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 microvolume capability of a Thermo Scientific™ NanoDrop™ Spectrophotometer, the assay provides an accurate means of protein quantitation with minimal consumption of sample.

Dynamic range

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

Supplies

Equipment:

- NanoDrop 8000 Spectrophotometer
- Low volume 8-channel pipettor for loading samples onto measurement pedestals (low retention tips)
- 37O °C water bath or heat block
- Vortex mixer

Materials:

- Low lint laboratory wipes
- 0.5 ml Eppendorf tubes (for stock reagent)
- 0.2 ml mini-centrifuge strip tubes and caps or 96 well PCR plate (for standards and sample reactions)

Recommended Reagents:

- BCA reagent, Thermo Scientific™ Pierce™ product numbers 23225, 23227, 23250
- Pierce pre-diluted BSA standards Pierce product number 23208 (optional) or other protein standard
- PR-1 Reconditioning Kit, part number CHEM-PR1-KIT

Assay recommendations

- Measure 2 µL standard and sample aliquots.
- Making triplicate measurements for both standards and samples is good practice. Use fresh aliquots for all replicate measurements.
- Use an 8-channel pipettor to simultaneously load all 8 measurement positions. **Note: The use of a single channel pipettor to load multiple positions may result in erroneous results**.

Sample ID entry

The NanoDrop 8000 Spectrophotometer offers several options for entering sample IDs. When making only a few measurements, it is easy to type in sample names prior to measurement or use the Manual Plate Setup. When measuring several samples, the user may load a list of predefined sample IDs. The lists may be created in Excel or Notepad but lists must be saved as a .txt file. It is recommended that a list file be generated prior to starting the assay if many samples are to be measured.

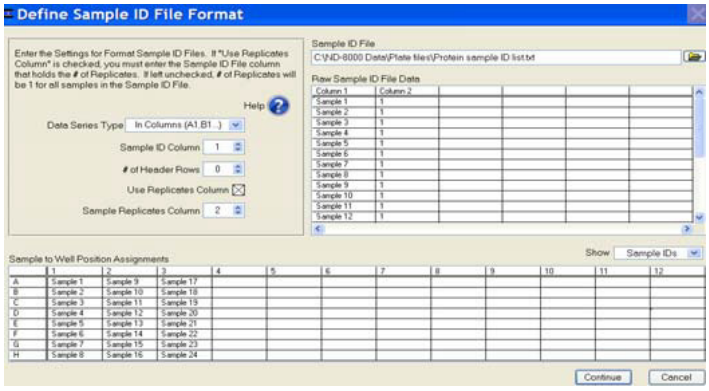

Figure 1. The NanoDrop 8000 software allows the use of predefined sample ID lists

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BCA Protein Assay sample preparation

1. Equilibrate all reagents and samples to room temperature, then mix each 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:** Add 10 µL of working reagent to each standards and sample tube/well.

   – **High range assay:** Add 200 µL of working reagent to each standards 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, then cool to room temperature.

Protocol

1. Clean pedestals by pipetting 2 µL of dH_2O onto each all 8 lower pedestals, and lowering the arm so that the water makes contact with both the upper and lower measurement surfaces. Raise the arm and wipe all pedestals with a dry laboratory wipe.

2. Launch the NanoDrop 8000 software and open the **Protein BCA** module.

3. Initialize the instrument by loading 2 µL of dH_2O to all 8 lower pedestals, lower the arm and click **OK**. When initialization is complete, use a dry laboratory wipe to wipe the water from all measurement surfaces.

4. From the **Standards Choose Source** window select the standard curve source. It is recommended that new standard absorbance values be measured each time the assay is run. Manually enter standard concentration values or enter a concentration series using a previously stored standard curve. If using a preloaded standard curve, continue to step 7.

Measurements Table — Double Click on any row to change the concentration or delete replicates.

	Standard	mg/ml	Ave Abs.	Abs. 1	Abs. 2	Abs. 3	Abs. 4	Abs. 5
Active	A - Reference	0.000						
Active	B - Standard 1	125.0						
Active	C - Standard 2	250.0						
Active	D - Standard 3	500.0						
Active	E - Standard 4	750.0						
Active	F - Standard 5	1000						
Active	G - Standard 6	1500						
Active	H - Standard 7	2000						

Figure 2. The NanoDrop 8000 software allows the use of a predefined standard curve or the user may build a new curve

5. Use an 8-channel pipettor to transfer 2 µL of dH_20 onto each of the 8 lower pedestals. Lower the arm and click **Blank**. When the measurement is complete wipe the pedestals with a lab wipe.

6. Gently mix the standards, then use a 8-channel pipettor to simultaneously load the reference (reagent and buffer, no protein) and standards to generate a new standard curve. Use fresh 2 µL aliquots to measure additional replicates.

7. Select the sample ID loading mode when prompted. Refer to page 1 for additional details.

8. Gently mix the samples and use an 8-channel pipettor to simultaneously load multiple pedestal positions. Use fresh 2 µL aliquots for each replicate.

9. After completing all the measurements, recondition the pedestals with PR-1.

Performance data

BSA (µg/mL)	A562 (n=5)	St Dev	%CV
0	.001	.002	NA
125	.011	.003	NA
250	.03	.001	3.28
500	.062	.002	3.13
750	.092	.002	2.14
1000	.119	.002	1.35
1500	.169	.003	1.67
2000	.218	.002	0.89

Table 1. Typical performance data for the high range BCA protein assay using a BSA Standard Curve

Typical BCA sample spectra

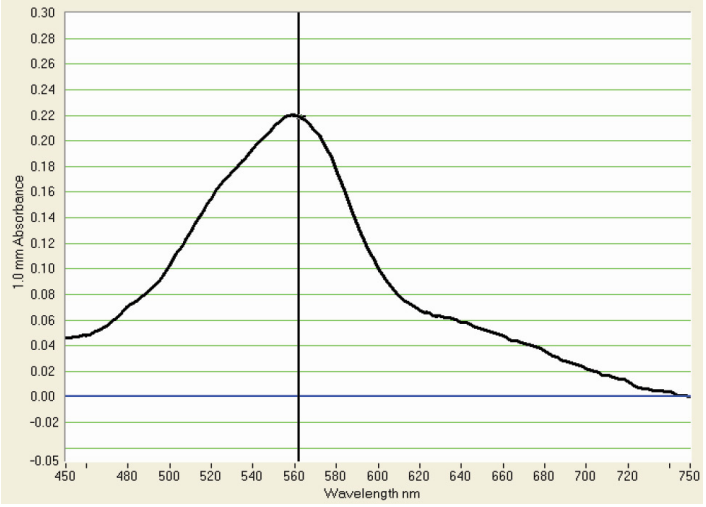

Figure 3. Example spectrum of BCA reagent-Protein sample

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