Document Number: **SASoM/METHOD/056.v6**

Title: **Use of 'Cell Tracker' Viability Dye in Cell Culture**

Version: **v6**

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Effective from:	04/05/2024
Valid to:	04/05/2026

SOP History		
Number	Date	Reason for Change
v1	18/12/2013	Original
V2	10/11/2015	Update
V3	10/11/2017	Update
V4	10/11/2019	Update
V5	04/05/2022	Update
V6	04/05/2024	Update

1.0 Purpose –

This SOP describes the current procedure for using Cell Tracker Viability Dye in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in cell culture work in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff involved in cell culture 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 –

CellTracker™ is a fluorescent probe which stains living / viable cells. Probes are inherited by daughter cells after cell fusion and are not transferred to adjacent cells in a population. The product can be obtained in a range of different forms and colours – this SOP relates to the following product:

CellTracker Green BODIPY (Life Technologies; C2102 - 5mg; mwt = 296.5536)

The optimal concentration of the probe for staining varies depending upon the application. Testing at least a tenfold range of concentrations is recommended. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells requires 5–25 µM dye. Less dye (0.5–5 µM) is needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, keep the dye concentration as low as possible.

Preparation of Stock 'Cell Tracker' reagent (10mM):

1. Before opening the dye vial, allow the product to warm to room temperature. Dissolve the lyophilized product in high-quality DMSO to a final stock concentration of 10mM (1.686mL). Aliquot (33 x 50µL) and store at -20°C.

Labeling of cells with 'Cell Tracker' reagent (5-25µM final concentration):

1. Plate out cells into appropriate 96-well / 24-well / 6-well trays / small petri dishes and allow to grow to sub confluence.
2. Remove media from the cells and wash the cells with fresh media containing NO ADDITIVES.
3. Dilute the Cell Tracker stock solution to a final working concentration of 5µM in serum-free medium (10µL stock / 20mL media). Warm the working solution to 37°C.
4. Add Cell Tracker (0.5mL per 24 well; 30µL per 96-well).
5. Incubate at 37ºc for 45min.
6. Remove Cell Tracker and wash with media (no additives).
7. Add fresh media and observe under microscope.
8. Photograph the image(s) as required.

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

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 white tissue, then disinfected with 70% ethanol.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents – Risk assessments – RA-BIOL-004 (Tissue culture)

9.0 Approval and sign off:

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 04/05/2024

Management Approval by:

Name: David Harrison

Position: Professor

Signature:  Date: 0405/2024

QA Release by:

Name: Peter Mullen

Position: QA Manager

Signature:  Date: 04/05/2024

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