Document Number:	**SASoM/METHOD/063.v4**
Title:	**Preparation of 2-fold Dilutions for 16-pad RPPA Antibody Optimisation Slides.**
Version:	**v4**
Author:	**Peter Mullen**

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

SOP History		
Number	Date	Reason for Change
v1	04/04/2013	Original
v2	21/03/2016	Update
v3	21/03/2018	Update
v4	21/03/2020	Biennial Update

1.0 Purpose –
This SOP describes the current procedure for preparing serial dilutions of protein samples prior to spotting onto Reverse Phase Protein Array (RPPA) Antibody Optimisation slides using the MGII Robotic Spotter in Laboratory 248 at the St Andrews School of Medicine (SASoM).

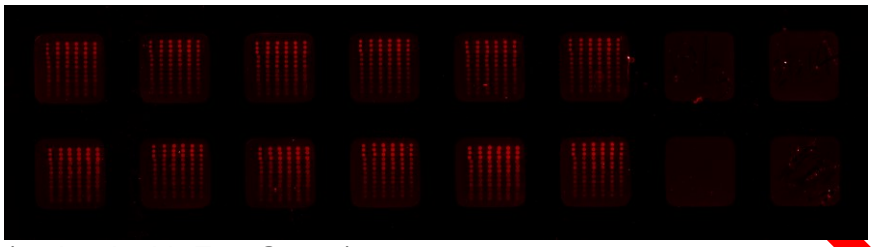
2.0 Scope –
This SOP applies to all staff in Laboratory 248 at the St Andrews School of Medicine (SASoM) involved in preparing Antibody Optimisation RPPA slides.

3.0 Responsibilities –
All staff preparing RPPA Antibody Optimisation slides 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 –

1. Antibody optimisation slides are prepared by spotting replicate protein samples onto 16-pad Fastslides using the MGII robotic spotter as illustrated below.



(stained with FastGreen)

2. Select a small panel of five or six cell lines which are representative of the samples you will ultimately be using for RPPA analysis. A suitable panel may comprise of BT549 (breast), HCC1954 (breast), HT29 (colorectal), MCF7 (breast), PANC1 (pancreatic) and RCC30 (renal) cancer cell lines. Keep samples on ice at all times.

3. Prepare protein lysates (from a 14cmϕ petri dish) in the usual manner using 400µl of 'Complete' Lysis Buffer including phosphatase inhibitors, protease inhibitors, aprotinin and Triton X-100 (SASoM/METHOD/006).

4. Determine the protein concentration of the lysates using the BCA Protein assay (SASoM/METHOD/023).

5. Since the MGII robot will spot samples onto the 16-pad slides using four pins, we need to make up four aliquots of each dilution from each sample – one for each pin (hence the need for more protein lysate material).

6. *For optimal spot recognition, sample dilutions should ideally start at 2mg/ml. All samples should therefore contain 2000µg (2mg) of total protein in a final volume of 1mL, of which 250µl will be 4X Mercapthoethanol Reducing Buffer. The difference in volume from sample to sample is made up with fully supplemented Lysis Buffer (see above).*

7. Using 1.5mL microcentrifuge tubes, make up the first dilution of each sample containing 2000µg of total protein (differing volumes) and 250µl (1/4 volume) of 4X Mercapthoethanol Reducing Buffer. Make up the total volume of each sample to 1000µl with fully-supplemented Lysis Buffer. Label each sample as 1A, 2A, 3A etc.

8. Denature all samples (1A, 2A, 3A, 4A, 5A, 6A) at 60ºC for 60mins.

9. Make up serial dilutions in 0.6mL microcentrifuge tubes as follows;
   1A = 240 µl neat (taken directly from denatured tubes above)
   1B = 240µl A + 240µl complete lysis buffer
   1C = 240µl B + 240µl complete lysis buffer
   1D = 240µl C + 240µl complete lysis buffer

1E = 240µl D + 240µl complete lysis buffer

10. Transfer 50µl of each sample dilution (1A-E, 2A-E, 3A-E etc) to appropriate wells of a 96-well ROUND-BOTTOM tray, taking into consideration the positioning of the pins. ENSURE you mark which is to be the 'front' and 'rear' of the tray for when it gets loaded into the MGII Biobank.

11. Seal the tray with Parafilm and store in covered boxes at -80ºC prior to spotting on the MGII Robotic spotter.

12. As a rough guideline, 20 sets of slides can be prepared from these trays.

13. Trays can be placed back in the freezer after spotting and subsequently re-spotted at a later date with no significant loss of activity.

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

8.2 SOP SASoM/METHOD/022
Protein Extraction from Human Tissues and Primary Xenografts

8.3 SASoM/METHOD/023
Protein Determination by the Bicinchoninic Acid (BCA) Assay.

8.4 SASoM/METHOD/006
Preparation of Protein Lysates from Adherent Cell Cultures

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