Document Number: **SASoM/METHOD/094.v3**

Title: **2D Cell Viability Assay using Calcein AM, Propidium Iodide and Hoescht 33342.**

Version: **v3**

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Effective from:	18/04/2020
Valid to:	17/04/2022

SOP History		
Number	Date	Reason for Change
v1	18/04/2016	Original
v2	18/04/2018	Update
v3	18/04/2020	Biennial Update

1.0 Purpose –

This SOP describes the current procedure for analysing cell viability on 2D adherent cells grown in 96F well trays using Calcein AM in lab 248 at the St Andrews School of Medicine (SASoM). Calcein AM is used as a measure of enzymatic activity in live cells, Propidium iodide is used as a measure of dead cells with compromised membrane integrity and Hoescht is used as a marker of all nucleated cells (alive or dead). Calcein AM was purchased from Fisher Scientific (C3100MP).

2.0 Scope –

The scope of this document is to describe the procedure for looking at cell viability of 2D adherent cells in 96 well trays using Calcein AM (live cells), Propidium iodide (dead cells) and Hoescht 33342 (all cells).

3.0 Responsibilities –

All staff involved in looking at cell viability of adherent cells in 96 well trays using Calcein AM, Propidium iodide and Hoescht 33342 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 – Cell Culture:

1. Set up and treat cell lines in 'Celigo Supported' 96-well flat bottom trays in the usual manner. This assay is a sacrificial endpoint assay designed to measure (i) live cells using Calcein AM (Fisher Scientific; C3100MP), (ii) dead cells using Propidium Iodide (Sigma, P4864), and all nucleated cells (dead and / or alive) using Hoescht 33342 (Sigma H3570).

2. Reconstitute Calcein AM by adding 50.2µL of DMSO into one of the 50µg vials of lyophilized Calcein AM. Mix gently by inversion.

3. Prepare a 'Mixed Dye Solution' of Calcein AM, Propidium Iodide, and Hoescht – 12mL of the mixed dye solution will be enough for all the wells of a 96-well tray. Adjust accordingly if not all the wells are being used. Dyes should be made up in F12 media (with no FCS) in order to reduce autofluorescence on the green channel.

4. The following table can be used to prepare the mixed dye solution:

Dye	Stock Concentration	Recommended Concentration	Dilution Factor	**Volume in 12mL media**	Conc Range for other cell types
Calcein AM	1mM	1 µM	1:1000	12 µL	0.1 - 10 µM
Propidium Iodide	1mg/mL	2 µg/mL	1:500	24 µL	0.1 - 5 µg/mL
Hoescht 33342	10mg/mL	5 µg/mL	1:2000	6 µL	1 - 10 µg/mL

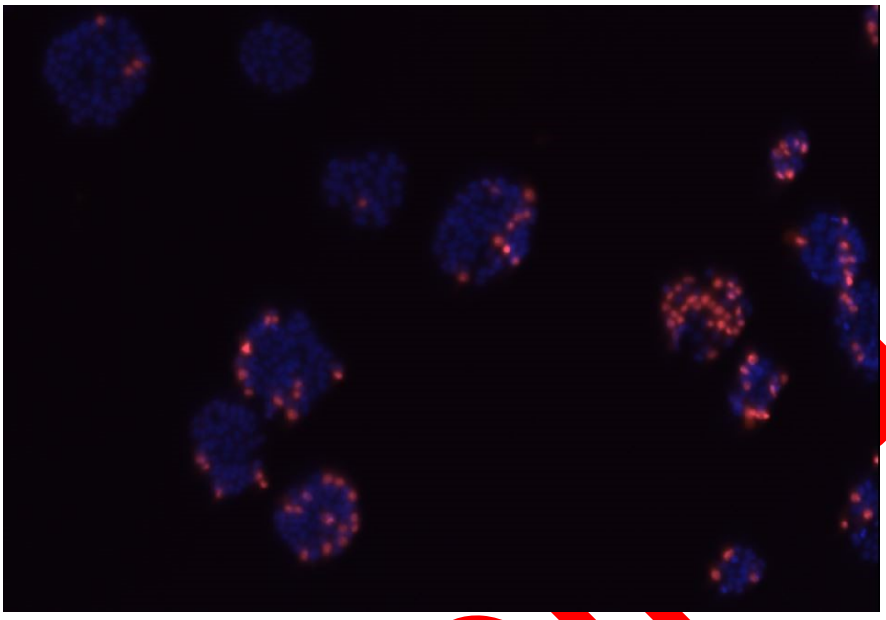
5. At the endpoint of your growth assay, remove experimental media and then wash cells with F12 media.

6. Remove all media from wells and add 100µL of mixed dyes solution.

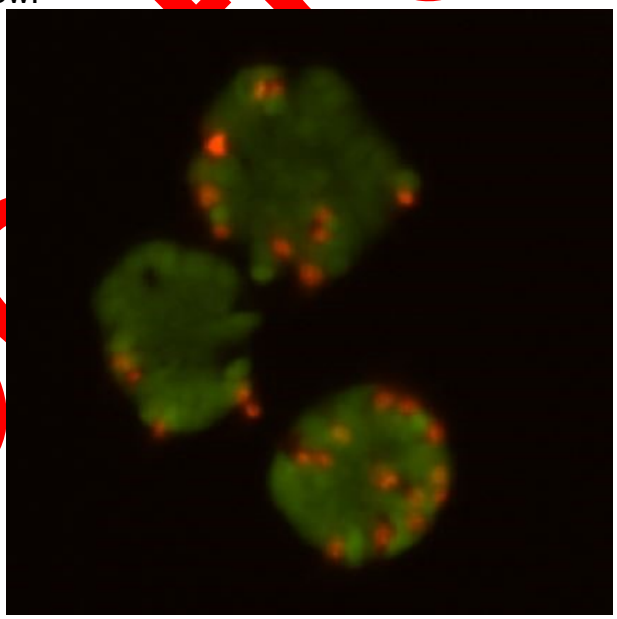
7. Incubate cells for 30 min at 5% CO2, 37°c.

8. Since cells in the wells are still 'live', the plate should be read immediately on the Celigo.

9. All cells (nuclei) stained with Hoescht 33342 will appear blue whilst those stained with Propidium Iodide (the dead cells) will appear red as illustrated below:



10. All cells stained with Calcein AM (live cells) will appear green whilst those stained with Propidium Iodide (the dead cells) will again appear red as illustrated below:



11. All liquid waste form this assay should be placed inside a sealable bag containing paper towels or sawdust to soak up the liquid, securely sealed and then disposed of in the red 'Chemical Waste' bins.

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 -
All staff should undergo training in this technique before performing procedure.

8.0 Related documents –

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