Protocol

Protein Lowry Assay

Introduction

The Bio-Rad® *DC* Protein Assay[1] is a colorimetric assay for protein concentration following detergent solubilization. The reaction is similar to that described in the "Lowry Assay: Protein by Folin Reaction" by Lowry et al in the *Journal of Biological Chemistry*, 1951.

The assay is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent in a two-step process leading to color development. The first step is the reaction between protein and copper in an alkaline medium and then the reduction of Folin reagent by the copper-treated protein. Color development is due primarily to the amino acids tyrosine and tryptophan, but also to a lesser extent by cystine, cysteine, and histidine. The reaction will have a blue color with maximum absorbance at 750 nm using Thermo Scientific™ NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer and the Protein Lowry Applications.

Dynamic Range

The assay has a range of 0.2 to 2.0 mg/mL.

A standard curve should be prepared each time the assay is performed. We recommend choosing a second order polynomial curve type for the standard curve.

Supplies, Materials & Reagents

- NanoDrop One/One^{C} Spectrophotometer
- 0.5 – 2 µL pipettor tips, low retention tips recommended
- 50 µL pipettor tips, low retention tips recommended
- 400 µL pipettor tips, low retention tips recommended
- Low lint laboratory wipes
- 0.6 mL micro-centrifuge tubes (Fisherbrand™ catalog number 05-408-120)
- *DC* Protein Assay (Bio-Rad product number 500-0116)
- Pierce pre-diluted BSA standards (P/N 23208) or other commercially prepared protein standards
- PR-1 Kit
- PBS (Thermo Fisher P/N 28372)

Assay Recommendations

- Measure 2 µL sample aliquots.
- Making standard and sample measurements in triplicate is good practice.
- Re-condition pedestals with PR-1 upon completion of assay.

Sample Preparation

The sample preparation outlined below is for pedestal measurements on the NanoDrop One/One^{C} Spectrophotometer. Follow the manufacturer's protocol for a standard assay when making measurements in a cuvette.

1. Equilibrate all reagents, unknowns and protein standards to room temperature. Mix thoroughly but gently to avoid introducing micro bubbles.

2. If unknown samples contain detergent, prepare a working Reagent A, by adding 20 µL of Reagent S to each mL of Reagent A that will be required for the run. Label this as "Working Reagent A" with an expiration date of one week post preparation. If unknown samples do not contain detergent, omit this step and use Reagent A as supplied in the kit.

3. Prepare standards to cover the range of the assay (0.2 to 2.0 mg/mL). A standard curve should be prepared each time unknown samples will be tested. If not using pre-diluted standards, for best results, the standards should be diluted in the same buffer as the unknown samples.

4. Label all tubes and pipet 10 µL of standards and unknown samples into appropriately labeled micro-centrifuge tubes. Pipet 10 µL of PBS into the reference or zero standard.

5. Add 50 µL of either Working Reagent A or Reagent A supplied in the kit (see step #2) to each tube. Mix well.

6. Add 400 µL of Reagent B to each tube. Mix well, but not so vigorously as to introduce micro-bubbles into the tubes.

7. Incubate all tubes at room temperature for 15 minutes.

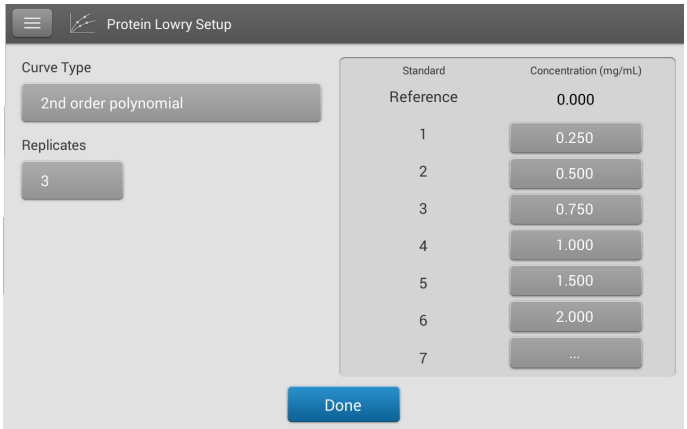
Protocol

1. From the NanoDrop One/One^{C} *New Experiment* screen, tap the **Proteins** tab and then tap the "Protein Lowry" icon to open the application.

