Technical Note

#9 Bradford Assay in Microvolume

Introduction

Rapid and accurate estimation of protein concentration is essential in laboratory practice. Depending on the protein sample and buffers utilized, the optimal spectrophotometrical method should be performed. Direct protein UV measurements at 280 nm require pure proteins solved in buffers without absorbance interference at 280 nm. If these conditions are unable to be met, it is recommended to use a protein assay: Bradford, Biuret, BCA or Lowry.

The optimal colorimetric assay should be chosen depending on the linear range of the assay and the interference of buffer components with the assay reagents. For more details please refer to the manufacturer's directions.

The focus of this technical note is the Bradford assay (Bradford 1976). The Bradford assay is a spectroscopic analytical procedure for rapid detection and quantification of total protein in a solution. The method relies upon the formation of protein-dye complexes. The dye, Coomassie Brilliant Blue, exists in three forms: cationic, neutral, and anionic (Compton and Jones 1985). In an acidic environment, the dye binds protein in solution, resulting in a change from its cationic (red) form to a protein bound anionic (blue) form. The anionic form has a maximum absorbance at 590 nm (Amax = 590 nm). The protein-dye complex can best be detected using a spectrophotometer at 595 nm. Accordingly, the quantity of protein can be estimated by detection of the amount of anionic dye formed (Kruger 2002).

The binding of an unknown protein can then be compared to a standard curve prepared from a set of known concentration protein standards at 595 nm. The assay is performed at room temperature (RT) and does not require special equipment.

Material

Coomassie Plus – The Better Bradford Assay Reagent (Thermo Scientific #23238) and Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set (Thermo Scientific #23208) were used for all Bradford measurements. Measurements were done in the Protein Assay method of a NanoPhotometer® NP80/N60/N50.

Assay Protocol

This protocol focuses on NanoVolume measurements. For Bradford assays in cuvette mode please refer to the manufacturer's directions.

The linear range for Bradford assays on the NanoPhotometer® NP80/N60 is 0–2000 µg/ml and for the NanoPhotometer® N50 0–1000 µg/ml BSA.

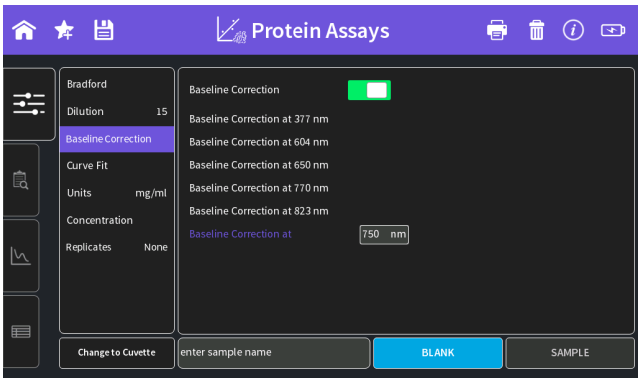
A 50:1 sample volume to assay reagent was used and incubated for 10 minutes at room temperature.

Preparation of standards and samples with unknown concentration:

1. Equilibrate the Bradford reagent, BSA standards and unknown samples to RT.
2. Add 250 µl of Bradford reagent in reaction tubes. Note: For each replicate prepare a separate tube. Note: it is recommended to add a zero reference to the standard curve. Prepare the zero standard with Bradford reagent and ddH_2O.
3. Add 5 µl of standard or unknown sample to each reaction tube.
4. Mix well/vortex the reaction tubes.
5. Incubate for 10 minutes at RT.

Parameter settings in Protein Assay method:

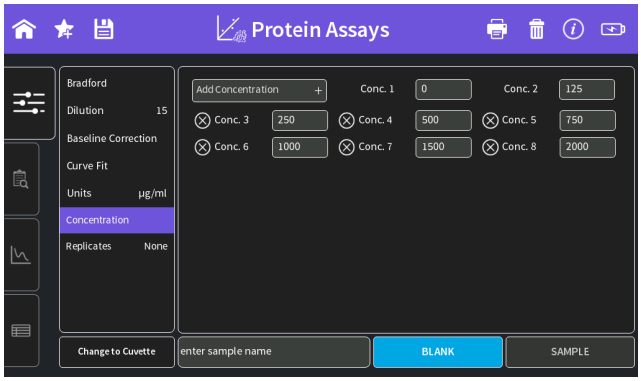
- **Bradford** (595 nm)
- **Dilution** 15/0.67 mm path
- **Baseline correction**:
  NanoPhotometer® NP80/N60 to 750 nm
  NanoPhotometer® N50 to 350 nm

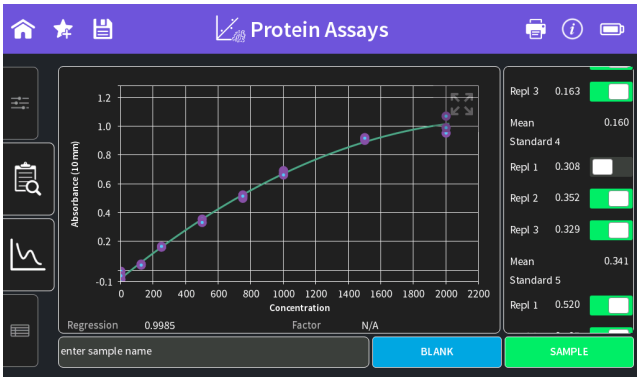


- **Curve Fit**: Regression 2nd order
- **Units**: Depends on the standards used
- **Concentration**: Enter the concentrations of the used standards. Concentrations can be added by clicking on the "Add Concentration" button. Pre-diluted BSA standards of Thermo Scientific: 0 µg/ml; 125 µg/ml; 250 µg/ml; 500 µg/ml; 750 µg/ml; 1000 µg/ml; 1500 µg/ml and 2000 µg/ml.

