Document Number:	**SASoM/METHOD/034.v5**
Title:	**Western Blot Antibody Detection Using Licor Odyssey Scanner.**
Version:	**V5**
Author:	**Peter Mullen**

Effective from:	01/02/2021
Valid to:	01/02/2023

SOP History		
Number	Date	Reason for Change
V1	01/02/2013	Biennial review. Changes to sections 3, 4, 5, 6 & 8.
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 Western Blot antibody detection using LiCor Odyssey in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM involved in visualising western Blots with the Licor Odyssey scanner.

3.0 Responsibilities –
All staff involved in performing Western Blot antibody detection using LiCor Odyssey 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 –

SDS-PAGE and Transfer onto nitrocellulose membranes.

Prepare samples for western blot and run down an SDS-PAGE gel as per usual protocols. Transfer samples onto nitrocellulose membrane in the usual manner (30V overnight at 4ºC).

PBS is made up by dissolving 10 x PBS tablets (Oxoid; BR0014G) in 1 litre of DW.

TBS can be made up as follows:

	TBS (x1)	**TBS (x10)**
Tris Base, pH7.5	6.05g	60.5g
NaCl	8.76g	87.6g
DW	1000mL in total	1000mL in total

[A] Fastgreen Protein Normalisation

NOTE: if performing protein normalisation with FastGreen (rather than using antibodies to actin, GAPDH etc), it is important to carry out this step BEFORE the membrane is exposed to 'Blocking Buffer'.

After removing the membrane from the transfer buffer, ...

[B] Primary Antibody Detection

After transfer and / or Fastgreen normalisation, block the membrane in Li-Cor 'Intercept' Blocking Buffer (diluted 50:50 in PBS or TBS) for 1 hour at room temperature. Licor 'Intercept' Blocking Buffer is available in ether PBS or TBS formulations. The PBS formulation should generally be avoided if using primary antibodies targeted against phosphorylated proteins (e.g pERK).

Dilute the primary antibodies in Li-Cor 'Intercept' Blocking Buffer (diluted 50:50 in PBS / TBS) using recommended / previously optimised dilutions. Membranes can be incubated with a cocktail of primary antibodies providing they have (i) different molecular weights or (ii) were derived from different host species (which would use a different secondary antibody).

Incubate membrane in primary antibodies overnight at 4ºC as per standard Western Blotting protocol. Shorter incubation times can be used but specificity / sensitivity can be compromised (e.g.1hr at room temperature).

[C] Secondary Antibody Detection using LiCor Scanner

Make up either (i) 0.1% PBS-Tween 20 (PBS-T; 1ml Tween 20 / 1L PBS) or (ii) 0.1% TBS-Tween 20 (TBS-T; 1ml Tween 20 / 1L TBS) depending on which formulation of Licor 'Intercept' blocking buffer you are using.

Wash membrane in PBS-T / TBS-T at room temperature for 5 mins (x3).

Dilute fluorescently-labelled secondary antibodies at 1:10,000 dilution (1.5µL/15ml) in Licor 'Intercept' Blocking Buffer (diluted 50:50 in PBS / TBS) containing 0.01% SDS in the first instance - this may require optimisation between 1:5,000 and 1:25,000 depending on the antibodies being used. Mouse-derived primary antibodies are detected using an anti-mouse fluorescently-labelled secondary antibody (either 680nm or 800nm wavelength) whilst rabbit-derived primary antibodies are detected using an anti-rabbit fluorescently-labelled secondary antibody (again of either 680nm or 800nm wavelength). By combining a mouse primary and a rabbit primary along with their respective secondary antibodies (one of 680nm and the other of 800nm), dual-labelled Westerns can be obtained. The 800nm channel is preferable (in terms of sensitivity) and so should be used for the antibody whose expression is likely to be lower. Secondary antibodies should not be exposed to the light.

Incubate the membrane in secondary antibodies at room temperature for 45mins with gentle shaking – it is important to protect the membrane from the light until such time as it has been finally scanned.

[D] Detection and Visualisation

Wash membrane in PBS-T / TBS-T at room temperature for 5 mins (x3), keeping the membrane in the dark.

Wash membrane in PBS / TBS at room temperature for 5 mins (x3) to remove residual Tween 20, again keeping the membrane in the dark.

Lie the membrane flat on a piece of filter paper in the dark and allow to air dry – allowing the membrane to dry will enhance the signal but render it useless for stripping and re-probing.

Scan the membrane on the LiCor Odyssey scanner. Keep the membrane in the dark until such time as it has been scanned.

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/013
General RA 49

8.2 SOP SASoM/METHOD/027
Polyacrylamide Gel Electrophoresis.

8.3 SOP SASoM/METHOD /050
Western Blotting.

8.4 SOP SASoM/EQUIP/037
Use and Maintenance of the Licor Odyssey.

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature:  Date: 11/02/2021

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