Document Number:	**SASoM/METHOD/051.v4**
Title:	**Amplified Immunofluorescence**
Version:	**v4**
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Effective from:	01/01/2019
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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update

1.0 Purpose –
This SOP describes the current procedure for staining cells and tissue with antibodies with fluorescently labelled conjugates in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved with immuno staining of cells and tissue.

3.0 Responsibilities –
All staff involved in staining cells and/or tissue using antibodies 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 –
<u>For staining cells</u>
Bring slides up to room temp after removing from freezer

Wash slides in PBS-(0.05%) Tween 20, 2 x 5 minutes.

Treat slides with hydrogen peroxide/Water (3mls + 97mls) for 10 minutes in coplin jar.

Wash slides with PBS-T.

Block slides using Vector Avidin/ Biotin blocking kit – 15 minutes each. At this stage if required, not all tissues/cells require this step.

Wash with PBS-T.

Block with blocking serum 10-15 minutes as suggested by blocking solution spec. sheet.

Incubate slides in primary antibody diluted with blocking solution as required for one hour.

Wash with PBS-T.

Incubate section with appropriate biotinylated secondary antibody diluted as required for 30 - 45 minutes.

Wash slides with PBS-T.

Incubate with Streptavidin Alexa conjugated antibody diluted 1 in 200 for 30 minutes.

Wash again with PBS-T.

Allow slides to air dry in dark.

Mount and counter stain as required.

For staining tissues
Tissue sections must first be dewaxed to remove paraffin and rehydrated which is done by treating sections in xylene and then taken down to water through graded alcohols. Slides should be passed through two changes of xylene, five minutes in each and 2 minutes in each graded alcohol solution.

Antigen retrieval is then performed on slides by treating them with either heated 0.1M Sodium Citrate/0.1M Citric Acid pH6 or Tris/EDTA pH9.0 or a commercially available solution for 5 minutes in a microwavable pressure cooker in a microwave. Allow 20 minutes for slides to cool.

Wash slides with PBS-T and carry on SOP as described above

5.0 Personal protection –
A Howie coat must be worn at all times and gloves when required as advised by material safety data sheets

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 be trained by an experienced person before undertaking this procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/008 & 019
RA/GEN/006 and 015

8.2 SOP SASoM/METHOD/063
Dewaxing and rehydration of paraffin embedded sections

8.3 SOP SASoM/METHOD/015
Use of the Pressure Cooker for Antigen Retrieval

9.0 Approval and sign off –

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