Document Number:	**SASoM/METHOD/070.v4**
Title:	**Protein Determination in 1.5mL microcentrifuge tubes using the Biorad RC-DC Protein Assay.**
Version:	**v4**
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SOP History		
Number	Date	Reason for Change
v1	09/09/2014	Original
v2	03/09/2106	Update
v3	03/09/2018	Update
V4	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for determining protein concentration using the Biorad RC-DC Protein Assay (#500-1212) 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 –

The Biorad RC-DC Protein Assay is a colorimetric assay for protein quantitation based on the original Biorad DC (Lowry) Protein assay but is now reducing agent-compatible (RC) and detergent-compatible (DC).

The following abbreviated list of reagents are deemed compatible with the RC DC Protein Assay (but NOT necessarily the previous DC Protein Assay):

10% SDS	2% Triton X-100
2% Tween 20	2% NP40
0.5M HCL	0.05% Sodium Azide
2% CHAPS	1% CHAPSO
100mM Dithiothreitol	5% ß-Mercapthoethanol
100mM EDTA	500mM Imidazole
2.5M NaOH	4M Urea
Laemmli Buffer (5% ß-Mercapthoethanol)	

This protein assay is carried out in standard 1.5mL microcentrifuge tubes.

A standard curve is created using 1.5mg/ml bovine gamma globulin (Standard I, Biorad #500-0005) *or bovine serum albumin (Stanbdard II, Biorad #500-00007)* diluted to create an 8-point standard curve. Results are then extrapolated using a pre-defined Excel file.

Prepare the protein standard solution by adding 19.2mL (rather than 20mL) of DW to create a 1.5mg/mL (rather than 1.44mg/mL) solution. Dispense into 25 x 0.75mL aliquots and store at -20°c for up to 6 months.

Label Microcentrifuge tubes for the standard curve (A-H) and test samples (1-x).

Label eight SEPARATE borosilicate glass tubes (A-H) and prepare the protein standards as shown in the table below:-

	Final concentration [µg/ml]	Volume of 1.5mg/ml protein standard (µl)	Volume of distilled water (µl)
Tube A	0	0	150
Tube B	50	5	145
Tube C	100	10	140
Tube D	250	25	125
Tube E	500	50	100
Tube F	750	75	75
Tube G	1000	100	50
Tube H	1500	150	0.00

Transfer standards (25µL) and test samples (10µL sample + 15µL DW) into pre-labelled clean dry microcentrifuge tubes.

Calculate the volume of each subsequent reagent required using the table below:

n=	S (µL)	A (mL)	I (mL)	II (mL)	B (mL)
1	5	0.25	0.125	0.125	1
5	25	1.25	0.625	0.625	5
10	50	2.5	1.250	1.250	10
15	75	3.75	1.875	1.875	15
20	100	5	2.5	2.5	20
25	125	6.25	3.125	3.125	25
30	150	7.5	3.75	3.75	30
40	200	10	5	5	40

In a separate tube, add 5µl of DC 'Reagent S' to each 250 µl of DC 'Reagent A' as required - see table above for calculations (each standard or sample will subsequently require 125µL of 'complete' Reagent A).

Add 125µl RC Reagent I into each tube. Vortex. Incubate the tube for 1 minute at room temperature.

Add 125µl RC Reagent II into each tube. Vortex. Centrifuge the tubes at 15,000g for 5 minutes.

Carefully aspirate and discard the supernatants from each tube using a pipette.

Add 125µl RC '*complete*' Reagent A into each microcentrifuge tube. Vortex. Incubate tubes at room temperature for 5 minutes, or until precipitate is completely dissolved. Vortex before proceeding to the next step.

Add 1mL of DC Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 minutes. The absorbance will be stable for at least 1hr.

Make up the standard curve in the 96-well tray starting in column 2 (going from top to bottom) by adding the following.

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
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 absorbance at 750nm (650nm – 750nm).

Read the plate at 750nm (650nm – 750nm is acceptable) on the Biohit BP800 Microplate Reader and download the data to the '*Biorad RC DC Protein Assay Template-690nm'* file on the desktop to plot the standard curve and show the extrapolated data. Please note that the BCA protein Assay Template must NOT be used as it has a different standard curve!

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 (Protein Isolation)
RA/GEN/019 (Biohit BP800 Platereader)

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

9.0 Approval and sign off –

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