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Title:	**Alamar Blue Growth Assay**
Version:	**v5**
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Effective from:	28/09/2020
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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
v3	01/01/2017	Update
v4	01/01/2019	Update
v5	28/09/2020	Amendment

1.0 Purpose –
This SOP describes the current procedure for performing an Alamar Blue HS Assay 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 Alamar Blue Assays.

3.0 Responsibilities –
All staff involved in performing Alamar Blue assays 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 –

This Alamar Blue assay uses 'Alamar Blue HS' reagent (Thermo Fisher; A50100) – this is a higher sensitivity version of the standard Alamar Blue assay and after optimisation was found to be superior.

Plate cells into 96-well trays and then leave to settle down for an appropriate time period (usually 48hrs). NOTE: It is necessary to also include a number of wells which contain culture media alone (ie NO CELLS) in order to obtain the background fluorescence for the plate. Alamar Blue HS will be added to half of these control wells and half will be left as media alone (no Alamar Blue HS).

Treat cells with inhibitor (e.g. chemotherapeutics, Protides, etc) or stimulus (e.g. Epidermal Growth factor) as required. Return the plate(s) to the incubator for the remainder of the experiment (e.g. four days, seven days, etc).

Add Alamar Blue HS stain in a quantity which is equal to 10% of the total volume in each well. If cells have been left in test media made up in a volume of 150uL, then 15uL of Alamar Blue HS should be added to each well. It is important to also remember the control wells containing only media (half of which will receive Alamar Blue HS an the other half will not).

Return the plate(s) to the incubator for a further 4 hours – the end point of the Alamar Blue HS incubation should therefore correspond with the time at which cells were originally treated with test agents (ie 96hrs, etc).

Scan the plate on the Biohit BP800 plate reader 4 hours after addition of Alamar Blue HS stain. The plate should be read at (i) 570nm and (ii) 600nm. The lid of the plate should be removed before reading the plate. Use of the Biohit BP800 plate reader is covered under a separate Standard Operating Procedure (SASoM-EQUIP-029-Biohit BP800 Microplate Reader).

Scan results can be imported into a pre-prepared Microsoft Excel template for final analysis.

5.0 Personal protection –

All staff should complete an in-house induction to the tissue culture area and be trained in sterile TC techniques before starting any TC work.

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 –

Risk assessments
CHARM RA20156 - Use of Chemotherapy Drugs and their Protides.
CHARM RA20158 - In-vitro Cell Proliferation Assays

SOPS
SASoM-EQUIP-029-Biohit BP800 Microplate Reader

9.0 Approval and sign off –

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