Document Number:	**SASoM/METHOD/057.v4**
Title:	**Use of 8-Chamber Slides for Cell Culture / Immunohistochemistry**
Version:	**v4**
Author:	**Peter Mullen**

Effective from:	10/11/2019
Valid to:	09/11/2021

SOP History		
Number	Date	Reason for Change
v1	19/12/2013	Original
V2	10/11/2015	Update
V3	10/11/2017	Update
V4	10/11/2019	Update

1.0 Purpose –
This SOP describes the current procedure for using 8-Chamber Slides 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 cell culture work.

3.0 Responsibilities –
All staff involved in cell culture 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 –

This SOP has been optimised for use of Lab-Tek II 8-Chamber Slides with covers (RS Glass Slides). These chamber slides can however be purchased in number of different formats from eg. Thermo Fisher as below:

- 154453 (1-chamber slides)
- 154461 (2-chamber slides)
- 154526 (4-chamber slides)
- 154534 (8-chamber slides)

Protocol:

1. Plate out an equal number of cells (4000 in the first instance) into each well of the chamber slide. This cell number will require optimisation depending on the cell line being used and the duration of the experiment in question.
2. Place the chamber slides back in the incubator with the protective cover in place and leave for the desired time period. Slides should generally be left for 48hrs prior to any experimental procedure.
3. Transfer Chamber Slides to Hypoxystation for 24hrs if required.

Fixation of 8-Chamber Slides

4. Remove media from the Chamber Slides and aspirate dry with a pastette.
5. Add excess 4% Paraformaldehyde (200µL) and leave for 10mins.
6. Discard the 4% Paraformaldehyde and snap off the slide 'Chamber' using the special tool provided. Transfer the slides to PBS and wash for 3 x 5mins.
7. Leave the cells in PBS prior to immunohistochemistry, Haematoxylin and Eosin (H&E) Staining, etc.

If carrying out experiments under hypoxic conditions, steps 3-5 MUST be performed within the Hypoxystation. Once fixed, all subsequent experimental procedures can be performed on the open bench in line with normal protocols.

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 white tissue, then disinfected with 70% ethanol.

7.0 Training -

All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments – RA-BIOL-004 (Tissue culture)

8.2 SOPS
SASoM-METHOD-025-Immunohistochemistry
SASoM-METHOD-041-Haematoxylin and Eosin (H&E) Staining

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

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