Protocol_BCA assay_Protein Concentration

Product: Thermo Scientific™ Pierce™ BCA Protein Assay Kit, Cat. no. PI23227

- Working range for BSA equals 20 to 2000µg/mL. Down to 5µg/mL with the enhanced protocol. Below this use MicroBCA kit
- Not compatible with SDS, DTT, beta-merc
- The Thermo Scientific™ Pierce™ BCA Protein Assay Kit is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu+2 to Cu+1 by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu+1) using a unique reagent containing bicinchoninic acid (see reference 1 on page 4). The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20–2000 µg/mL). T

Reagents:

- BCA Reagent A, 500 mL
- BCA Reagent B, 25 mL
- Albumin Standard Ampules, 2 mg/mL, 10 x 1 mL

  Store at room temperature

Other materials

- 96-well plates
- Plate reader that measures 562nm

Microplate method	
Prepare standards	Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20–2,000 µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

Preparation of the BCA working reagent (WR)	Use the following formula to determine the total volume of WR required:
	
	1. (# standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required
	    1. Example: for the standard test-tube procedure with 3 unknowns and 2 replicates of each sample: (9 standards + 3 unknowns) × (2 replicates) × (200 uL) = 4.8 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).
	    1. For the above example, combine 5.0 mL of Reagent A with 100ul of Reagent B

96-well assay	1. Record standard and sample positions using a 96-well template 2. Add 25 µL of each standard or unknown sample replicate into a microplate well 3. Add 200 µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds 4. Cover plate and incubate at 37°C for 30 minutes 5. Cool plate to RT. Measure the absorbance at or near 562 nm on a plate reader
Plate reader in CMK lab	1. Open software, MPM.6.exe 2. Choose “new experiment” 3. Click on template icon. Enter standards by typing in the concentration (e.g “2000” for 2000ug/mL), enter samples b typing in “S#”, e.g. S1 for sample 1 4. Select read new plate and set the endpoint to 562nm 5. Use curve fit plot icon (f(x)) to change the curve fit to four-parameter, which provides more accurate results than a purely linear fit 6. Record sample concentrations or use the export to excel option to save data (must use USB) 7. For more details see instruction manual on BioRad website- Microplate Manager® 6 Software Instruction Manual | Version 6.1