BCA Protein Assay

Introduction

The BCA Protein Assay combines the protein-induced biuret reaction with the highly sensitive and selective colorimetric detection of the resulting cuprous cation ($Cu1^+$) 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 the increase in absorbance is linear across a wide protein concentration range. 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 presented below are specific for the pedestal mode of the NanoDrop One/One^C instruments. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the NanoDrop One^C.

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 One/One^C Spectrophotometer
- 0.5 - 2 µL pipettor (and low retention tips) and 10 – 1000 µL pipettors

Materials:

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

Reagents:

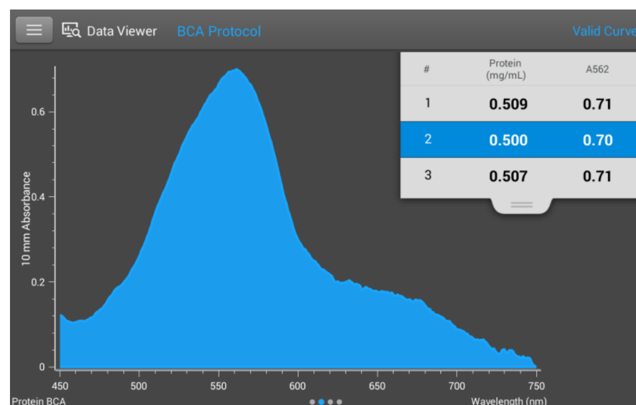
- BCA reagent, Pierce Product # 23225, 23227, 23250
- Pierce pre-diluted BSA standards Pierce Product #23208 (Optional) (or other protein standard)
- PR-1 Reconditioning kit Part #CHEM-PR1-KIT

Assay Recommendations

- Measure 2 µL sample aliquots
- All standards and samples should be measured within 10 minutes
- 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 introducing 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 PCR strip well.
   - **Micro-assay (1:1 sample to working reagent ratio):** Add 10 µL of working reagent to each standard and sample tube/well.
   - **High range assay (1:20 sample to working reagent ratio):** Add 200 µL of working reagent to each standard and sample tube/well.
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. Incubate the standard and sample tubes at either 37º C for 30 minutes or 60º C for ~ 5 minutes. Cool the tubes to room temperature.



Typical absorbance spectrum for a BCA protein assay sample measurement.

Once printed this document is no longer controlled

Protocol

1. Select the **Protein** tab from the Home screen. Tap the **Protein BCA** application button.
2. Enter the values for each standard concentration in the table on the right. The software allows for the reference and up to 7 additional standards. The zero reference and/or 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.
3. On the left side of the screen, select the Curve Type and number of replicates to measure. We recommend selecting the **Linear** curve type and 3 replicates. Tap **Done**.

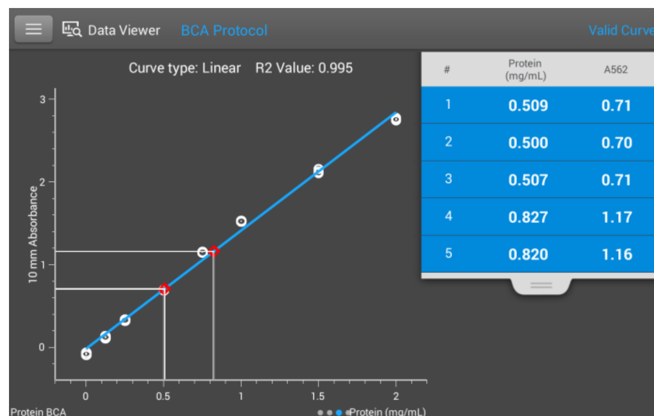
   Note: If the instrument self-test begins, do not touch the instrument.
4. Establish a blank using diH_2O. It is advisable to use the dye reagent and protein buffer without any protein added as the zero reference sample for this assay.
   - Pedestal Option: Pipette 2 µL of blank solution onto the bottom pedestal, lower the arm and tap **Blank**.
   - Cuvette Option (Model One[C] only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

   Note: The arm must be down for all measurements, except those made with cuvettes. 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.
5. 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. After your last standard measurement, you can choose to load more standards or run samples.
6. After all of the Standards have been measured, click on the **Run samples** radio button. Enter a sample ID and load a 2 µL aliquot of sample when using the pedestal. Tap **Measure**.
7. After completing all Standard and Samples measurements it is good practice to re-condition the pedestals using PR-1.
8. 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.

Standard Curve Data

BSA (µg/mL)	A562 (n=3)	Std. dev.	%CV
0	-0.077	0.016	NA
125	0.120	0.013	NA
250	0.327	0.010	NA
500	0.691	0.002	0.3
750	1.148	0.005	0.4
1000	1.524	0.008	0.5
1500	2.130	0.025	1.2
2000	2.759	0.013	0.5



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: www.thermoscientific.com/pierce