Document Number:	**SASoM/METHOD/103.v2**
Title:	**Culture, fixation and immunocytochemistry of cells on coverslips for immunofluorescent imaging**
Version:	**v2**
Author:	**Peter Mullen**

Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
V1	21/05/2018	Original
V2	11/11/2020	Original

1.0 Purpose –

This SOP describes the current procedure for the culture, fixation and immunocytochemistry of cells on coverslips for immunofluorescent imaging 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 culture, fixation and immunocytochemistry of cells on coverslips for immunofluorescent imaging.

3.0 Responsibilities –

All staff involved in the culture, fixation and immunocytochemistry of cells on coverslips for immunofluorescent imaging 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 –

Poly-L-lysine coating of sterile coverslips (if using Sigma P4707)
In tissue culture hood, fill a 14cm petri dish with 0.01% poly-l-lysine (sigma). Place sterile (autoclaved) 22x22 mm coverslips in dish and rock gently to cover. Place in 37°C incubator for 5 minutes. Using sterile forceps, place coverslips in a coverslip drying rack and leave in hood overnight or until dry. PLL can be reused so place back in bottle. Store coverslips in a sealed sterile container.

Culturing cells on cover slips in 6-well plates
Place one PLL-coated coverslip in each well of a 6-well plate. Prepare cells in media to desired cell density. Add 3-4 mL cell suspension to each well and treat as desired.

Fixation and storage of coverslips (**4% PFA**, but alternatively methanol-acetone 1:1 or 100% methanol. Fixation method will depend on protein of interest)
In the fume hood, dilute 16% Paraformaldehyde (PFA) in PBS to 4% immediately prior to use. Label a fresh 6-well plate, add 1-2 mL PBS to each well and transfer cover slips using forceps. Dispose of media and old plate appropriately. Rinse coverslips in fresh plate in PBS and aspirate. In the fume hood, add 4% PFA to each well (1-2 mL is more than sufficient) and leave in hood for 20 minutes to fix. Aspirate PFA into aldehyde waste bottle and replace with PBS. Wash 3x5 minutes with PBS on rocker (still in 6-well plate). Note that 1st wash should also be placed in aldehyde waste. Store in PBS at 4°C until use. Use within 1 month (this can be extended if PBS is supplemented with sodium azide to preserve).

Permeabilisation and blocking (in a 6-well plate)
Place desired coverslips in a labelled 6-well plate. Permeabilise cells in 0.1% Triton-X-100 in PBS for 10 minutes then tip off. Wash 3x5 minutes in PBS on rocker. Block with 0.1M glycine in PBS for 5 mins (blocks aldehyde groups – otherwise serum may bind to free aldehydes and possibly block specific staining close to antigenic sites). Wash 3x5 minutes in PBS. Block non-specific binding sites with 5-10% goat serum in PBS for 10 mins (NB: blocking serum should be from the same species that secondary antibodies are raised in).

Primary and Secondary Antibody probing and mounting
Prepare 50 µL primary antibody cocktail (dilution according to manufacturer instructions) per coverslip (in blocking solution- 2% goat serum in PBS or serum from whatever species secondary antibody is raised in). Stretch parafilm paper side up on large petri dish or alternative. Label parafilm with coverslip conditions and aliquot 50 µL primary antibody cocktail per coverslip. Drain excess liquid from coverslips with lint-free tissue on the corner and drop cell-side down onto drops of antibody on parafilm. Do not press down, just drop. Incubate for 1 hour at room temperature. Wash 3x5 minutes in PBS on rocker in 6-well plate. Prepare secondary antibodies (generally 1:750-1000 for Alexa Fluor) in same blocking solution as used for primary antibodies. Drop 50 µL per coverslip on fresh parafilm, drain excess PBS from cover slips with lint-free tissue and place coverslips cell-side down on droplets. Incubate for 1 hour at room temperature in the dark. Wash 3x5 minutes in PBS. Prepare 1:10,000 Hoechst (33342) in PBS. Drain excess liquid with lint-free tissue from corner of coverslips. Repeating

the same process, incubate Hoechst for 10 minutes at room temperature in the dark. Wash 3x 5 mins in PBS on rocker. Rinse 2x in Elga water and refill. Drain corner with lint-free tissue and mount with prolong gold onto labelled slides. Leave to dry overnight in a slide book. The following day, seal edges by painting clear nail polish around the edge of the coverslips.

Store in the dark and image as desired.

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training –
All staff should undergo training in this technique before performing procedure

8.0 Related documents –

8.1 Risk assessments –
8.2 SOP -

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date: 11/11/2020
Management Approval:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date: 11/11/2020
QA release by:		
Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date: 11/11/2020