Document Number: **SASoM/METHOD/143.v1**

Title: **Use of the checkpoint inhibitor Nivolumab in cell culture experiments.**

Version: **v1**

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Effective from:	24/08/2021
Valid to:	24/08/2023

SOP History		
Number	Date	Reason for Change
v1	24/08/21	Original

1.0 Purpose –

This SOP describes the current procedures for using the checkpoint inhibitor Nivolumab in cell culture experiments in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff using the checkpoint inhibitor Nivolumab in cell culture experiments in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff using the checkpoint inhibitor Nivolumab in cell culture experiments 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 Nivolumab (Selleckchem #A2002) which is an anti-PD-1 antibody which serves as a checkpoint inhibitor to block interaction of PD-1 and PD-L1 on the surface of cells. This reagent should be used in co-cultures involving PBMCs, natural killer cells, and/or dendritic cells. The protocol should therefore be read in conjunction with (i) SASoM-METHOD-104-Isolation of PBMCs from whole blood samples, (ii) SASoM-METHOD-106-PBMC culture, and (iii) SASoM-METHOD-142-Generation of dendritic cells from PBMC's for in-vitro experiments.

Reagents should be stored at 4 degrees C.

1. Plate out and incubate e.g. HCT116 cancer cells at the desired cell density and leave to settle down for an appropriate time period (e.g 48hrs).
2. After 48 hrs, separately prepare sufficient immune cells (PBMCs, natural killer cells and / or dendritic cells) at an appropriate concentration for co-culture experimental conditions.
3. To those dishes / wells receiving Nivolumab, add a sufficient volume of reagent to the immune cell suspension in order to achieve a final Nivolumab concentration of 10 ug /ml (1:500 dilution from base reagent). You may need to titre the Nivolumab concentration if desired.
4. Remove spent media from the petri dishes containing your cancer cells and then replace with fresh media containing your drug of interest (e. g. gemcitabine).
5. Begin co-culture by adding the immune cell suspension (+ or – Nivolumab) and incubate for the desired time period. For immune activation you can use a positive control of anti CD3/28 which typically causes upregulation of PD-L1 on immune cells.
6. Incubate for experiment specific duration (e.g. 4 days).
7. Proceed with downstream applications.

Note: If performing flow cytometry, you cannot use additional anti-PD-1 since the epitope might be blocked by Nivolumab and so potentially disrupt any fluorescent signal.

5.0 Personal protection –

A Howie coat must be worn at all times.

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.
SASoM-METHOD-142-Generation of dendritic cells from PBMC's for in-vitro experiments.
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: ORead Date: 06/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature: Peter Mullen Date: 06/09/21

QA release by:

Name: John O'Connor

Position: QA Manager

Signature: Date: 08/09/21

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