Document Number:	**SASoM/METHOD/107.v3**
Title:	**"Oil Red O" staining of cultured cells.**
Version:	**v3**
Author:	**Peter Mullen**

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

SOP History		
Number	Date	Reason for Change
v1	01/11/18	Original
V2	26/11/2018	Optimisation to the protocol
V3	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for staining cells with "Oil Red O" in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies all staff /students in the SASoM staining cells with "Oil Red O".

3.0 Responsibilities –
All staff / students involved in staining cells with "Oil Red O" 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 –

"Oil red O" is a lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids (and some lipoproteins) on frozen / paraffin sections but can also be used in-vitro. In histology, a supersaturated solution of oil red O in isopropanol may be used to stain fat in tissue. It can also be utilised to identify fats / lips using in-vitro cell cultures. It has deep red/orange, and clearly visible.

Oil Red O Staining for Cultured Cells

1. Culture and treat cells in tissue culture plates, petri dishes etc as per normal experimental protocols.
2. Remove the media from the culture plate (the volumes used below are appropriate for 6-well plates).
3. Add 2 ml of PBS to wash the cells and remove PBS completely.
4. Add 2 ml of 4% paraformaldehyde (at room temperature) and incubate for 10 min (again at room temperature).
5. Safely discard 4% paraformaldehyde (to aldehyde waste) and add 2 ml fresh PBS. *If you are not ready to continue with the staining protocol, wrap the plates up with parafilm and then store for up to a month at 4˚C.*
6. Remove PBS.
7. Rinse cells in 60% Isopropanol
8. Prepare Saturated oil Red 'O' (Sigma Cat#O0625) in 100% isopropanol
9. Dilute the saturated oil Red 'O' to 60% (i.e. 6ml Saturated Oil Red O to 4mL distilled water (Working solution lasts for 1-2 hours).
10. Filter 2mL (per 6-well) of Oil Red O Working solution to the cells and incubate for 20minutes.
11. Wash cells with 2 ml of 60% isopropanol for 5 min IF NEEDED at room temperature (this may need adjusting).
12. Transfer to water to stop the differentiation of the stain (this step may also need adjusting).
13. Let the cells dry completely at room temperature. DO NOT use XYLENE!
14. If using cells on coverslips, mount to a microscope slide using DPX mountant.
15. Acquire images under the microscope for analysis

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 undergo training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments RA/BIOL/004: RA/COSHH/004.

9.0 Approval and sign off –

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Position: Research Fellow

Signature: Date: 11/11/2020

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