Document Number:	**SASoM/METHOD/023.v5**
Title:	**Protein Determination by the Bicinchoninic Acid (BCA) Assay.**
Version:	**v5**
Author:	**Peter Mullen**

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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

1.0 Purpose –

This SOP describes the current procedure for determining protein concentration using the Bicinchoninic Acid assay (Sigma BCA-1) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved with carrying out a protein assay using this method.

3.0 Responsibilities –

All staff involved in protein extraction are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

This assay is carried out in 12 x 75mm borosilicate glass tubes (Fisher; 14-961-26). A 1mg/ml protein standard (Sigma; P0914-5AMP) in 0.15M Sodium Chloride to create an 8-point standard curve. Results are calculated using a pre-defined Excel file.

Turn on a water bath and set at 60ºC.

Label eight tubes (A-H) and make up protein standards as shown in the table below:-

	Final concentration [µg/ml]	Volume of 1mg/ml protein standard (µl)	Volume of distilled water (µl)
Tube A	0	0.00	150 (50 x 3)
Tube B	50	2.50	47.50
Tube C	75	3.75	46.25
Tube D	100	5.00	45.00
Tube E	250	12.50	37.50
Tube F	500	25.00	25.00
Tube G	750	37.50	12.50
Tube H	1000	50.00	0.00

Label sample tubes 1 – onwards.

Add 45µl of distilled water to all sample tubes. Add 5µL of each test sample to respective tubes (remember samples are therefore diluted 1:10).

Make up the BCA solution by adding 1 volume (e.g. 1ml) of Copper Sulphate solution (Sigma C2284) to 50 volumes (e.g. 50ml) of Bicinchoninic Acid solution (Sigma B9643). Mix thoroughly to give a green-coloured solution.

Add 1ml to ALL tubes (i.e., standards and samples). Add a further 2ml to tube 'A' as this will also be used as a '*blank*' as well as the lowest standard.

Vortex all tubes and incubate in the water bath for 15 mins at 60ºC.

Remove samples and allow to cool. Both 'standards' and 'samples' should appear purple in colour.

Take a flat-bottom 96-well plate and aliquot 200µl of standard 'A' into each well of the first column on the plate (A1-H1). This will be used to 'blank' the plate in the spectrophotometer.

Make up the standard curve starting in column 2 (going from top to bottom) by adding 200µl of standard 'A' to wells B1 & B2, 200µl of standard 'B' to wells B3 & B4, 200µl of standard 'C' to wells B5 & B6, 200µl of standard 'D' to wells B7 & B8, 200µl of standard 'E' to wells C1 & C2, 200µl of standard 'F' to wells C3 & C4, 200µl of standard 'G' to wells C5 & C6 and 200µl of standard 'H' to wells C7 & C8.

Add 200µl of sample '1' to wells D1 & D2, followed by duplicates for each of the remaining samples.

Check that there are no air bubbles in the wells - if so, burst with a needle.

Read the plate at 540nm on the Biohit BP800 Microplate Reader and download the data to 'BCA Template' file on the desktop to plot the standard curve and show the extrapolated data.

5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should under go training in this technique before performing procedure

8.0 Related documents –

8.1 Risk assessments COSHH/011
RA/GEN/019

8.2 SOP SASoM/EQUIP/029
Use and Maintenance of the Biohit BP800 Microplate Reader

9.0 Approval and sign off –

Author:

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Signature: Date:

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