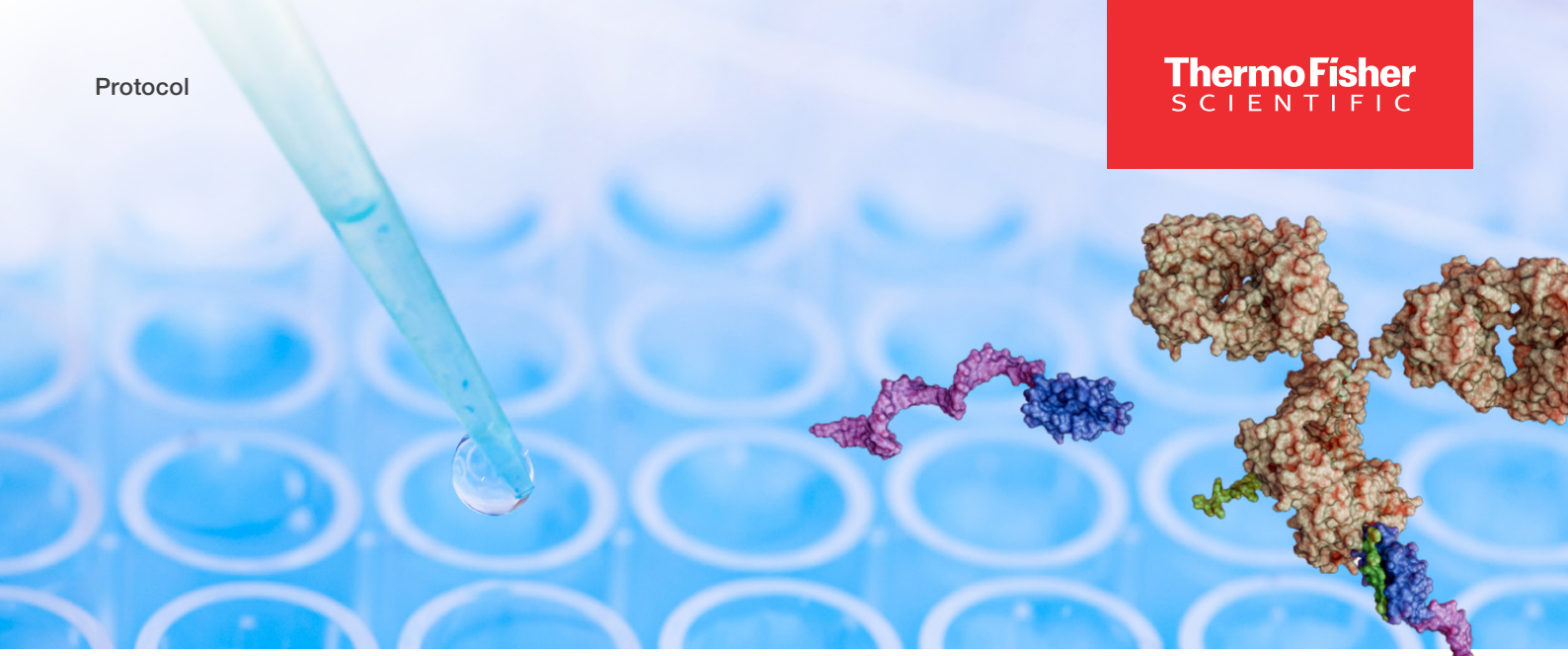
Protocol



NanoDrop Ultra Spectrophotometers and Fluorometers

Lowry Protein Assay

Introduction

The Thermo Scientific™ Pierce™ Modified Lowry Protein Assay is a colorimetric assay which uses a stable form of a traditional, two-component, Folin phenol- and copper-based reagent system for protein concentration. 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, and it can be measured from 650–750 nm using any of the Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometers and the Protein Lowry Applications.

