Document Number:	**SASoM/METHOD/035.v5**
Title:	**Sulforhodamine B (SRB) Growth Assay**
Version:	**v5**
Author:	**Peter Mullen**

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SOP History		
Number	Date	Reason for Change
V1	01/02/2013	Original
V2	01/02/2015	Update
V3	01/02/2017	Update
V4	01/02/2019	Update
V5	01/02/2021	Update

1.0 Purpose –

This SOP describes the current procedure for performing a SRB (Sulforhodamine B) assay 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 measuring cell growth using SRB assays

3.0 Responsibilities –

All staff involved in performing SRB assays 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 –

Seed cells in a 96-well plate. Number of cells required in each well should be determined for individual cell lines by the user.

Treat cells with inhibitors/stimulus and incubate them for the appropriate period of time.

Take cells out of incubator and to each well add 50µl of the cold 25% Trichloracetic acid (TCA) solution – each well should go cloudy as you add solution! Place plate in the cold room at 4ºC for 60mins.

Carefully remove the plate from the cold room and tip the TCA solution down the sink. Small quantities of TCA may be disposed off down the sink and if necessary neutralise with bicarbonate.

Wash each row of wells under gently running tap water, holding the plate as near to the tap as possible to minimise splashing. Wash each tray 10X.

Blot the plate dry by banging upside down on a layer of towels.

Place plate in the warm oven (50ºC) to dry.

Once dry add 50µl of the SRB dye solution to each well containing cells. Tap each plate to ensure the surface of each well is covered. Leave on the bench at room temperature for 30min.

Drain off SRB solution by tipping the solution down the sink and wash each row of wells with 1% glacial acetic acid at room temperature, again holding the plate as near to the beaker as possible to minimise splashing. Wash each tray 4X.

Blot the plate dry by banging upside down on paper towels.

Place plate in the warm oven to dry.

Once dry add 150µl of the Tris buffer solution. Tap the plate a few times on each of the four sides to ensure the remaining SRB dye has become completely dissolved in the Tris buffer. Leave on a shaker/rocker at room temperature for 60min.

Read plate at 540nM on the Biohit BP800 Platereader.

Solutions

Cold 25% Trichloracetic acid (TCA) solution: Dilute 100% TCA stock solution in water. Store at 4°C.

1% acetic acid solution: Dilute 20mL of glacial acetic acid stock into distilled water to a final volume of 2 litre.

SRB 0.4% solution in 1% acetic acid: Dissolve 2g of SRB in 1% acetic acid solution and make up to 500mL. Store at 4°C.

10 mM Tris solution pH 10.5: dissolve 1.21 g of Tris base in water. Adjust the pH to 10.5 with 2-3 drops of 2M sodium hydroxide and add water to 1litre. Store at 4°C.

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 undergo training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/006 & 007
RA/GEN/042 & 051

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

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 13/01/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 13/01/2021

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature:  Date: 13/01/2021