Document Number: **SASoM/METHOD/100.v3**

Title: **Phalloidin / Hoescht / DAPI Staining of cells in vitro.**

Version: **v3**

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Effective from:	09/09/2021
Valid to:	09/09/2023

SOP History		
Number	Date	Reason for Change
v1	01/06/2017	Original
V2	01/06/2019	Update
V3	09/09/2021	Update

1.0 Purpose –

This SOP describes the current procedure for staining cells in vitro with Phalloidin / Hoescht 33342 / DAPI.

2.0 Scope –

The scope of this document is to describe the step by step procedures for preparing cells and staining with Phalloidin / Hoescht 33342 or DAPI to identify actin filaments along with cell nuclei.

3.0 Responsibilities –

All staff preparing cells and staining with Phalloidin / Hoescht 33342 by this protocol 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 –

Sterilise some microscope cover slips by either (i) autoclaving or (ii) by soaking in ethanol and then air-drying.

Place a drop of tissue culture media into the bottom of a 6-well tray and then place the cover slip on top – the cover slip should then stick to the well by capillary action.

Add cells to the well in the usual manner and place in the incubator for the desired time period. 1000 cells in 0.2mL (for a 96-well tray) equates to 30,000 cells in 4mL for a 6-well tray.

Remove media from wells and wash the cells in PBS (2 x 5mins).

Fix the cells in 4% Paraformaldehyde (PFA) for 10 mins; if fixed with methanol the staining will not work. A 16% stock solution can be purchased from Sigma () and diluted 1 in 4 with PBS.

Wash in PBS (1 x 5mins)

Permeabilise in 0.2% Triton X-100 made up in PBS (20µL/10mL) for 5min.

Block in 5% BSA (0.5g/10mL) / 0.1% Triton (10µL/10mL) made up in PBS for 30min.

Add Phalloidin-647 at a dilution of 1:20 (60ul Phalloidin / 1200ul) made up in 3% BSA / PBS (0.3g BSA in 10ml PBS). Hoescht 33342 at a dilution of 1:2000 (1ul Phalloidin / 2000ul) can also be added at this point if required.

Protect form light.

Incubate for 1 hour at room temperature.

Wash with PBS (4 x 5mins) and then allow to air dry.

Place one drop of DPX mountant on a microscope slide, flip over the coverslip and then drop it onto the slide, allowing the mountant to spread across the cover slip. If Hoescht 33342 dye has not been used, DPX mountant containing DAPI can be used as an alternative.

Leave to dry overnight at room temperature and then seal the edges of the cover slip with nail varnish.

Visualise on the Leica DM5500 microscope. Phalloidin will appear in the Cy5 channel and Hoescht in the DAPI channel.

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

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

7.0 Training –

8.0 Related documents –
8.1 Risk assessments –
CHARM_RA20138_Immunofluorescence detection of antibodies.

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 09/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 09/09/2021

QA release by:

Name: John O' Connor

Position: QA Manager

Signature:  Date: 09/09/2021

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