

Genova

Protocol: P09-004A

## Bicinchoninic Acid Protein Assay

### ■ Introduction

The principle of the bicinchoninic acid (BCA) protein assay<sup>1</sup> relies on the formation of a  $\text{Cu}^{2+}$ -protein complex under alkaline conditions, followed by reduction of the  $\text{Cu}^{2+}$  to  $\text{Cu}^{1+}$ . The amount of reduction is proportional to the amount of protein present. BCA forms a purple-blue complex with  $\text{Cu}^{1+}$  in alkaline environments, thus providing a basis to monitor the reduction of alkaline  $\text{Cu}^{2+}$  by proteins. This assay can be used to quantify proteins in the concentration range from 0.2 to 1.0mg/ml. It is compatible with many detergents but not compatible with reducing agents such as dithiothreitol above 1mM. It is always advisable to prepare the standard in the same buffer as the sample to minimise any interference effects. BCA assays are routinely performed at 37°C. Colour development begins immediately and can be accelerated by incubation at higher temperatures. Higher temperatures and/or longer incubation times can be used for increased sensitivity.

### ■ Materials required

Bicinchoninic Acid Protein Assay Kit (Sigma BCA1)  
Suitable tubes with caps to hold and mix 2.1ml samples and to heat at up to 60°C  
Plastic disposable cuvettes (Jenway 060 084)  
Standard protein solution of known concentration (1mg/ml)  
Incubator or block heater to heat sample tubes

### ■ Method

#### Preparation of the BCA working reagent

BCA reagents A and B are available commercially from a number of different sources. Instructions given here are for the kit supplied by Sigma Aldrich, part code BCA1, although other methods will be similar. Please refer to the manufacturer's instructions.

1. Mix 50 parts of Reagent A (a solution containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1N NaOH, pH 11.25) with 1 part of Reagent B (4% (w/v)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), preparing sufficient reagent for all the standards and samples. 2ml of working reagent is required for each sample.
2. Mix until the solution is a uniform light green colour. The solution is stable for 1 day.

#### Preparation of samples and standards

1. Turn on the Genova and allow it to warm up. See below for set up instructions.
2. Prepare a series of protein standards ranging in concentration from 0.2 to 1.0mg/ml such that the final volume for the assay is 0.1ml. Examples of dilutions are given in Table 1 below. Note that the Genova can accept up to 6 standards, not including the blank.
3. Prepare the unknown samples in a similar way such that the final volume is 0.1ml.
4. Add 2.0ml of the BCA working reagent to each sample and standard, vortex gently and follow one of the following incubation parameters:
  - a. 60°C for 15 minutes or:
  - b. 37°C for 30 minutes or:

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- c. 25°C (room temperature) from 2 hours to overnight
- 5. If required, allow the tubes to cool to room temperature.
- 6. Transfer the samples to cuvettes and measure the absorbance at 562nm using the BCA protein mode on the Genova as detailed below.

Final concentration (mg/ml)	Volume of a 1 mg/ml standard (µl)	Volume of water or dilution buffer (µl)
0 (blank)	0	100
0.2	20	80
0.4	40	60
0.6	60	40
0.8	80	20
1.0	100	0

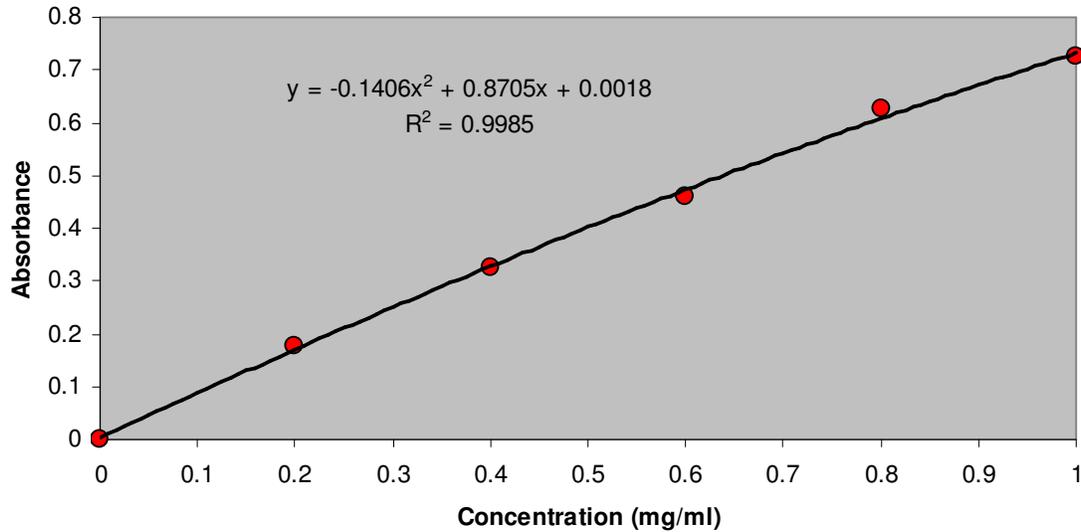
**Table 1:** Examples of standard dilutions for preparation of a calibration curve.

### Setting up the Genova for the BCA assay

1. From the main screen of the Genova select PROTEIN MODE and then BCA.
2. Select SETUP. The wavelength should already be set to 562nm. Adjust the units to mg/ml or other units as appropriate.
3. Select the required resolution. Note that at high resolution i.e. 0.001, the concentrations will appear to fluctuate more.
4. Select EXIT and then CURVE. Select a METHOD number (0-9); the standard curve data generated will be stored with the method number displayed.
5. Enter the number of standards (maximum of 6).
6. In the table under the unit heading (mg/ml), enter the concentration values of each of the standards.
7. Place the sample blank into the Genova and press CAL to begin the calibration procedure. Press CAL again to zero the instrument on the blank.
8. Following the instructions on the screen, insert each standard as requested pressing CAL each time. Once all standards have been measured, the ABS column of the table will be updated with the actual absorbance.
9. To view the standard curve, select VIEW. To obtain the equation of the curve, select STATISTICS. The curve fit is quadratic of the form  $y = ax^2 + bx + c$ .
10. Press any key to clear the statistics then exit back to the main measurement screen.
11. Place the sample blank in the Genova and press CAL to zero.
12. Remove the blank and insert each sample. Read the concentration from the screen in the chosen units.

An example calibration curve is shown in Figure 1.

## BCA assay standard curve



**Figure 1:** A typical BCA assay standard curve with samples ranging from 0.2 to 1.0mg/ml protein.

### ■ References

1. Smith, P.K. et al., *Anal. Biochem.* **150**: 76-85, (1985).

The protocols described here are for guidance only. Be aware of any hazardous compounds, take precautions where necessary and dispose of any waste in the appropriate manner.