Document Number:	**SASoM/METHOD/110.v2**
Title:	**BrdU Incorporation of cells grown in Chamber Slides or on Cover Slips.**
Version:	**v2**
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	11/11/2020	Updated

1.0 Purpose –
This SOP describes the current procedure for BrdU incorporation of cells grown in Chamber Slides or on Cover Slips 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 BrdU Incorporation of cells grown in Chamber Slides or on Cover Slips.

3.0 Responsibilities –
All staff involved in the preparation of protein lysates from adherent cell cultures in this manner 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 –

Materials:

BrdU Stock Solution (10mM) (Life Technologies; B23151)
Mouse Anti-Brdu antibody (BD; 347580)
Protein Block (Dako; X0909) / antibody diluent (Dako; S0809)
'Alexfluor 555' Goat-anti-Mouse-IgG-H-L Secondary Antibody (Life Tech; A-21422)
Hoescht 33342 (Life Tech; H3570)
ProLong™ Gold Antifade Mountant (Life Tech; P36930)
1% H_2O_2 (made from 30% stock (3mL/90mL DW); Sigma H1009-500ML)
80% Ethanol
PBS and 0.1% PBS-T
1N HCl (85 ml of HCl into 1000 ml of dH_2O, or 8.5mL in 100mL dH_2O)

Preparation:

Set up cells in either Chamber Slides or on Cover slips using the usual cell culture protocols. Cells should be made up at a concentration of 20,000/mL (80,000 in 4mL of media) if leaving them for 24hrs, or 30,000/mL (120,000 in 4mL of media) if leaving them for 48 hrs prior to BrdU incorporation.

Aliquot cell suspension (4mL) into either (i) a 1-pad chamber Slide, or (ii) a 6-well tray containing a poly-d lysine coated coverslip (see SASoM-METHOD-103). Leave for 24/48 hrs (or longer if treatment is required).

Make up BrdU working solution at 10µM (2.5µL in 2.5mL) and 25µM (6.25µL in 2.5mL) using fully supplemented media. Remove culture media from the cells and then replace with BrdU-containing media. Replace in the incubator and leave for 2hrs. *NOTE; The concentration of BrdU being used, as well as the incubation period, may need optimising depending on how quickly the cell line of interest is proliferating / incorporating BrdU.*

Fixation:

- Remove culture media from the wells / chamber slides and wash in ice-cold PBS. If using chamber slides, snap off the top chamber so as to effectively be left with microscope slides.
- Fix the cells in ice-cold 80% ETOH for 30 min (or up to 4 weeks). This can be done in the coldroom but samples may need sealing with parafilm to prevent evaporation over a longer time period.

Detection and Visualisation:

- Remove ethanol and wash cells in 0.1% PBS-T for 10min.
- Incubate cells in 1% H_2O_2 for 10min.

- Wash cells in 0.1% PBS-T for 10min.
- Incubate cells in 1N HCl for 20min.
- Wash cells in PBS for 5min.
- Incubate cells in Dako Protein Block for 10min.
- Incubate cells in Anti-BrdU (1:50; 40µL/2mL of antibody diluent) for 30min. Making up 150µL of solution should cover the central portion of the slide.
- Wash cells in PBS for 5min (X2).
- Incubate cells in anti-mouse/rabbit Alexa Fluor® 555 secondary antibody (1:100 in antibody diluent) for 30min.
- Wash cells in PBS for 5min (X2).
- Incubate in Hoescht 33342 counterstain (1:1000 in PBS) for 10min.
- Wash cells in PBS for 5min (X2).
- Dehydrate in 80% ETOH for 1min.
- Leave to air dry and then mount in Prolong Gold anti-fade.
- Leave to dry o/n and then scan on the Zeiss Axioscan or the Leica DM5500 microscope.

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 under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments –
RA-COSHH-007-Fixation and staining of cells;
RA-COSHH-008- Immunohistochemistry-fluorescence

8.2 SOP -
SASoM-METHOD-103-Preparation, culture, fixation and immunocytochemistry of cells on Poly-D-lysine coated coverslips

9.0 Approval and sign off –

Author:

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Signature: Date: 11/11/2020

Management Approval:

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