section A) as a control sample.

C. Prepare Micro BCA Working Reagent (WR)

1. Calculate the total volume of Working Reagent (WR) needed for the assay. Each tube (test and standard duplicate) requires 500µL of WR. Therefore, the volume of WR required (*X*) equals the total number of tubes times 0.5mL (then add 1mL extra). Next calculate the volume of each Micro BCA Reagent needed to make *X*mL of WR:
   - Reagent A: *X*mL × 0.50 = _____ mL
   - Reagent B: *X*mL × 0.48 = _____ mL
   - Reagent C: *X*mL × 0.02 = _____ mL
2. Combine Reagents A, B and C (per above calculations) in a 50mL conical tube. Mix end-over-end by hand or on a rotator.

D. Perform the Protein Assay

1. Add 500µL of WR to each tube. Cap all tubes and vortex to mix.
2. Incubate tubes for 50 minutes in a water bath equilibrated to 55°C.
3. Remove tubes from the water bath, vortex to mix and then centrifuge the tubes for 15 minutes at 10,000 × *g*.
4. Add 200µL of each standard or sample, in triplicate, into a 96-well plate.
5. Transfer the plate to a plate reader and measure the absorbance of the samples at 562nm.
6. Subtract the average absorbance of the blank standard replicates from the absorbance readings of all other standards and unknown samples.

E. Calculate Coupled Protein Concentration

1. Plot a standard curve based on the absorbance vs. concentration of the protein Test standards. Plot the absorbance measurements against the concentration values given in the right-most column of the table in Section B (i.e., 50 to 0µg/mL).
2. By direct reference to the plotted standard curve, calculate the concentration of protein in the TEST samples. To express the protein-specific concentration, obtain the average of replicate-wells and duplicate-samples, and then subtract the result of the quenched control beads sample.
3. Finally, because the TEST samples contained 0.5mg beads/mL (See Section B), the concentrations of protein per milligram of bead are twice the values of the TEST samples. Final result = ____ µg protein/mg beads
4. Alternatively, the concentrations of protein per milliliter of original 10mg/mL beads are twenty (20) times the values of the TEST samples. Final result = ____ µg protein/mL of beads

Related Thermo Scientific Products

23235	**Micro BCA Protein Assay Kit**, sufficient for 480 tube assays or 3200 microplate assays
23208	**Bovine Serum Albumin Standard Pre-diluted Set,** 7 × 3.5mL aliquots in the range of 125-2000µg/mL
23213	**Bovine Gamma Globulin Standard Pre-Diluted Set,** 7 × 3.5 mL aliquots in the range of 125-2000µg/mL
23209	**Bovine Serum Albumin Standard Ampules, 2mg/mL,** 10 × 1 mL
23212	**Bovine Gamma Globulin Standard Ampules, 2mg/mL,** 10 × 1mL

General References

Brown, R., *et al*. (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. *Anal. Biochem.* **180(1):**136-9.

Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. **150 (1):**76-85.

Current versions of product instructions are available at *www.thermoscientific.com/pierce*.. For a faxed copy, call 800-874-3723 or your local distributor.

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