Document Number:	**SASoM/METHOD/038.v5**
Title:	**RPPA Antibody Detection on 1-Pad Fast©Slides Using LiCor Odyssey.**
Version:	**V4**
Author:	**Peter Mullen**

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

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 RPPA antibody detection on 1-Pad Fast©Slides using the 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 RPPA antibody detection on 1-Pad Fast©Slides using the LiCor Odyssey scanner.

3.0 Responsibilities –
All staff involved with RPPA antibody detection on 1-Pad Fast©Slides using the 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 –

Blocking and Primary antibody incubation on 1-PAD FastSlides (Day 1).

Remove slides from sealed box in coldroom and place in a suitable container. Add excess LiCor Blocking Buffer (diluted 50:50 in PBS) and incubate at RT for 1 hour on a rocking platform.

Prepare primary antibodies in LiCor Blocking Buffer (diluted 50:50 in PBS) at the desired concentrations and keep on ice.

Mount the slides in either (i) the single frame Chip Clip or (ii) the 'FastFrame' four bay slide holder so that a tight seal is formed between the slide and the incubation chamber.

Remove residual buffer from wells and add 700µl primary antibody to respective wells.

Place the slide and chamber into a sealed wet box and incubate on rocking platform overnight at 4ºC.

Secondary antibody incubation / visualisation on 2-PAD FastSlides (day 2).

Make up 0.1% PBS-Tween20 (PBS-T; 100µl Tween20 /100ml PBS).

Remove slides from coldroom and carefully remove the primary antibodies from each well.

Add 700µl of PBS-T and wash slides on rocking platform at RT for 5 minutes (X3).

Prepare fluorescently-labelled secondary antibodies by diluting in Odyssey Blocking Buffer (diluted 50:50 in PBS) at 1:2000 dilution (1µl/2ml) in the first instance along with 0.01% SDS (50µl/50mL from a 10% stock solution) - this may require optimisation between 1:1,000 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 RPPA Fast©Slides can be obtained.

Remove PBS-T buffer from wells and add 700µl fluorescently-labelled secondary antibodies to respective wells. Incubate 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.

Remove secondary antibodies from well and briefly wash (x3) in 700µl PBS/T at room temperature.

Remove slide from carrier, transfer to a suitable container and wash in excess PBS/T for 15mins, keeping the membrane in the dark.

Remove PBS/T and further wash membrane with PBS at room temperature for 15mins to remove residual Tween 20, again keeping the membrane in the dark.

Dry the Fastslide in 50ºC oven for 10mins and then scan on the Li-Cor Odyssey scanner. Keep the slide in the dark until 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 under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/028

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