3. Once all standards are measured, the standard curve is shown and the Regression is calculated. It is possible to exclude single standard or replicate measurements from the standard curve calculation by switching the toggle switch off.

Please note that changes to the standard curve are only possible prior to measurement of the first sample (first unknown sample).





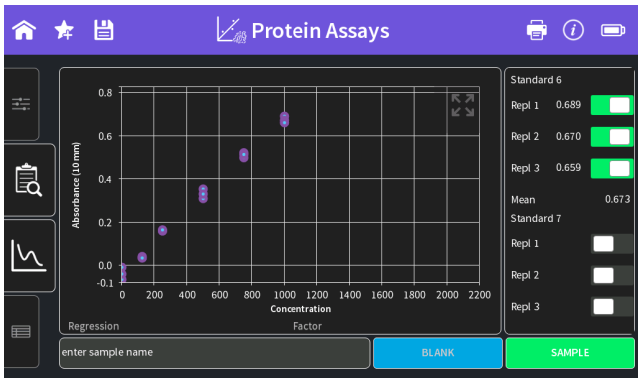
- **Replicates**: Three replicates recommended

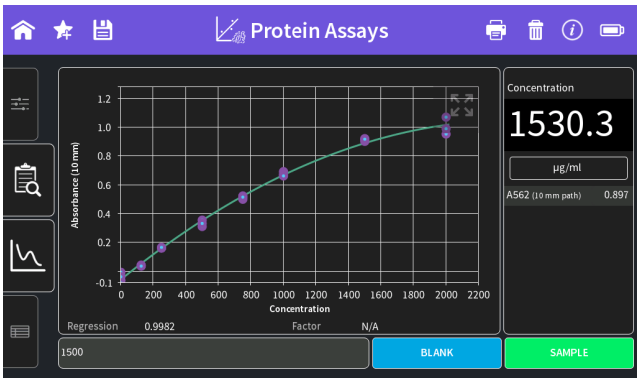
Create Standard Curve:

1. Use 2 µl of ddH_2O for the Blank measurement
2. Measure 2µl of each standard/standard replicate step by step

Measure unknown samples:

Use 2 µl sample volume for measurements of unknown samples. Concentration will be shown in the results and table view.





Cleaning:

Clean the measurement head and the mirror in the sample arm carefully after each blank/standard/sample measurement with a slightly wet fluff-free tissue.

Results

Typical standard curves for Bradford assays are shown in Figure 1 and 2.

Figure 1 shows a standard curve in the range of 0–2000 µg/ml BSA measured on the NanoPhotometer® N60 showing a coefficient of determination (R^2) of 0.9978.

Figure 2 shows a standard curve in the range of 0–1000 µg/ml BSA measured on the NanoPhotometer® N50 showing a coefficient of determination (R^2) of 0.9992.

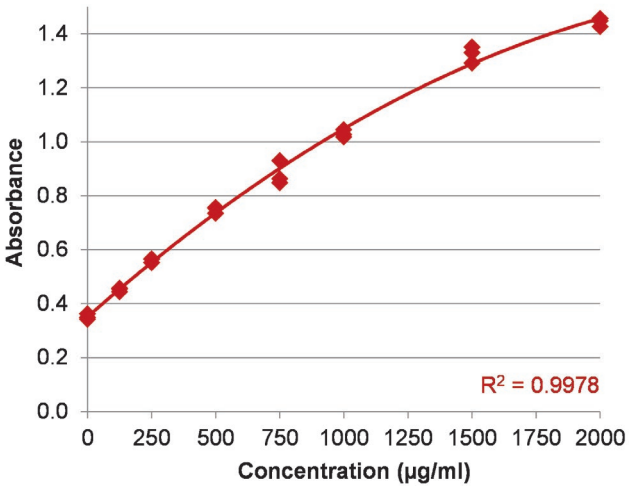


Figure 1: BSA standard curve in the range of 0–2000 µg/ml using a 50:1 sample to assay reagent ratio.

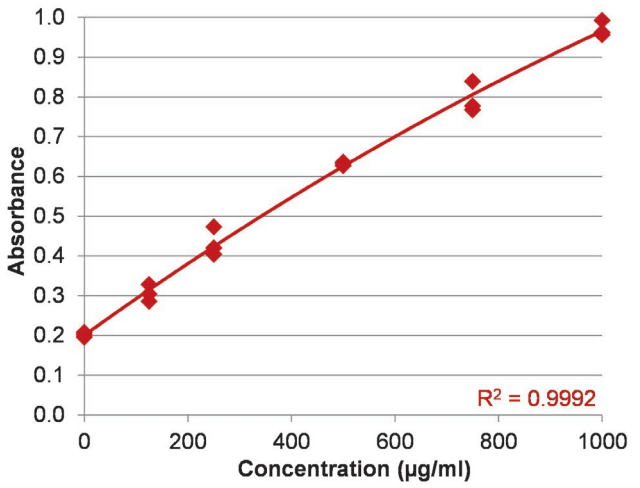


Figure 2: BSA standard curve in the range of 0–1000 µg/ml using a 50:1 sample to assay reagent ratio.

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72, 248–254.

Compton, S. J. and Jones, C. G. (1985). Mechanism of dye response and interference in the Bradford protein assay. Analyt. Biochem. 151, 369–374.

Kruger N.J. (2002). The Bradford Method for Protein Quantitation. In: Walker J.M. (eds) The Protein Protocols Handbook. Humana Press

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