

STANDARD OPERATING PROCEDURE

Title: Protein concentration measurements using Bio-Rad protein assay kit

SOP#: M-101

Version #: 1

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1. PURPOSE

This procedure is to be used for the determination of the protein concentration.

2. SCOPE

This document describes the procedure for measuring protein concentration of a sample.

3. RESPONSIBILITIES

It is the responsibility of the person(s) performing this test to be familiar with lab safety procedures and to have basic laboratory skills. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- Perkin Elmer Lambda20 spectrophotometer

5. MATERIALS

- Polystyrene semi-microcuvettes; Sarstedt Cat. # 67.742
- Parafilm

6. REAGENTS

- Protein to be tested
- BioRad Protein Assay concentrated dye reagent; BioRad Cat. #500-0006
- Bovine Serum Albumin (BSA) protein standard; Bio-Rad Cat. #500-0007

7. PROCEDURE

7.1. Preparation of BSA standard curve

- 7.1.1. Take vial of lyophilized BSA standard from BioRad and reconstitute using Milli-Q water to a final concentration of 8mg/ml.
- 7.1.2. This material is stored in aliquots of 100 μ l at -20° C.
- 7.1.3. Take 37.5 μ l of the 8mg/ml stock of BSA standard and add 12.5 μ l of milli-Q water for a final volume of 50 μ l and a concentration of 6mg/ml.
- 7.1.4. Take 25 μ l of the 8mg/ml stock of BSA standard and add 25 μ l of milli-Q water for a final volume of 50 μ l and a concentration of 4mg/ml.
- 7.1.5. Take 12.5 μ l of the 8mg/ml stock of BSA standard and add 37.5 μ l of milli-Q water for a final volume of 50 μ l and a concentration of 2mg/ml.

7.2. Dilution of unknown protein and BSA standards

- 7.2.1. Add 800 μ l of milli-Q water into 1ml semi-microcuvettes. Enough cuvettes are needed so that each standard is assayed in duplicate and each unknown is assayed in triplicate.
- 7.2.2. Add 1 μ l of milli-Q water to a cuvette containing 800 μ l of milli-Q water. This will be used as the blank.
- 7.2.3. Add 1 μ l of each BSA standard into a separate cuvette. Test each concentration in duplicate.
- 7.2.4. Add 1 μ l of each unknown protein into a separate cuvette. Test each unknown protein in triplicate.
- 7.2.5. Then add 200 μ l of undiluted Bio-Rad Protein Assay concentrated dye reagent to each cuvette.
- 7.2.6. Place a piece of parafilm over the top of the cuvette and mix by inverting.
- 7.2.7. Incubate at room temperature for at least 10 minutes and no more than 30 minutes.

7.3. Measurement of absorbance at 595nm

- 7.3.1. The Perkin Elmer Lambda20 spectrophotometer should be turned on 30 minutes before taking measurements to ensure the lamp is stable.

- 7.3.2. Using the UV WinLab software open the concentration application.
- 7.3.3. Set the wavelength to 595nm.
- 7.3.4. Enter the sample descriptors in the menu.
- 7.3.5. Place the blank cuvette in the sample position and blank the instrument.
- 7.3.6. Once the instrument is blanked, the absorbance at 595nm for each standard or unknown can be determined.
- 7.3.7. Print or record the absorbance values measured.

7.4. Determination of the protein concentration

- 7.4.1. Enter the data into an excel spreadsheet.
- 7.4.2. Calculate the mean of the standards and plot a calibration curve.
- 7.4.3. The standard curve should be linear between 1 - 8mg/ml BSA. Any curve with a fit R^2 of less than 0.9 should be rejected and repeated.
- 7.4.4. Calculate the mean of all the unknown samples.
- 7.4.5. Using the trendline function in excel select a linear regression analysis.
- 7.4.6. Using the values for the gradient and intercept on the y axis calculate the protein concentration in the unknown samples
- 7.4.7. If the samples fall below 1mg/ml or above 8mg/ml the protein determination should be repeated using more or less of the sample as required.

8. REFERENCED DOCUMENTS

- 8.1. Biorad Protein Assay instructions for use.
- 8.2. Perkin Elmer Lambda 20 spectrophotometer operator's manual.
- 8.3. Bradford, MM "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Analytical Biochem.* **72**: 248-254. 1976
- 8.4. Stoscheck, CM "Quantitation of Protein." *Methods in Enzymol.* **182**: 50-69. 1990
- 8.5. Microsoft Excel instructions for use.