Document Number: **SASoM/METHOD/138.v1**

Title: **Monitoring Cell Division using the CFSE Cell Division Tracker Kit (Biolegend #423801)**

Version: **v1**

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Effective from:	19/08/2021
Valid to:	19/08/2023

SOP History		
Number	Date	Reason for Change
v1	19/08/21	Original

1.0 Purpose –

This SOP describes the current procedures for monitoring cell division using the CFSE Cell Division Tracker Kit (Biolegend #423801) in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM monitoring cell division using the CFSE Cell Division Tracker Kit (Biolegend #423801) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff monitoring cell division using the CFSE Cell Division Tracker Kit (Biolegend #423801) 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 kit (Biolegend #423801) uses Carboxyfluorescein Succinimidyl Ester (CFSE) which passively enters live cells and fluoresces green (excitation 492 nm, emission 517 nm). CFSE is able to passively diffuse into cells. Inside the cell, its acetate groups are cleaved by intracellular esterases, and the molecules are converted to fluorescent esters. CFSE is retained within the cell and covalently couples to intracellular molecules via its succinimidyl group. Due to this covalent coupling reaction, fluorescent CFSE can be retained within the cell for an extremely long period. Once the dye has been incorporated within the cell, it is not transferred to adjacent cells. CFSE is widely used for cell proliferation assays and in vivo cell tracking.

CFSE is heritable by daughter cells without affecting cell viability and therefore can be used to monitor the amount of cell division in-vitro as the degree of fluorescence diminishes as more cellular division occurs.

The kit comes as 5 vials of 100ug CFSE and 500uL anhydrous DMSO and should be stored at -20 degrees C.

Reagents:

CSFE Kit, DMSO, PBS, RPMI, DMEM, MEM,

Method:

1. Grow cells under treatment conditions / time-course as desired.
2. Bring CSFE vial and DMSO to room temperature.
3. Spin down CSFE vial to collect the contents at the bottom of the vial before reconstituting.
4. Add 36 uL of DMSO to 1 vial to make a 5 mM stock solution.
5. Prepare a 5 uM working solution by diluting 1 ul of 5 mM CSFE stock solution in 1 ml of PBS for every 1 ml of cell suspension.
    a. The working concentration may need to be optimised for specific cells or time-course.
    b. Reagent can be used at concentrations ranging from 0.5 uM – 10 uM for cell labelling.
6. Spin down cells and resuspend them at 10-100 x 106 cells/ml in CSFE working solution.
7. Incubate cells at room temperature or 37 degrees C for 20 mins in the dark.
8. Quench staining by adding 5 times the original staining volume with cell culture medium.
9. Pellet cells and resuspend in cell culture medium.
10. Incubate cells for 10 minutes.
11. After incubation CSFE labelled cells are ready for downstream applications or immediate analysis using e.g. the CytoFlex flow cytometer.

Note: on the CytoFlex Flow cytometer, CFSE can be visualised using the FITC filter (bandwidth 525/40).

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 –
CHARM_RA22141_Preparation of Cell Culture Media and Additives
CHARM_RA23757-Monitoring Cell division using the CFSE cell tracker kit.

9.0 Approval and sign off –

Author:

Name: Dr Oliver Read

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Signature:  Date: 06/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 06/09/2021

QA release by:

Name: John O'Connor

Position: Research Technician

Signature:  Date:08/09/2021

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