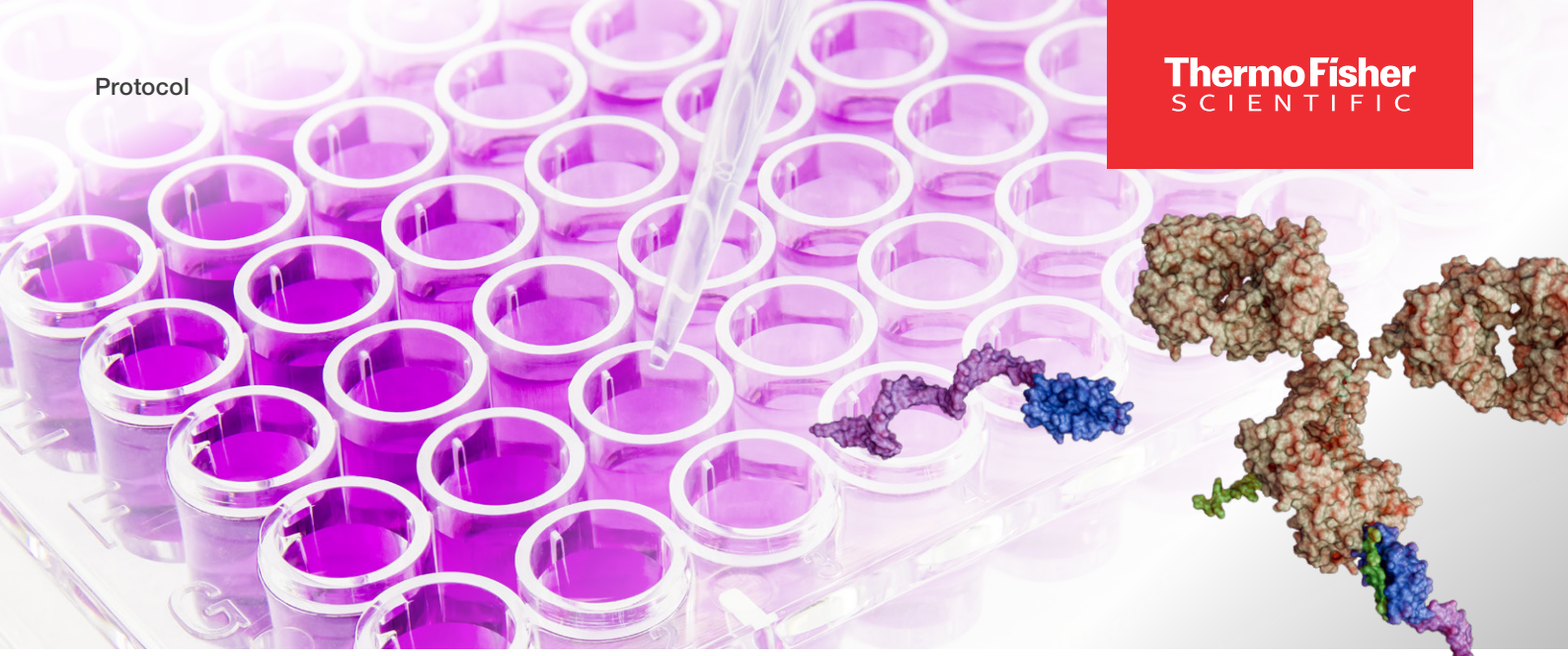
Protocol



NanoDrop Ultra Spectrophotometers and Fluorometers

BCA Protein Assay

Introduction

The Thermo Scientific™ Pierce™ BCA™ Protein Assay combines the protein-induced biuret reaction with the highly sensitive and selective colorimetric detection of the resulting cuprous cation (Cu^{1+}) by bicinchoninic acid (BCA). A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion. The BCA/copper complex is water-soluble and exhibits a linear absorbance at 562 nm over a broad range of protein concentrations. In conjunction with the micro-volume capability of a Thermo Scientific™ NanoDrop™ Spectrophotometer, the assay provides an accurate means of protein quantitation with minimal consumption of sample.

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 micro-assay has a linear range of 20–200 µg/mL using a 1:1 sample to reagent ratio. A higher range of 125–2000 µg/mL may be obtained using a 1:20 sample to reagent ratio.

