Document Number:	**SASoM/METHOD/135.v1**
Title:	**Culture of Peripheral Blood Mononuclear Cells (PBMCs).**
Version:	**v1**
Author:	**Oliver Read**

Effective from:	15/03/2021
Valid to:	15/03/2023

SOP History		
Number	Date	Reason for Change
v1	15/03/2021	Original

1.0 Purpose –

This SOP describes the current procedure for culturing PBMCs (Peripheral Blood Mononuclear Cells) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in primary PBMC cell culture.

3.0 Responsibilities –

All staff involved in PBMC isolation and culture 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 –

The following protocol refers to culturing PBMCs for immediate use. Any experiments that require differentiation / isolation of specific cell sub-populations may require different reagents e.g. media / serum concentration and cytokine supplements.

a) Refer to previous SOP for isolation of PBMCs (SASoM/METHOD/104_Isolation of PBMCs from whole blood samples).
b) PBMCs can either be cultured fresh from isolation, or set up from from frozen aliquots.
c) After removal from liquid nitrogen, thaw the cells and resuspend in RPMI 1640 media supplemented with 10% FBS and 1% Pen/Strep.
d) Dispense the cells at the desired densities into either flasks, dishes, or plates.
e) Place cells in "primary culture" incubator (37°C, 5% CO_2) for 24 hours to allow cells to acclimatize to ex-vivo culture conditions before proceeding with experiments.
f) After 24 hours incubation, proceed with downstream experiments e.g. qPCR, western blot, flow cytometry, etc.:

Points to Note:
PBMCs are a mixture of suspension and adherent cells. Whilst monocytes will adhere to the surface of any plastic they come into contact with, other cell types will remain in suspension. This needs to be taken into account when harvesting cells.

Some experiments may require you to stimulate cells in order to encourage proliferation e.g. T-cell proliferation.

I. T-cell proliferation can be achieved by adding PHA (lectin from Phaseolus vulgarus – Sigma L2769) and LPS (liposaccharide – Sigma L4391)
II. NK (Natural Killer) cell stimulation can be achieved by adding PMA (Phorbol 12-myristate 13-acetate – Sigma P1585) and Ionomycin (Sigma I0634)

NOTE 1: All manipulations with chemicals of hazard rating 4/5 should be done with SHIELDskin? Nitrile / polychloroprene gloves. These gloves are Category III PPE registered and offer a higher level of protection when working with DMSO and other biohazards (AQL 0.65 - EN 374-2:2014 Level 3) .

NOTE 2: Phorbol 12-myristate 13-acetate was purchased in the lowest quantity available (1 mg). This was taken into the TC hood and resuspended in 2 mL of DMSO to give a final concentration of 0.5 mg/mL. Aliquots of 50uL were then transferred to 0.6mL PCR tubes and frozen at -20°C. The container with the tubes inside it was clearly labelled with appropriate hazard stickers.

NOTE 3: A working solution for NK stimulation would be 50 ng/ml (1 in 10,000 dilution of the stock into cell culture media = 50uL in 500mL). As the amount used in

the application is so little the PMA is disposed through regular TC waste after having been neutralised in HazTab for 30mins.

NOTE 4: UNUSED aliquots must be discarded through the University Waste Disposal scheme.

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times. Sharps should be disposed of in sharp-bins

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training –
All personnel using this method need to have received cell-culture and sterile lab-practice training

8.0 Related documents –
8.1 Risk assessments –
RA28660_Isolation and Culture of Peripheral Blood Mononuclear Cells (PBMC)

8.2 Standard Operating Procedures –
SASoM-METHOD-104-Isolation of PBMCs from whole blood samples
SASoM-METHOD-135- Culture of Peripheral Blood Mononuclear Cells (PBMCs)

9.0 Approval and sign off –

Author:

Name: Oliver Read

Position: Post Doc

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