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Title: Pierce™ BCA Protein Assay	**Ref.:** BCA_002	**Version:** 2

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** See both Important information and Hazards sections at the end of the protocol.*

1. PURPOSE

The Pierce BCA (bicinchoninic acid) protein assay allows colorimetric detection and quantitation of total protein in a sample. It combines the well-known reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium (biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+}) by BCA.

2. REFERENCE TO DOCUMENTS

- Pierce BCA Protein Assay Kit User Guide (Doc. Part N° 2161296, Pub. N° MAN0011430, Rev. B.0): https://www.thermofisher.com/order/catalog/product/23227#/23227
- Smith, P.K. *et al.* Measurement of protein using bicinchoninic acid. *Anal Biochem* 150 (1985), 76-85. https://doi.org/10.1016/0003-2697(85)90442-7
- "Tech Tip # 8 – Eliminating interfering substances from samples for BCA protein assays". ThermoScientific: https://www.thermofisher.com/order/catalog/product/23227#/23227

3. MATERIAIS

Caution: see Section 5 for appropriate handling of materials marked with <!>.

Equipment and reagents

- Plate reader (BioTek Instruments)
- Centrifuge (ThermoScientific Heraeus Megafuge 16R)
- Laboratory drying oven
- Sample/plate mixer
- Multichannel pipette (8 channels; 200 µL)
- Pipette tips (200 µL)
- 96-well plate (Corning, cat# 3359)
- 0.6 mL Eppendorf tubes
- Falcon 50mL conical centrifuge tube (x1)
- Ice
- Pierce™ BCA Protein Assay Kit (ThermoScientific, cat# 23227) <!>
- Bovine Serum Albumin (BSA) Standard (ThermoScientific, cat# 23209; 2 mg/mL) <!>

4. PROCEDURE

A- Preparation of diluted albumin (BSA) standards

1) If necessary, prepare 80 µL aliquots (in 0.6 mL Eppendorf tubes) from one Albumin Standard (BSA) ampule (2 mg/mL). Store the aliquots in a -20 °C freezer until use. Otherwise, take a 2 mg/mL BSA aliquot from the freezer.
2) Dilute the contents of one Albumin Standard (BSA) aliquot (80 µL; 2 mg/mL) sequentially into several clean vials (0.6 mL Eppendorf tubes), preferably using the same diluent as the samples (ddH_2O). Use the following table as a guide.

Table 1 – Preparation of diluted albumin (BSA) standards.

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (mg/mL)
A	120	80 of stock (2 mg/mL)	0.8
B	100	100 of vial A dilution	0.4
C	100	100 of vial B dilution	0.2
D	100	100 of vial C dilution	0.1
E	100	100 of vial D dilution	0.05
F	100	100 of vial E dilution	0.025
G	100	0 - blank	0 - blank

** There will be sufficient volume for three replications of each diluted standard (3 x 25 µL).*

*** Vortex each solution before diluting it. Keep on ice until used.*

B- Preparation of the BCA working reagent (WR)

1) 200 µL of WR are necessary per sample. Use the following formula to determine the total volume of WR required:

total volume WR required = (# standards + # unknowns) × (# replicates) × (volume of WR per sample)

Example: for 4 unknowns and 3 replicates of each sample:

(8 standards + 4 unknowns) × (3 replicates) × (0.2 mL) = 7.2 mL WR required

2) Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B) in a Falcon tube.

Example, combine 10 mL of Reagent A with 0.2 mL of Reagent B.

Prepare sufficient volume of WR based on the number of samples to be assayed.

The WR is stable for several days when stored in a closed container at room temperature (RT).

C- Sample preparation and colorimetric assay

1) Dilute the samples (with ddH_2O), if necessary.

Prepare sample blanks. The buffer in which samples were prepared should always be run as sample blank (in the same dilution as the sample).

If you don't know the protein concentration, test different dilution ratios.

For samples obtained from usual cell culture procedures, we suggest diluting them 10x.

Beware of interfering substances. Refer to the manufacturer's Tech Tip #8 for guidance.

2) Pipette 25 µL of each standard or unknown sample replicate into a microplate well.

If sample size is limited, 10 µL of each unknown sample and standard can be used (sample to WR ratio = 1:20).

However, the working range of the assay in this case is limited to 125 - 2000 µg/mL.

3) Using a multichannel pipette for better reproducibility, add 200 µL of the WR to each well. Avoid bubble formation and cross-contamination between samples.
4) Mix plate thoroughly on a plate shaker for 30 seconds.
5) Cover plate and incubate at 37 °C for 30 minutes.
6) Cool plate to RT. Measure the absorbance at 562 nm on a plate reader.

Important information

- Lab coat and gloves (powder-free) should be worn at all stages to avoid contamination of samples by human epidermal proteins (keratins).
- The working rang for this assay is 20‑2000 µg/mL, according to the manufacturer.
- Increasing the incubation time or ratio of sample volume to WR increases the net 562 nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standards and unknowns are treated identically, such modifications may be useful.
- Calculations: subtract the average 562 nm absorbance measurement of the blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates. Prepare a standard curve by plotting the average blank-corrected 562 nm measurement for each BSA standard *vs.* its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample. Note: if using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve provides more accurate results than a purely linear fit.
- Refer to the manufacturer's *User Guide* and *Tech Tips* for additional instructions, including information on interfering substances.

5. HAZARDS[1]

Bicinchoninic Acid solution (trade secret) may be harmful if swallowed. Causes serious eye irritation. Wear personal protective equipment (PPE).

Bovin Serum Albumin standard contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution. Wear personal protective equipment (PPE).

Copper(II) Sulfate is harmful if swallowed. Causes skin irritation and serious eye irritation. Very toxic to aquatic life with long lasting effects. Wear personal protective equipment (PPE).

Discard all residues accordingly.

For further safety information, refer to the respective Material Safety Data Sheet (MSDS).

[1] Sources:
CAMEO Chemicals: <https://cameochemicals.noaa.gov/>
PubChem: < https://pubchem.ncbi.nlm.nih.gov/compound/Copper-sulfate#section=GHS-Classification>
ThermoFisher: <https://thermofisher.com/support>