

Bradford ULTRA Reagent From Novexin

Available at: <http://www.biocompare.com/review/1056/Bradford-ULTRA-Reagent-From-Novexin.html>

May 14 '08

Review Synopsis

Product

Bradford ULTRA Reagent From Novexin

The Good

Easy to use. Fast. Highly sensitive and reproducible. Tolerates a wide range of detergents. Nearly protein independent.

The Bad

The reagent shows excellent linearity, but within a narrow range of protein concentrations that needs to be well-defined with standard spectrophotometers. Polynomial fitting is required to analyze broader low-range standard curves. Most importantly, the procedure is highly reagent consuming given the sample/reagent ratio. However, the use of microplate readers might address this limitation.

The Bottom Line

This is a quick and easy reagent solution for protein quantification if you are using a detergent (at a moderate concentration) in your preparation. It's extremely easy to use and the protocol is fast and reproducible. The absorbance is stable for at least 30 minutes, making the timing of the measurements not absolutely critical. There is a significantly lower protein to protein variability.

Full Product Review

The Bradford ULTRA Reagent is a quick Coomassie-binding method used for the detection and the quantification of protein in presence of up to 1% w/v detergent (1% in high protein range, 0.1% in low protein range). The detection is based on a colorimetric assay wherein a one-step reaction yields a color change from brown to blue - corresponding to a shift in absorption maximum from 465 to 595 nm - that occurs when the Coomassie dye binds protein in an acidic medium. The entire

procedure from start to finish takes no longer than 30 minutes and should always be performed at room temperature.

The advantage of using this reagent is that it is not affected by detergents, a limitation of the traditional Bradford assay. This greater tolerance of detergents eliminates the requirement for removal of the detergent from detergent-solubilized protein before use. Alternatively, if you are using a detergent concentration incompatible with the Bradford ULTRA limits, you can dilute your samples so that the concentration of detergent is reduced to 1% or 0.1%, according to the concentration range required to assay. The reaction remains instantaneous like with the standard Bradford formulation and the color remains stable for 30 minutes, making the reaction extremely stable for accurate measurements.

The assay is compatible with many detergents including Tween 20, Brij 35, SDS, Triton X-100, CTAB present up to 1% in standard assays or 0.1% in microassays. Indeed, this formulation is not subject to the high background noise obtained at 595 nm (as in classical Bradford assays); this results in a higher sensitivity with accurate results. The intensity of the color formation is protein dependent and correlates to the basicity (in particular the number of positive charges, especially on lysine, arginine and histidine residues) and the hydrophobicity of the protein. The chosen model protein (a suitable standard should have a similar percentage of basic residues as the target protein) used to create the standard curve necessary to estimate the protein concentration should be prepared in the same buffer as the sample. Protein standards can vary in their readout, so to ensure reproducibility, it's imperative to conserve the same protein model (BSA, bovine gamma immunoglobulin, bovine beta lactoglobulin, etc.) each time a standard curve is realized. The standards are not included with the 500 ml reagent solution which must be stored at + 4°C.

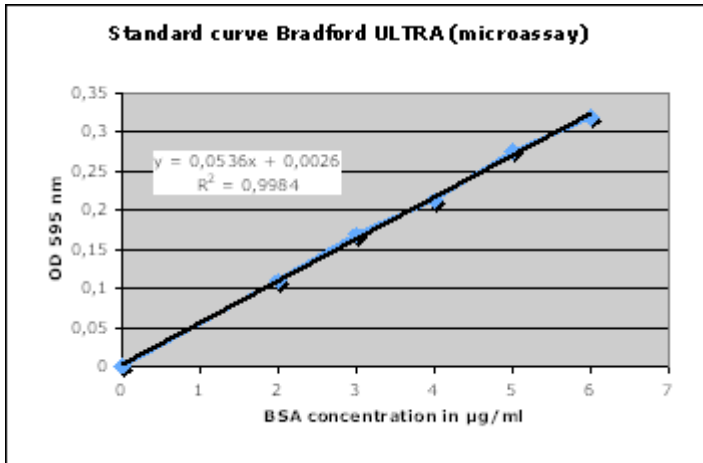
Before performing the assay, mix the reagent solution by gently inverting the bottle several times without shaking, then remove the amount of reagent needed and equilibrate it to room temperature. Protein standards must be created by preparing a dilution series of the model protein; for low concentrations, a standard curve covering 1 µg/ml to 25 µg/ml is recommended, whereas for high concentrations, it should cover 0.1 mg/ml to 1.5 mg/ml. However, the best linearity interval often depends on the dynamic range of the spectrophotometer used and so should be optimized before running a full assay. Instead of this optimization, a second order polynomial regression can be applied.

The assay is very easy to perform: once equilibrated to room temperature, again mix the Bradford ULTRA reagent that was removed aside, then dispense it to all samples, standards and blanks; each preferably realized in triplicate. According to the protein range, the sample/reagent ratio is 1/1 (low) or 1/15 (high). Vortex each tube before start to read absorbances at 595 nm in a spectrophotometer or a microplate reader (you can allow the reactions to stand for 5-15 minutes at room temperature before measuring the absorbance). A color change should occur. Subtract the average 595 nm measurement for the blanks (if the spectrophotometer is not automatically zeroed with a blank value) from the average 595 nm obtained for the standard and unknown samples. Create a standard curve plotting average blank-corrected 595 nm absorbance (y-axis) obtained for each dilution of the standard protein vs. concentration in ug/ml or in mg/ml according to the range (x-axis) and finally estimate the unknown protein concentration by reference to the slope of this curve (with a linear or a polynomial interpolation).

Regarding our low range assays, we have used 1.6 ml semi-microcuvettes containing 500 ul of sample + 500 ul of Bradford ULTRA and read absorbances with the [Eppendorf BioPhotometer](#) where the standards; signal was established to be strictly linear between 2 and 6 ug/ml. In this way, we have successfully utilized this reagent for yeast protein quantification after detergent solubilization in 0.1% SDS final. To obtain a concentration value, the sample absorbance must fall within the limits of the standard curve. If the unknown protein concentration used yields an A^{595} value higher than that observed with the highest concentration of the standard tested, you can either dilute the unknown protein or generate another standard curve using a higher concentration range of the standard. If the unknown samples are diluted, remember to adjust the final concentration of the unknown samples by multiplying by the dilution factor.

I have found that this product works very well and gives very reproducible results (CV \leq 3% using the Eppendorf BioPhotometer). It was especially nice with our yeast extracts. The price is reasonable compared to cost of the classic Bradford formulations. The ULTRA reagent when stored out of direct sun light at + 4°C is good for one year from date of receipt.

A BSA standard curve example for a 1 ml microassay procedure (absorbances were read with the Eppendorf BioPhotometer and a linear regression was applied to determine the slope and the intercept) is added as a figure to illustrate a typical result with this reagent.



The absorbance (or optical density) at 280 nm in a 1 cm pathlength microcuvette of a 10 mg/ml (= 1% w/v) solution of BSA should equal 6.61 (i.e., a 0.5 mg/ml solution will have an $A_{280} = 0.33$ and a 1 mg/ml, 0.66).

Sylvie Sinapah
Graduate Student
Département de Biologie Joliot Curie
Commissariat à l'énergie atomique
France