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 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 presented below are specific the pedestal mode for NanoDrop™ 2000/2000c instruments. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the NanoDrop 2000c.

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 2000/2000c Spectrophotometer
- 0.5-2 µl pipettor (low retention tips)

Materials:

- Low lint laboratory wipes
- 0.5 ml Eppendorf 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
- It is recommended that a new standard curve be generated for each assay
- 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.

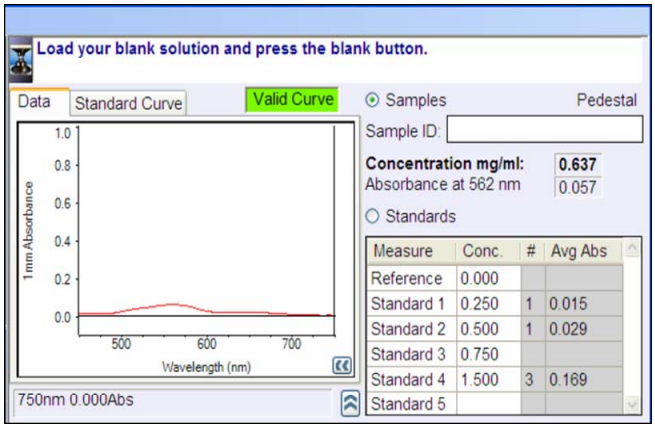
3. Add the appropriate reagent volume to each tube of a PCR strip or each well of a PCR plate.

   - **Micro-assay (1:1 sample to working reagent ratio):** Add 10 µl of working reagent to each standards and sample tube/well.

   - **High range assay (1:20 sample to working reagent ratio):** Add 200 µl of working reagent to each standards and sample tube/well.

4. Add 10µl 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 37°C for 30 minutes or 60°C for ~ 5 minutes, then cool to room temperature.



Typical absorbance spectrum for a BCA protein assay sample measurement.

Protocol

1. Select the **Protein BCA** application from the Home page. If the wavelength verification window appears, ensure the arm is down and click **OK.**

2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The Reference and/or standards can be measured in 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. In the left window pane select **Add to report** to automatically include all measurements in the current report. The default setting is for all samples to be added to reports. The **Add to report** checkbox must be selected prior to a measurement to save the sample data to a workbook.

4. Select the file drop-down option **Use current settings as default** as a convenient way to limit set-up time for each new workbook.

5. Select **Overlay spectra** to display multiple spectra at a time.

6. Establish a blank using dH_2O. 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.

   – Pedestal Option: Pipette 2 µL of blank solution onto the bottom pedestal, lower the arm and click **Blank**.

   – Cuvette Option (Model 2000c 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, including 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.

7. Under the Standards tab, highlight a standard and load as described for the blank above. Click **Measure**. Measure all standards prior to measuring samples.

8. After all of the Standards have been measured, click on the **Samples** radio button. Enter a sample ID. Load 2 µL of sample when using the pedestal. Click **Measure**.

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

10. 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.

After the measurement:

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

– Cuvette Option: Remove the cuvette, rinse thoroughly and dry between samples.

Standard Curve Data

BSA (µg/mL)	A562 (n=5)	St dev	%CV
0	-.009	.001	NA
125	.010	.001	NA
250	.026	.001	NA
500	.057	.001	1.5
750	.084	.001	1.4
1000	.113	.002	1.5
1500	.166	.001	.5
2000	.213	.001	.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, please refer to the Pierce Website (http:www.piercenet.com/).