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Quantitate immobilized protein

TR0009.2

Introduction

With many affinity chromatography techniques, there is a need to assess the amount of protein that has been successfully bound to a solid support. Often, the amount of immobilized protein can be estimated by subtracting the assayed amount of unbound protein (e.g., void and wash fractions) from the known amount of starting (or pre-bound) protein. However, for a variety of reasons, this strategy is not always feasible. In such cases, it is possible to use the Pierce® Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236) to estimate the amount of protein immobilized on a support.

When coomassie dye binds to protein in an acidic medium, the bound molecules of dye exhibit a shift in absorbance maximum from 465 nm to 595 nm with a concomitant color change from brown to blue. Usually, one assays directly for protein by measuring the increase in absorbance at 595 nm caused by the presence of the protein. However, one can also assay inversely by measuring the decrease in absorbance at 465 nm caused by removal of unbound dye from solution in the presence of protein (Figure 1). This inverse measurement strategy allows one to assay for immobilized protein.

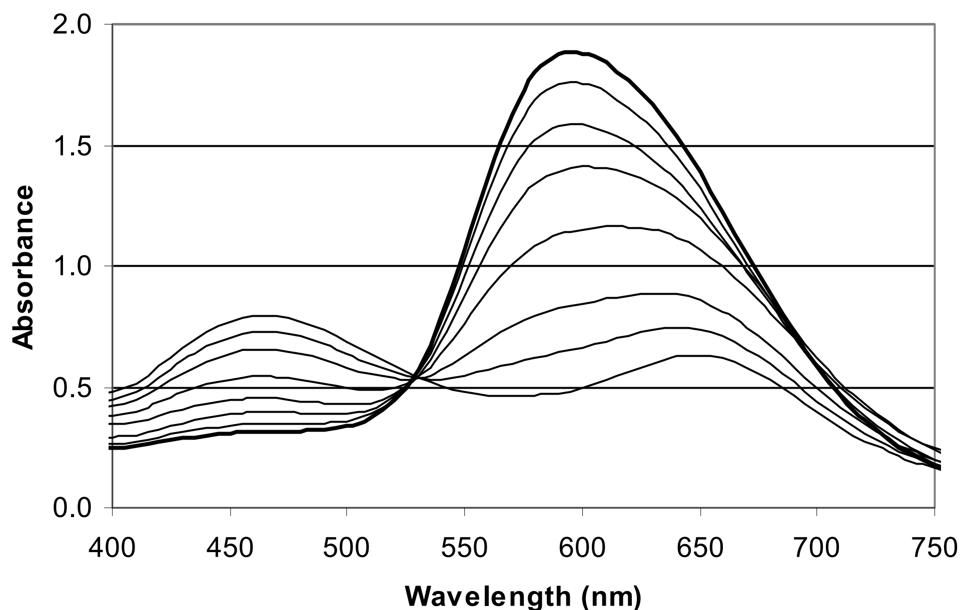

Figure 3. Absorbance spectra for BSA standards in the Pierce Coomassie Plus (Bradford) Assay (standard test tube procedure). BSA standards are 0, 125, 250, 500, 750, 1000, 1500, 2000 µg/ml, respectively. The 2000 µg/ml line is drawn thicker than the others to orient the sequence. Notice that an inverse relationship between protein concentration and absorbance occurs below 525 nm (maximum at 465 nm); concentration of immobilized protein can be estimated by measuring the decrease in dye absorbance at 465 nm.

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Materials Required

- Immobilized protein sample: the method presented here assumes use of protein immobilized onto a beaded agarose or other similar resin prepared in a 50% slurry with buffer. Except for the immobilized protein, the resin and slurry buffer is assumed to be free of chemical groups that would bind or otherwise interfere with coomassie dye detection of protein.
- Control solid support (optional): resin that was never activated (e.g., plain 4-6% beaded agarose) or activated affinity support that has been quenched instead of coupled to the test protein. When preparing quenched resin, ensure that the quenching functional group is compatible with the Coomassie Plus Protein Assay.
- Pierce Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236)
- 1.5 ml microcentrifuge tubes and microcentrifuge
- Cuvettes and spectrophotometer, or microplate and plate reader

Procedure

1. For each standard dilution to be assayed, add 50 µl of buffer (same buffer as was used to make the 50% slurry of the test immobilized protein) to appropriately labeled microcentrifuge tubes.

   Note: If using a solid support control, add 100 µl of mixed 50% control resin slurry to each designated empty standard tube, centrifuge briefly to pellet the resin, and remove the buffer supernatant (presumably 50 µl).
2. Pipette 50 µl of each standard into the microcentrifuge tubes prepared in Step 1.
3. Pipette 100 µl of mixed 50% resin slurry immobilized protein sample into a tube.
4. Add 1.25 ml of Coomassie Plus Assay Reagent to all tubes (standards and test).
5. Vortex tubes to mix reagent and resin. Incubate tubes for 2-5 minutes with periodic vortexing to keep resin suspended.
6. Centrifuge tubes for 1-2 minutes at low speed (e.g., 1500 × *g*) in a microcentrifuge.
7. Transfer supernatants to spectrophotometric cuvettes (or 300 µl to microplate wells).
8. Measure the absorbance of supernatants at 465 nm (not 595 nm as in a Bradford-style assay).
9. Plot a standard curve based on absorbance vs. concentration (µg/ml) of the protein standards, then determine the protein concentration of the immobilized protein (in µg/ml of resin) by reference to this curve.

   Note: the standard curve will be an inverse curve (high absorbances for the blank and low standards and low absorbances for the high standards.

Related Products

23236	**Pierce Coomassie Plus (Bradford) Protein Assay Kit**, working range of 1-1,500 µg/ml
23209	**Albumin Standard Ampules, 2 mg/ml**, 10 × 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide.
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**, 7 × 3.5 ml aliquots in the range of 125-2,000 µg/ml
23212	**Bovine Gamma Globulin Standard Ampules**, 2 mg/ml, 10 × 1 ml
23213	**Pre-Diluted Protein Assay Standards**, Bovine Gamma Globulin Fraction II (BGG) Set, 7 × 3.5 ml aliquots in the range of 125-2,000 µg/ml

References

1. Bonde, M., Pontoppidan, H. and Pepper, D.S. (1992). Direct dye binding — a quantitative assay for solid-phase immobilized protein. *Analytical Biochemistry* **200**, 195-198.

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

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