Document Number: **SASoM/METHOD/084.v5**

Title: **Colony Formation Assay with Celigo Visualisation**

Version: **v5**

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SOP History		
Number	Date	Reason for Change
V1	02/11/2015	Original
V2	2/11/2017	Update
V3	02/11/2019	Update
V4	04/05/2022	Update
V5	04/05/2024	Update

1.0 Purpose –

The purpose of this SOP is to describe the Colony Formation Assay (CFA) with visualisation on Celigo in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all those performing a Colony Formation Assay within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users carrying out a Colony Formation Assay within the SASoM to comply with this SOP. All users must read and then sign the SOP before using it and comply with all safety instructions.

4.0 Procedure –

This SOP describes the methodology for setting up Colony Formation Assays (CFA) which can be analysed on the Celigo scanner rather than manually counting the resulting colonies in the conventional manner.

CFAs are an in-vitro cell-survival assay based on the ability of a single cell to grow into a unique colony. Cells are normally stained with a dye such as crystal violet (which binds to DNA) and then manually counted. A colony is, by convention, defined as a group comprising of at least 50 cells.

Since this is laborious and time consuming, the experiment will be set up in 6-well trays and scanned using the Celigo scanner. Analysis on Celigo will be done using basic brightfield images and therefore there is no need to stain cells with crystal violet. Crystal violet staining can however be done after scanning if required for comparison / recording purposes. The analysis settings on Celigo have been optimised to identify colonies of a size approximating 50 cells in size but will require further optimisation depending on the cell lines being studied.

Preparation of PBS

10 Phosphate Buffered Saline tablets (Fisher Scientific; 10209252) were dissolved in Elga water to make 1L of PBS. The PBS was then autoclaved prior to use.

Preparation of Crystal violet stain

Crystal violet (0.5g) is *carefully* weighed and dissolved in 20ml of methanol before being made up to 100ml with milli-Q water. (NB: crystal violet and methanol are hazardous substances). Store at room temperature protected form light.

Optimisation of cell seeding density

The optimum cell density required for the cells to grow over a 1-3 week period must first be determined. This involves seeding plates with a range of different cell numbers and allowing them to grow for 2-3 weeks. Usually, the cell number range is between 50-500 cells per well depending on the doubling time of the cell line. The optimum plating density will need to be established for each cell line of interest.

PSN1 (fast growing cells) were plated out at 125 cells / 6 well plate
MiaPaCa2 (medium growing cells) were plated out at 250 cells / 6 well plate

Procedure

The Colony Formation Assay is usually performed with cells which have previously been treated with eg cytotoxic agents. Label 6-well plates, making sure that duplicates or triplicates are included as required.

Remove and discard media from the petri dish(es) / flask(s) in the initial drug screen and then wash with sterile PBS. Remove the PBS, add Trypsin / EDTA and then place in the incubator until the cells have released from the plastic. Calculate cell concentration with a haemocytometer.

Add the appropriate number of cells to each well (eg. 125 cells per well) and make up to 4mls with fresh medium. Each well should therefore contain the same number of cells in the same volume (although the cells will have come from different source dishes). Place the cells in the incubator and leave for 2-3 weeks.

Allow the cells to grow and change the culture medium every 3-5 days. Monitor cell growth and when the number of cells in a colony has reached 50 or more, the cells can be scanned, stained and counted. Since different cell-lines have different doubling times, growth should be monitored accordingly as the experiment will require terminating different times.

Scanning of plates using Celigo:

At the end of the experiment (usually 2-3 weeks after plating out) the plate should be read on the Celigo scanner. The plate should be read with the media in-situ and the lid left in place. Users must consult SOP document 'SASoM/EQUIP/061.v1 - How to Acquire a Scan using the Celigo Cytometer' before proceeding any further.

Analysis should be performed using the 'Single Colony Verification' model with the following settings:

Image Resolution	3
Well Mask	ticked
Well Mask Mode	Automatic
% Well Mask	85-100%
Algorithm	Texture
Intensity Threshold	10
Saturated Intensity	0
Precision	High
Diameter	5-10
Background	un-ticked
Separate Touching Colonies	un-ticked
Thickness	15
Minimum Colony Size	37,500*
Aspect Ratio	0.00
Intensity	0 – 255

(* This seems a good correlation with colonies of approximately 50 cells in size)

Crystal Violet staining Procedure:

Having scanned the plate, the plate can be stained in the conventional manner using Crystal Violet (previously made up as described above).

Aspirate the cell-culture medium from the 6-well plates and then wash each well with 2mls of PBS. Discard PBS. Add 2mL of 4% paraformaldehyde – leave for 15mins and then remove for safe disposal. Add 1mL of Crystal Violet, leave for 10 min, and then wash in

excess tap water (best done by immersing petri dishes in a plastic container submerged under running tap water).

Air dry plates and then read manually by eye! Count the number of 'darkly' stained colonies (containing 50 or more cells) under the white light or phase-contrast microscope at low magnification.

Plating efficiency (PE) calculations:

PE = (no. of colonies) / (no. of original cells seeded)

5.0 Personal protection -
Howie coat must be worn at all times.

6.0 Spillages -
It the Celigo is contaminated by spills it must be cleaned ***immediately after the spill has occurred***. A final wipe-down should be performed with 70% ETOH.

7.0 Training –
All users have to be trained by a designated person before using the Celigo Instrument.

8.0 Related documents –

8.1 Equipment Manual –
Celigo Cytometer User Guide
Celigo Cytometer Expression Analysis Application Guide
Celigo Cytometer Administration Guide.

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: 04/05/2024

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