Document Number: **SASoM/METHOD/127.v1**

Title: **Cell Cycle progression using BrDU and Propidium Iodide by Flow Cytometry**

Version: **v1**

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Effective from:	03/06/2020
Valid to:	02/06/2022

SOP History		
Number	Date	Reason for Change
v1	03/06/2020	Original

1.0 Purpose –

This SOP describes the current procedure for preparing samples for cell cycle progression using BrDU and propidium Iodide by flow cytometry in Laboratory 248/9 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved preparing samples for cell cycle progression using BrDU and propidium Iodide by flow cytometry in the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff involved in preparing samples for cell cycle progression using BrDU and propidium Iodide by flow cytometry 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 –

The following protocol is designed for the assessment of cell cycle progression using Bromodeoxyuridine (BrdU) and Propidium Iodide (PI). This protocol can also be performed in combination with gamma-H2AX to assess levels of DNA damage (although care needs to be taken when it comes to spectral overlap between the three channels when commencing flow).

- It should be noted that (i) Fixation and (ii) Permeabilization is different to standard flow protocols.
- Cells MUST be in single cell suspensions BEFORE fixation takes place.

Methodology:

1. Perform any prior cell culture and treatment regimen relevant to your experiment.
2. Add BrdU to cells at a final concentration of 10µM and incubate for 30 mins in a TC incubator - as a positive control for DNA damage you can add H2O2 (final concentration of 10 µM) 15-20 minutes prior to the end of the incubation period.
3. If using adherent cells; wash with PBS and detach cells using trypsin or mechanical means.
4. Spin cells down using a centrifuge then resuspend cells in ice-cold PBS as single cell suspensions.
5. Count and aliquot out desired numbers of cells in cold PBS.
6. Fix cells by adding them drop-wise to a 5 ml aliquot of 70% ethanol with gentle vortexing. Incubate on ice for 30 mins (or leave at -20°C until ready to proceed further).
7. Spin cells at 500g for 10 mins at 10°C. Aspirate supernatant then loosen the pellet by gentle vortexing.
8. Slowly add 1 ml of 2N HCl / 0.5% Triton X-100 dropwise with gentle vortexing to denature DNA and expose BrdU epitope for antibody binding. Incubate for 30 mins at RT.
9. Spin cells at 500 g for 10 mins, aspirate supernatant, and resuspend in 0.1 M sodium tetraborate (1mL).
10. Spin cells at 500 g for 10 mins, aspirate supernatant. Resuspend sample in excess incubation buffer (0.5% BSA in PBS) to wash cells.
11. Spin cells at 500 g for 5 mins, aspirate supernatant, and resuspend in incubation buffer containing appropriate dilution of primary anti-BrdU ab (+ any other primary antibodies provided no species cross-reactivity). Incubate at RT for 30 mins. Make sure you also have some unlabeled samples for flow calibration and as a control.
12. Spin cells at 500 g for 5 mins, aspirate supernatant, wash 2X by centrifugation with excess incubation buffer.
13. Resuspend cells in appropriate dilution of secondary antibody in incubation buffer. Incubate at RT in the dark for 30 mins. Again make sure you have samples that are not labelled with secondary antibody for calibration and as a control.

14. Spin cells at 500 g for 5 mins, aspirate supernatant, wash 2X by centrifugation with excess PBS.
15. Resuspend cells in PBS containing a 1:500 dilution of Propidium Iodide. Incubate for 5 mins.
16. Aliquot 200 ul of cell suspension onto a flat-bottomed 96 well plate (ideally in triplicate) for flow cytometry using the Guava in Lab 249
17. Cell cycle analysis can be performed using FlowJo7 software.

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
RA20270 – Cell Cycle Analysis by Flow Cytometry

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8.2 SOP SASoM/METHOD/018
Flow Cytometric DNA Analysis of Cell Lines.

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
Management Approval:		
Name:	Peter Mullen	
Position:	Research Fellow	
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