Product Data Sheet

Bradford Assay Kit for PiCOEXPLORER™

Catalog Number: 0209

AthenaES®
a division of Athena Environmental Sciences, Inc.

Athena Enzyme Systems™
1450 South Rolling Road
Baltimore, MD 21227
USA

T (MD): 410-455-6319
T (USA): 888-892-8408
F: 410-455-1155
aesinfo@athenaes.com

Product Description

The Bradford Assay is used to quantify the amount of protein in aqueous buffer solutions. First described in 1976, the Bradford assay is one of the most widely used techniques for protein quantitation due to its simplicity and speed. The basis of the assay is the binding of Coomassie Blue dye. The initial binding of the non-polar portion of the dye is to hydrophobic pocket brings the negatively charged dye in close proximity to the positively charged amine groups of the polypeptide. When bound under acidic conditions, the dye is blue in color with a λmax of 595 nm. The amount of bound dye is directly proportional to the amount of protein present.

Protocol

Materials:

Bradford Dye Reagent - Cat. No. 0209R
BSA Calibrator, 1 mg/ml - Cat. No. 0209C
0.2 ml Microtubes - Cat. No. 0208
Unknown Samples
Dilution Buffer (1x PBS pH 7.3) - Cat. No. 0209B
PiCOEXPLORER Absorbance Reader - Cat. No. 0207

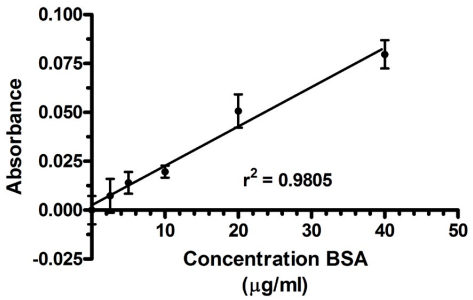
Methods:

1. Prepare a calibration curve using the 2 mg/ml BSA Calibrator.
   - 1.1 Dispense 50 µl Dilution Buffer into a duplicate set of five 0.2 ml microtubes (10 tubes total. Label duplicate tubes 20, 10, 5, 2.5 and 0 µg/ml.
   - 1.2 To duplicate tubes, labeled 40 µg/ml, dispense 96 µl Dilution Buffer. Add 4 µl 1 mg/ml BSA Calibrator to each of the two tubes and vortex.
   - 1.3 Serially dilute 50 µl from the 40 µg/ml tube to the 20 µg/ml tube and through to the 2.5 µg/ml tube. Mix well after each dilution. Dispose of the remaining 50 µl. Each tube should have 50 µl liquid.
2. Dilute the unknown samples in Dilution Buffer to give a protein concentration about 10 µg/ml. To demonstrate dilution linearity, prepared dilutions above and below the 10 µg/ml.
3. Dispense 50 µl of the diluted unknown samples into duplicate microtubes.
4. Add 150 µl of Bradford Dye Reagent to each tube, cap and mix well.
5. Incubate at room temperature for 5 min.
6. Record the absorbance of each tube using the PiCOEXPLORER.
   - 6.1 Record the absorbance of the calibrator curve tube in the Standard Curve function with the zero control in the first position. Select the desired calibration curve.
   - 6.2 Record the absorbance of the unknow samples in the Measure function.

Ordering Information

Cat. No.	Description	Unit Size
0209	Bradford Assay Kit	Kit
0209R	Bradford Dye Reagent	100 ml
0209B	Dilution Buffer	100 ml
0209C	BSA Calibrator	0.5 ml
0208	0.2 ml Microbubes	bag 1,000

Example Calibration Curve



References

Bradford, M.M. (1976), "Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Anal. Biochem., 72: 248–254, doi:10.1016/0003-2697(76)90527-3, PMID 942051

Zor, T.; Selinger, Z. (1996), "Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies", Anal. Biochem., 236: 302–308, doi:10.1006/abio.1996.0171, PMID 8660509

Noble, J.E.; Bailey, M.J.A. (2009), "Quantitation of Protein", Methods Enzymol., 463: 73–95, doi:10.1016/S0076-6879(09)63008-1

Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.