Document Number:	**SASoM/METHOD/013.v4**
Title:	**Use of Fast Green FCF for Reverse Phase Protein Array (RPPA) protein normalisation.**
Version:	**v4**
Author:	**Peter Mullen**

Effective from:	21/03/2020
Valid to:	20/03/2022

SOP History		
Number	Date	Reason for Change
v1	03/04/2014	Original
v2	21/03/2016	Update
v3	21/03/2018	Update
v4	21/03/20	Biennial Update

1.0 Purpose –
This SOP describes the current procedure for using Fast Green FCF for Reverse Phase Protein Array (RPPA) protein normalisation in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in RPPA work in Laboratory 248 at the St Andrews School of Medicine (SASoM).

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

4.0 Procedure –
Fast Green FCF dye is used for the general staining of proteins on porous nitrocellulose films for sample protein quantification. It is therefore particularly suited to Reverse Phase Protein Arrays (RPPA) where it can be used to quantify the 'total protein' deposited on nitrocellulose-coated 'Fastslides'.

Fast Green was purchased from Sigma (F7258-25G).

Be careful with the Fast Green though as it is hygroscopic so even a small amount left on the bench will attract water very quickly and the whole bench will look like St.Patrick's day in Dublin!

Make up 1 litre of Destaining Buffer as follows:

100ml Glacial Acetic Acid
300ml EtOH
600ml Distilled Water (green Elga tap)

Make up 50mL of Fast Green FCF dye (0.005% w/v) as follows:

2.5mg (0.0025g) of Fast Green FCF
50mL of Destaining Buffer
Mix thoroughly until in suspension.

Procedure:

1. Rinse slides for 1 minute in PBS.
2. Incubate slides in 0.005% w/w Fast Green FCF dye solution for 2hrs at room temperature.
3. Rinse slides in Destaining buffer (3 x 5mins).
4. Incubate slides in Destaining buffer for 2hrs at room temperature, keeping well agitated throughout.
5. Air dry and read on the Licor Odyssey scanner. Images can be captured on both the 680nm (red) and 800nm (green) channels.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

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 distilled water and a damp cloth.

7.0 Related documents –

8.1 Risk assessments – RA/GEN/009 (General Laboratory Safety)

8.1 SOPs – SASoM/METHOD/038/039/040

8.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE