Document Number:	**SASoM/METHOD/113.v1**
Title:	**Revert™ 700 Total Protein Stain Normalization Protocol**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	01/12/2019
Valid to:	30/11/2021

SOP History		
Number	Date	Reason for Change
v1	01/12/19	Original

1.0 Purpose –
This SOP describes the current procedure for REVERT Total Protein Staining on western blot membranes in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved performing an REVERT Total Protein Staining in in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff performing REVERT Total Protein Staining 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 –

In quantitative Western blotting (QWB), normalization mathematically corrects for unavoidable sample-to-sample and lane-to-lane variation by comparing the target protein to an internal loading control. The internal loading control is used as an indicator of sample protein loading, to correct for loading variation and confirm that observed changes represent actual differences between samples.

Total protein detection is becoming the "gold standard" for normalization of protein loading. After transfer, but prior to immuno-detection, the membrane is treated with a total protein stain to assess actual sample loading across the blot. Because this internal loading control uses the combined signal from many different sample proteins in each lane, error and variability are minimized. This antibody-independent method corrects for variation in both sample protein loading and transfer efficiency, and monitors protein transfer across the blot at all molecular weights.

Revert 700 Total Protein Stain (licor.com/revert) is a near-infrared fluorescent membrane stain used for total protein detection and normalization. Revert staining is imaged at 700 nm, and fluorescent signals are proportional to sample loading. This protocol describes how to use Revert 700 Total Protein Stain for Western blot normalization and quantitative analysis and should be read in conjunction with the Licor REVERT Total Protein Stain Pack Insert. It can be used on either Nitrocellulose or PVDF membranes.

Reagents:

REVERT Total Protein Stain Solution (Methanol to be added prior to use):

REVERT Wash Solution (can be made in-house as detailed below):

- Add 33.5mL Glacial Acetic Acid
- Add 150mL Methanol
- Add 316.5mL ELGA Water (TOTAL VOLUME 50mL)

REVERT Reversal Solution (can be made in-house as detailed below):

- 150mL Methanol
- 2g Sodium Hydroxide
- 350mL ELGA Water (TOTAL VOLUME 50mL)

Procedure:

[A] Western Blot and Membrane Transfer:

Having prepared, run and transferred samples using standard SDS-PAGE / Western Blot and Transfer methods, the membranes can first be stained with Revert™ 700 Total Protein Stain using the protocol(s) described below:

[B] Single-Colour Western Blot (800nm target only).

1. Add methanol to the 'Total Protein Stain' reagent as indicated on the bottle.

2. After transfer, rinse the membrane in water, and incubate in 5-10mL of '**REVERT Total Protein Stain**' solution for 5 minutes, with gentle shaking.

3. Decant Total Protein Stain solution completely and save for future re-use. This solution can be used NO MORE than four times.

4. Rinse the membrane (two times for 30 seconds) with 5-10mL of '**REVERT Wash Solution'** and then briefly rinse the membrane in water. *[Revert Wash solution (P/N 926-11012): 6.7% (v/v) Glacial Acetic Acid, 30% (v/v) Methanol, in water].*

5. Image the membrane IMMEDIATELY in the 700 nm (red) channel with an Odyssey® imaging system. Adjust settings so that no saturation appears in the bands to be quantified.

6. Rinse the membrane briefly with water, and proceed immediately to blocking and immuno-detection steps using appropriate blocking buffers / IRDye® 800CW conjugated secondary antibody to detect the target (as per standard protocols).

[C] Two-Colour Western Blot (800nm target only).

1. Follow steps 1-4 above to stain and image the membrane.

2. After imaging of the Total Protein Stain, incubate the membrane in 5-10mL of '**REVERT Reversal** ' solution for 5-10 minutes, with gentle shaking. Reversal is complete when stain is no longer visible by naked eye. WARNING: Do NOT reverse for more than 10mins. *[REVERT Reversal Solution (P/N 926-11013: 0.1M Sodium Hydroxide, 30% (v/v) Methanol, in water]*

3. Rinse membrane briefly with water and proceed immediately to blocking and immuno-detection steps using appropriate blocking buffers / IRDye® conjugated secondary antibody to detect the target (as per standard protocols).

Notes:

1. *For weak or low-abundance targets, 800nm channel detection is recommended for best results.*
2. *After reversal, 1-3% residual fluorescence from REVERT may be seen during imaging in the 700nm channel; but this will NOT impact results.*

Use Licor Empiria Studio software to complete normalised western blot analysis.

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 the procedure.

8.0 Related documents –

8.1 Risk assessments –
RA-GEN-034-Licor Odyssey Scanner

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:	SOP Administrator	
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