Document Number:	**SASoM/METHOD/071.v3**
Title:	**Preparation of 2-fold Sample Dilutions from FFPE for RPPA Spotting.**
Version:	**v3**
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SOP History		
Number	Date	Reason for Change
v1	15/09/2014	Original
v2	15/09/2016	Update
v3	15/09/2018	Update

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

2.0 Scope –
This SOP applies to the staff in the SASoM involved in the preparation of 2-fold sample dilutions for RPPA spotting.

3.0 Responsibilities –
All staff involved with in the preparation of 2-fold sample dilutions from FFPE-derived protein samples for RPPA spotting 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 –
Prepare protein lysates from FFPE blocks as described in the relevant SOP (SASoM-METHOD/069/Preparation of protein from FFPE (Paraffin) blocks).

Determine protein concentration of all FFPE samples using the Biorad RC-DC protein Assay kit (SASoM-METHOD-070-Protein Determination by the Biorad RC-DC Protein Assay). Protein concentration should not be determined using a Bradford or BCA assay due to reagent incompatibilities. Keep samples on ice at all times.

A series of five 2-fold dilutions must be prepared for RPPA spotting onto Fast®slides in order that standard 4-parameter modelling with MicroVigene spot analysis software can be performed. (If 5-parameter modelling with MicroVigene is required, then at least six dilutions will be necessary).

For optimal spot recognition, sample dilutions should ideally start at 1mg/ml 1000µg/1000µl = 100µg/100µl including 25µl of RPPA denaturing buffer. All samples should therefore contain 100µg of total protein in a final volume of 100µl, of which 25µl will be 4X Mercapthoethanol Reducing Buffer. The difference in volume from sample to sample is made up with fully supplemented Lysis Buffer so as to ensure all tubes are of the same volume (100µl).

As a guideline, all FFPE protein lysates must therefore have a protein concentration greater than 133µg/100µl if 1mg/ml is to be adopted as the starting concentration for the serial dilutions.

Using small (0.6mL) centrifuge tubes, make up the first dilution of each sample with 100µg of total protein (differing volumes) and 25µl (1/4 volume) of 4X Mercapthoethanol Reducing Buffer. Make up the total volume of each sample to 100µl with fully-supplemented Lysis Buffer. Label each sample as 1A, 2A, 3A etc.

Denature all ‘neat’ samples at 60ºC for 60mins.

Make up serial dilutions as follows;

1A = 30 µl neat
1B = 50µl A + 50µl complete lysis buffer
1C = 40µl B + 40µl complete lysis buffer
1D = 30µl C + 30µl complete lysis buffer
1E = 20µl D + 20µl complete lysis buffer

Add 35µl 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.

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

Trays can be placed back in the freezer after spotting to be 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/069-Preparation of protein from FFPE (Paraffin) blocks

8.3 SASoM/METHOD/070-Protein Determination by the Biorad RC-DC Protein Assay

9.0 Approval and sign off –

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