Document Number: **SASoM/METHOD/128.v1**

Title: **Antibody Detection by Flow Cytometry.**

Version: **v1**

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Effective from:	03/06/2020
Valid to:	02/06/2022

SOP History		
Number	Date	Reason for Change
v1	03/06/2020	Original

1.0 Purpose –

This SOP describes the current procedure for measuring antibody expression by flow cytometry in Laboratory 248/9 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in measuring antibody expression by flow cytometry in the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff involved in measuring antibody expression by flow cytometry 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 –

Note: the following protocol is based on the one described by Cell Signalling Technologies (CST).

Suitable cell numbers are required for flow cytometry as there is considerable loss due to washing steps, especially with permeabilised cells. A rough guideline is 1 x 10^5 per technical replicate when plating cells at the end.

Cells MUST be in single cell suspension BEFORE fixing or use on the machine

[A] Sample preparation:

1. Perform any prior culture and/or treatment regimens.
2. If using adherent cells; wash wells with PBS then detach cells from plates by incubating with trypsin (gently scraping using a sterile spatula if needs be).
3. Spin down cells and resuspend in cold PBS as single cell suspensions. Count cells before aliquoting separate samples for flow for each condition.
4. If you are only looking for the expression of a fluorescent marker e.g. GFP in a cell line, cells can be directly inserted into the machine otherwise proceed with the rest of the protocol.
5. If you are only looking at cell surface antigens then proceed to "Surface antigen staining" (Part B). If intracellular staining is required then samples need to be fixed.
6. If fixation is required; fix samples with 4% PFA in PBS (15 mins RT incubation).
7. Take an aliquot of 16% PFA and dilute it with sterile PBS to 8%. Add 8% PFA solution to samples in a 1:1 ratio.
8. Wash cells 2 times with cold PBS.
9. Fixed samples can then be stored at 4°C so that later steps can be performed within 48 hours

[B] Surface antigen staining:

The following steps can either be done in Eppendorf tubes or U-bottomed 96-well plates. If live cells are required then all steps need to be performed ON ICE rather than at room temperature.

1. For surface antigen staining, spin down the desired number of cells and resuspend in 100 ul incubation buffer (0.5% BSA in PBS) containing the appropriate dilution of primary antibody and leave at RT for 30-45 mins.
2. Wash cells 2 times with incubation buffer.
3. If permeabilization is not required, proceed to "Secondary antibody labelling and DNA/viability dyes" (Part D).

[C] Permeabilization and intracellular staining:

4. Once all samples have been fixed, permeabilise cells with ice-cold 90% methanol for 30 mins (initially adding drop-wise with gentle vortexing, 100% ice-cold methanol can be found in the freezer leftmost of our bay).
5. Samples can be stored at -20°C in 90% methanol at this stage.
6. Wash with excess incubation buffer 2 times to get rid of methanol.
7. Incubate with 100 ul primary antibody for 30 mins at RT to label intracellular proteins.
8. Wash 2 times with incubation buffer

[D] Secondary antibody labelling and DNA / viability dyes:

9. Incubate cells with 100µL of diluted secondary antibodies (anti-rabbit AlexaFluor 488 and AlexaFluor anti-mouse 647) for 30-45 minutes.
10. Optional DNA/viability dye step
    a. Propidium Iodide – dilute stock Propidium Iodide solution 1:10 to get a working solution.
    b. Incubate cells with 200µL of PI working solution per technical replicate required for 5 mins.
11. Dispense samples onto a flat-bottomed 96-well plate.
12. Count cell samples using Guava EasyCyte 8HT Flow Cytometer.

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 –

8.1 Risk assessments
RA20270 - Flow Cytometry using FACSJazz or Guava

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