Note: All specifications and protocol instructions outlined below are for pedestal measurements on the Thermo Scientific™ NanoDrop™ Ultra/Ultra^{C}/Ultra FL/Ultra^{C} FL Spectrophotometers and Fluorometers. Follow the manufacturer's protocol for a standard assay when making measurements in a cuvette.

Dynamic Range

The assay has a range of 0.2 to 1.5 mg/mL.

Supplies

Equipment:

- NanoDrop Ultra/Ultra^{C}/Ultra FL/Ultra^{C} FL Spectrophotometer
- 0.5-2 µL pipettor (low retention tips)
- 10-1,000 µL pipettor (low retention tips)

Materials:

- Low lint laboratory wipes
- 0.6 mL microcentrifuge tubes (Fisherbrand™ catalog number 05-408-120)

Recommended Reagents:

- Pierce Modified Lowry Protein Assay Kit, #23240
- Pierce pre-diluted BSA standards, #23208 (or other commercially prepared protein standards)
- NanoDrop PR-1 Reconditioning kit, #CHEM-PR1-KIT

Assay Recommendations

- Measure 2 µL sample aliquots.
- It is recommended that a new standard curve be generated for each assay.
- Making standard and sample measurements in triplicate is good practice.
- Re-condition pedestals with PR-1 upon assay completion.

Sample Preparation

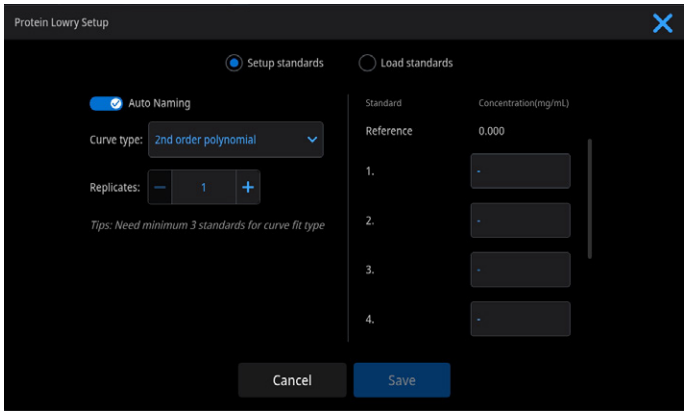
1. Equilibrate all reagents, unknowns and protein standards to room temperature. Mix thoroughly but gently to avoid micro bubbles.
2. Prepare enough 1X (1N) Folin-Ciocalteu Reagent by diluting the supplied 2X (2N) reagent 1:1 with ultrapure water. Each test replicate requires 20 µL of 1X Folin-Ciocalteu Reagent.

   Note: 1X Folin-Ciocalteu Reagent is unstable and should be prepared on the same day of use.
3. Prepare standards to cover the range of the assay (0.2 to 1.5 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 40 µL of standards and unknown samples into appropriately labeled micro-centrifuge tubes.
5. Add 200 µL of Modified Lowry Reagent to each of the standard and unknown sample tubes and mix well via gentle vortexing. If necessary, collect the solution at the bottom of the tube by a brief centrifugation.
6. Incubate the standard and sample tubes at room temperature (RT) for exactly 10 minutes.
7. Add 20 µL of 1X Folin-Ciocalteu Reagent to each tube. Mix well by gentle vortexing. If necessary, collect the solution at the bottom of the tube by a brief centrifugation.
8. Incubate the standard and sample tubes at room temperature (RT) for 30 minutes.

Protocol

1. From the home screen, select the **Proteins** tab, then select **Protein Lowry**.
2. Within the left panel of the screen, enable or disable Auto Naming (depending on your preference) using the provided toggle; select the Curve Type from the drop-down menu; and indicate the number of Replicates to measure using the -/+ buttons. We recommend selecting the **2nd Order Polynomial** curve type and measuring 3 replicates of each standard.

   Note: The curve type cannot be changed after the assay is in progress.
3. Enter the values for each standard concentration in the table within the right pane. The software allows for the reference and up to 7 additional standards. The zero reference and standards can be measured with up to 3 replicates.



