Document Number:	**SASoM/METHOD/132.v1**
Title:	**'Total Protein' Staining of Membranes using FastGreen FCF Solution**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	08/02/2021
Valid to:	08/02/2023

SOP History		
Number	Date	Reason for Change
v1	08/02/2021	Original

1.0 Purpose –
This SOP describes the current procedure for carrying out Total Protein' Staining of Membranes using FastGreen FCF Solution in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM carrying out Total Protein' Staining of Membranes using FastGreen FCF Solution in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff performing Total Protein' Staining of Membranes using FastGreen FCF Solution in this manner 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 –

Solutions:

0.002% FastGreen FCF Solution:

Weigh out 2mg of FastGreen FCF powder (Sigma; F7258-25G) into a 100mL bottle and then add (i) 26 mL of DW, (ii) 14 ml Acetic Acid (glacial) and (iii) 60 mL of Methanol (total volume 100mL).

Revert 700 Wash Solution:

Add 6.7mL of Glacial Acetic Acid (6.7% v/v) and 30mL of Methanol (30% v/v) to 63.3mL of water (final volume 100mL).

1M Sodium Hydroxide:

1M = 39.997g of NaOH in 1L
= 3.997g of NaOH in 100mL of DW.

Revert 700 Reversal Solution (0.1M sodium hydroxide / 30% (v/v) methanol).

Add 60mL of DW into a glass bottle and then add (i) 10mL of 1M NaOH and (ii) 30mL of Methanol (total volume = 100mL).

Procedure:

Total protein stain with FastGreen FCF solution.

1. After membrane transfer is complete, remove the membrane from the transfer tank and rinse briefly in DW.
2. Place the membrane in a container, add 5-10 mL of Fastgreen solution and then incubate at room temperature for 5 minutes, with gentle shaking.
3. Discard FastGreen FCF Solution to a suitable waste container (it must not go down the sink) and rinse the membrane for 30 seconds (x2) with approximately 5-10 mL of Revert 700 Wash Solution. Again the Revert 700 Wash Solution should be collected for waste disposal and not flushed down the sink. Briefly rinse the membrane with DW.
4. Image the membrane on the 700nm (red) channel using the Licor Odyssey® imaging system. Adjust the 'exposure' settings so that no saturation appears in the bands to be quantified.
5. Destain the membrane after imaging using 'Revert 700 Reversal Solution'. Rinse the membrane briefly with DW and then incubate with 5-10 mL of 'Revert 700 Reversal Solution' reversal solution for 5-10 min at room temperature with gentle agitation. If you do NOT need to use the 700 nm (red) channel for an antibody target there is no need to perform this step as the secondary detection will be carried out using the 800nm channel alone.
6. After de-staining, rinse the membrane briefly with DW and proceed immediately to the normal 'Blocking' and Immunodetection / visualisation steps.
7. Perform immunoblotting using IRDye® 680/800CW conjugated secondary antibodies to detect the target(s).
8. Image the membrane in the 680/800 nm channels with an Odyssey imaging system.

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.

8.1 **Related documents –**
8.1 Risk assessments
CHARM _ 22140_SDS-PAGE Western Blotting

8.2 SOPs
SASoM-METHOD-033-Western Blot Polyacrylamide Gel Electrophoresis
SASoM-METHOD-034-Western Blot Antibody Detection Using Licor Odyssey Scanner

9.0 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