Document Number:	**SASoM/METHOD/098.v3**
Title:	**Preparation of Cells for Annexin V Assay by Flow Cytometry**
Version:	**v3**
Author:	**Peter Mullen**

Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	15/10/2016	Original
v2	15/10/2018	Update
V3	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for performing an Annexin V assay on established cell cultures by flow cytometry using the R&D Systems kit.

2.0 Scope –
The scope of this document is to describe the procedures for harvesting and staining cells for for cytomtric analysis of Annexin V. .

3.0 Responsibilities –
All staff wishing to measure Annexin V levels in cells by this protocol 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 assay is designed to include the total number of apoptotic cells released into the media rather than looking a the rate at which cells are being committed into apoptosis; cells which have undergone apoptosis and which are subsequently floating around in the media are therefore included along with the early-stage apoptotic cells which have not yet become detached. The first step of the protocol can therefore be omitted if only the adherent cells are of interest.

1. Remove media from each petri dish and transfer to labelled FACS tubes. Spin all tubes at 1,700rpm for 4mins and then discard the media to leave a (small) cell pellet at the bottom of the tube.

2. Wash the petri dishes with PBS (2mL), discard and then add trypsin (1.5mL). Place in the incubator until cells have become detached (but no longer than necessary as this may artefactually induce high values).

3. Add DMEM / RPMI with 10% FCS (1.5mL) to stop the trypsin. Add the cell suspension to the tube containing the relevant cell pellet (pooling the two together).

4. Spin all tubes at 1,700rpm for 4mins.

5. Discard the media and resuspend the pellet in DMEM / RPMI with 10% FCS (1.5mL). Leave in the incubator for 5mins.

6. Spin all tubes at 1,700rpm for 4mins.

7. Discard the media and resuspend the pellet in ice-cold PBS (1mL).

8. Spin all tubes at 1,700rpm for 4mins.

9. Resuspend the pellet in ice-cold Annexin-V buffer (100µL). Add Propidium Iodide (10 µL) and FITC-Annexin (1 µL) to each tube as appropriate. Incubate in the dark on ice for 15mins.

10. Add a further 400 µL of Annexin buffer. Keep in the dark on ice.

11. Run samples on the Flow Cytometer within 1hr.

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 –
SOPS - SASoM-METHOD-018-Flow Cytometric DNA Analysis.

9.0 Approval and sign off –

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