Supplies

Equipment:

- NanoDrop Ultra/Ultra^{C}/Ultra FL/Ultra^{C} FL Spectrophotometer
- 1-10 µL pipettor (low retention tips)
- 100-1000 µL pipettor (low retention tips)

Materials:

- Low lint laboratory wipes
- 0.5 mL microcentrifuge tubes or 0.2 mL mini-centrifuge strip tubes and caps

Recommended Reagents:

- Pierce BCA reagent, #23225, 23227, 23250
- Pierce pre-diluted BSA standards, #23208 (optional) or other protein standard
- 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.

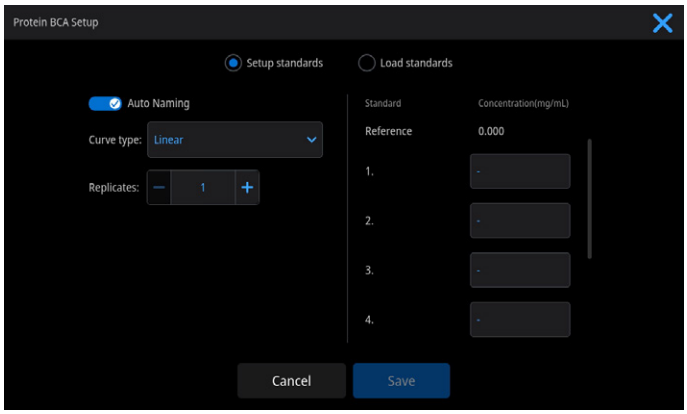
Sample Preparation

1. Equilibrate all reagents, unknowns and protein standards to room temperature. Mix thoroughly but gently to avoid micro bubbles.
2. Prepare enough fresh working reagent for all standards and samples to be measured using a 50:1 ratio of the kit reagents A:B.
3. Add the appropriate reagent volume to each microcentrifuge tube or mini-centrifuge strip well.
   - **Micro-assay (1:1 sample to working reagent ratio):** Add 10 µL of working reagent to each of the standards and sample tubes.
   - **High range assay (1:20 sample to working reagent ratio):** Add 200 µL of working reagent to each of the standard and sample tubes.
4. Add 10 µL of standards or samples to the appropriate tube. Mix well by gentle vortexing. If necessary, collect the solution at the bottom of the tube by a brief centrifugation.
5. It is advisable to use the dye reagent and protein buffer ("0" reference) without any protein added as the zero-reference sample for this assay.
6. Incubate the standard and sample tubes at either 37° C for 30 minutes, then cool to room temperature.

Protocol

1. From the home screen, select the **Proteins** tab, then select **Protein BCA**.
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. The Pierce protocol recommends using a **Linear** curve.

   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 two standards or the measurement of the zero reference and at least one standard. 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 diH_2O onto 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 BCA 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

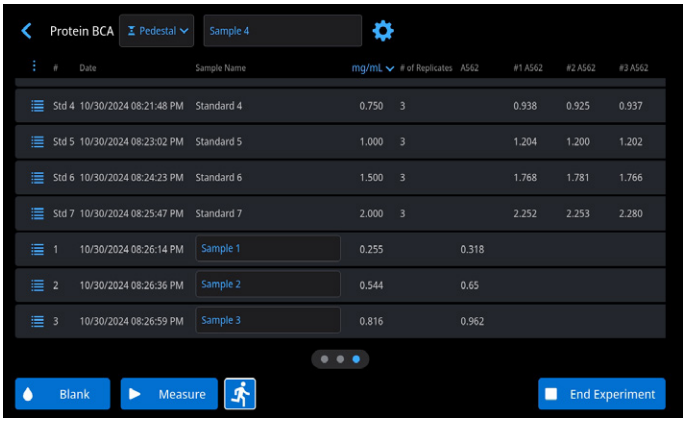
– Simply wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

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)	A562 (n = 3)	St dev	%CV
0	-0.145	0.204	NA
125	0.140	0.008	5.6
250	0.307	0.014	4.5
500	0.644	0.004	0.6
750	0.933	0.007	0.8
1000	1.202	0.002	0.2
1500	1.772	0.008	0.5
2000	2.262	0.016	0.7

Table 1. Typical absorbance values for a High Range assay using 1:20 sample to reagent ratio assay using the Pierce BCA reagent.

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

Learn more at **thermofisher.com/nanodrop**
or email us at **nanodrop@thermofisher.com**