Document Number: **SASoM/METHOD/142.v1**

Title: **Isolation of dendritic cells from PBMC's for in-vitro experiments using the CellXVivo Human Monocyte-derived DC Differentiation Kit.**

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Version: **v1**

Author: **Oliver Read**

Effective from:	24/08/2021
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SOP History		
Number	Date	Reason for Change
v1	24/08/21	Original

1.0 Purpose –

This SOP describes the current procedures for isolating dendritic cells from PBMC's for in-vitro experiments using the CellXVivo Human Monocyte-derived DC Differentiation Kit in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff isolating dendritic cells from PBMC's for in-vitro experiments using the CellXVivo Human Monocyte-derived DC Differentiation Kit in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff isolating dendritic cells from PBMC's for in-vitro experiments using the CellXVivo Human Monocyte-derived DC Differentiation Kit in this manner 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 protocol uses the Human Monocyte-Derived Dendritic Cell Differentiation Kit (R&D Systems #CDK004) for differentiation of PBMCs. The protocol should be read in conjunction with (i) SASoM-METHOD-104-Isolation of PBMCs from whole blood samples and (ii) SASoM-METHOD-106-PBMC culture.

The kit contains the following items, all of which should be stored at 2-8 degrees C under sterile conditions for up to 30 days, or at -20 degrees C to -70 degrees C in a manual defrost freezer for up to 3 months (with the exception of the Reconstitution buffer which should be stored at 2-8 degrees C under sterile conditions for up to 3 months).

- Serum-free dendritic cell base media (#390536).
- Recombinant human IL-4 (#967569).
- Recombinat human GM-MCSF (200x) (#967570).
- Recombinant TNF-a (200x) (#967571).
- Reconstitution buffer 2 (200x) (#967553).

Note: Whilst this kit has been donor screened for some infections agents, however as human sourced material testing cannot be guaranteed to detect all infectious agents, it should be treated as potentially infectious.

Procedure:

1. After isolating PBMCs from whole blood you can optionally attempt to isolate CD14+ cells using a magnetic bead system but this isn't strictly necessary.
2. Reconstitute the recombinant human IL-4 and GM-CSF by adding 500 ul of reconstitution buffer to each vial.
3. Make up an appropriate volume of Differentiation Media which consists of a user-determined volume of serum-free dendritic cell base media with added reconstituted IL-4 and GM-CSF (to a final concentration of 1x).
    - for every 10 ml of base media add 50 ul of each recombinant protein
    - Optional: can add antibiotics to this media at any time
4. Resuspend cells in 1 x 10^6 cells/ml in differentiation media and dispense into desired plate / petri dish/ flask. If going straight from PBMCs (without the initial isolation step) you may want to use a higher concentration of cells as only a small portion of PBMCs will be immature dendritic cells / monocytes (CD14+ cells).
5. Incubate cells in a 37 degrees C, 5% CO2 incubator for 3 days.
6. On day 3 remove half of the media and replace with fresh differentiation media. If cultures were set up straight from PBMCs you may notice floating lymphocytes that may be dead. Try and aspirate these when removing media (monocytes and dendritic cells should be adherent).
7. Incubate for a further 2 days.
8. On day 5 Repeat step 6.
9. On day 7 you should have immature dendritic cells which can then be used for downstream applications.
10. If you wish to mature your dendritic cells you can add recombinant TNF-a (reconstitute with 200 ul of buffer) to the differentiation media for an additional 4 days - this can be useful as a positive control for dendritic cell maturation.

5.0 Personal protection –
A Howie coat must be worn at all times. Suitable dispsable gloves should be worn when handling the contents of the kit since there may be a risk of infection.

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 –
SASoM-METHOD-104-Isolation of PBMCs from whole blood samples.
SASoM-METHOD-106-PBMC culture.
CHARM_RA22396_Preparation of Peripheral Blood Mononuclear Cells.
CHARM_RA22680_Isolation and Culture of Peripheral Blood Mononuclear Cells (PBMC).

9.0 Approval and sign off –

Author:

Name: Oliver Read

Position: Post Doctorate

Signature:  Date: 06/09/2021

Management Approval:

Name: Peter Mullen

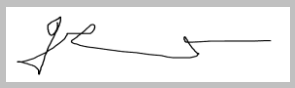
Position: SOP Administrator

Signature:  Date: 06/09/2021

QA release by:

Name: John O'Connor

Position: QA Manager

Signature:  Date:08/09/2021

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