   Note: The minimum requirement for standard curve generation is the measurement of the zero reference and at least one standard. The 2nd order polynomial curve type requires a minimum of 3 standards. It is recommended that additional standards be included as necessary to cover the expected assay concentration range.
4. After all standard concentrations have been added and settings have been updated, select **Save**.

   Note: Use the **Load standards** option at the top to load a previously run standard curve by selecting the curve and selecting **Save**. Only standard curves that were previously generated on the instrument will appear.
5. If using a NanoDrop Ultra^{C} or NanoDrop Ultra^{C} FL model, ensure that **Pedestal** is selected as the measurement pathway at the top of the screen.
6. Pipette 2 µL of DI H_2O onto the lower pedestal, lower the arm, and then select **Blank**.

   Note: It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.
7. Lift the arm and clean both pedestals with a fresh laboratory wipe.

8. Follow the direction at the top of the screen to measure the reference and standards. After each measurement, wipe the upper and lower pedestals using a dry laboratory wipe.

   Note: The arm must be down for all measurements.

9. After all standard measurements have been made, a pop-up box will indicate Standards Completed.

   – Select **Load More Standards** to add additional standards to measure.

     From the Protein Lowry Setup window, enter any additional standards and then select **Save**. You will then be prompted to measure the additional standards.

   – Select **Remeasure Standards** to remeasure a standard.

     From the measurement screen, open the Sample Details box for the standard you would like to remeasure. This can be done by pressing and holding the row of the desired standard; select **Remeasure**. Confirm by selecting **Yes** and you will then be prompted to remeasure the standard.

   – Select **Measure Samples** to continue with sample measurement.

     Note: Once a sample is measured, the standards can no longer be remeasured.

10. After selecting **Measure Samples**, enter a sample ID at the top of the screen. Load 2 µL of sample on the lower pedestal. Select **Measure**.

    It is not necessary to blank the instrument between the standard and the unknown sample measurements.

    Note: A fresh aliquot of sample should be used for each measurement.

11. After completing all Standard and Samples measurements it is good practice to re-condition the pedestals using PR-1.

Cleaning the instrument after a measurement

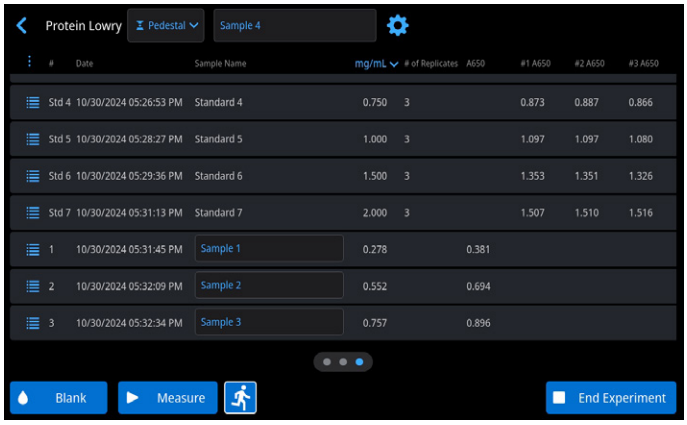
– Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

After the measurements:

1. Select each sample to display multiple spectra at a time.

2. Swipe the screen to the left to view the curve.

3. Swipe the screen again to the left to view your measurement results.



Standard Curve Data

BSA (µg/mL)	A650 (n = 3)	Std. dev.	%CV
0	-0.155	0.221	NA
125	0.192	0.012	6.0
250	0.369	0.004	1.0
500	0.683	0.006	0.8
750	0.875	0.011	1.2
1000	1.091	0.010	0.9
1500	1.343	0.015	1.1
2000	1.511	0.005	0.3

Table 1. Typical absorbance values and standard curve using the Pierce Modified Lowry Protein Assay.

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