2. Enter the mg/mL concentration values for each standard in the table on the right in the image below.

3. Enter the number of replicates for each standard. We recommend three replicates per standard, including the reference standard.

4. Select the curve type. We recommend a second order polynomial curve type for Protein Lowry Protein Assay.



5. Press *Done* when complete.

6. At the prompt, clean both pedestals and make a blank measurement using water as the blanking solution.

7. Follow the on-screen prompts to measure each replicate of each standard, taking care to wipe the upper and lower pedestal between each measurement. Each replicate measurement of the reference or standards should be made using a fresh aliquot on the pedestal.

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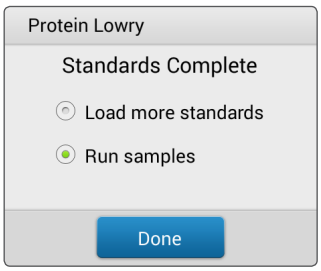
8. You will notice in the upper right corner of the screen the note in red: **Invalid Curve**. When enough data points have been measured to plot the curve type selected, this now will change from red to blue: **Valid Curve**. For a second order polynomial curve, at least three standards, including the reference must be measured before a curve can be constructed. This indication does not address the quality of the curve, it is a notification that enough standards have been measured to construct the curve type selected.

9. When the last replicate of the last standard has been read the following message will appear:



10. At this point, you may choose to run more standards or begin running samples. Please note that once samples are measured, you will not be able to make changes to the standard curve by measuring additional standards.

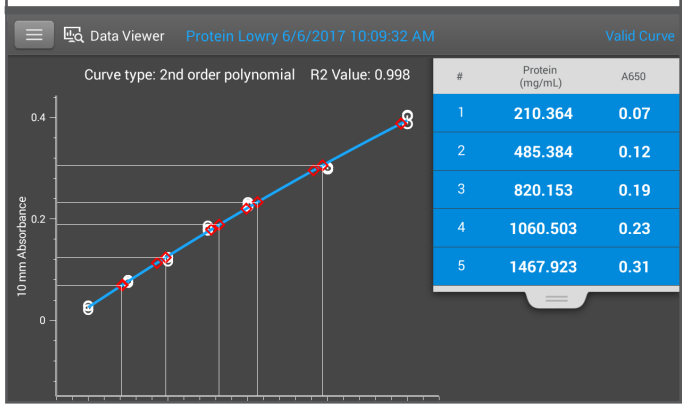
11. It is not necessary to blank the instrument between measurement of the standards and the unknown samples.

12. After each measurement, clean the upper and lower pedestals with a clean, dry laboratory wipe.

13. Upon completion of all measurements, we recommend cleaning and reconditioning the measurement pedestals using PR-1.

Standard Curve Data

BSA (µg/mL)	A(595) (n=3)	Std. dev.	%CV
0	0.44	0.003	NA
125	0.593	0.028	4.7
250	0.666	0.007	1.1
500	1.037	0.013	1.2
750	1.289	0.018	1.4
1000	1.445	0.015	1.0
1500	1.683	0.017	1.0
2000	1.783	0.012	0.7



The above data is typical of absorbance values and standard curve using the *DC* Protein Assay.

For additional information regarding the Lowry assay and reagents, please refer to the manufacturer's product literature supplied with the *DC* Protein Assay.

References

1. *DC* Protein Assay Instruction Manual, LIT449 Rev. D Bio-Rad Laboratories.

In the United States:
For customer service, call 1-800-766-7000
To fax an order, use 1-800-926-1166
To order online: thermofisher.com

In Canada:
For customer service, call 1-800-234-7437
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