Document Number: **SASoM/METHOD/139.v1**

Title: **Use of GolgiStop Protein Transport Inhibitor (BD #10716676) to detect proteins / cytokines typically released by cells in vitro**

Version: **v1**

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Effective from:	19/08/2021
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SOP History		
Number	Date	Reason for Change
v1	19/08/21	Original

1.0 Purpose –

This SOP describes the current procedures for using GolgiStop Protein Transport Inhibitor (BD #10716676) to detect proteins / cytokines typically released by cells in-vitro in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM using GolgiStop Protein Transport Inhibitor (BD #10716676) to detect proteins / cytokines typically released by cells in-vitro in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff using GolgiStop Protein Transport Inhibitor (BD #10716676) to detect proteins / cytokines typically released by cells in-vitro 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 –

GolgiStop Protein Transport Inhibitor (BD #10716676) contains monensin (0.26%) and comes as 2 vials of 0.7 ml solution and needs to be stored at 4 degrees C. It should be noted that monensin has been given the highest hazard rating of 5T under the University CHARM system.

When GolgiStop is taken up by cells it inhibits the golgi transport chain, thus preventing packaging and export of proteins/cytokines. Accumulation of these proteins in response to specific stimuli can be quantified by antibody labelling and flow cytometry after cells have been fixed and permeabilised.

Procedure:

1. Plate out suspension culture of (PBMC) cells in a volume of 3mL into 6-well trays at previously optimised cell densities and leave for x hrs / days.
2. Remove media and treat cells using the standard treatment regimens at the appropriate doses / time-points as desired.
3. At the end of the experiment, make up GolgiStop at a concentration of 2uL for every 3 mL of cell culture media containing FCS / Pen Strep (e.g.12uL /18mL media).
4. Remove spent media from each well and replace with diluted GolgiStop (3mL / well). Carefully mix the media in the well and return to incubator.
5. Incubate cells with GolgiStop at 37 degrees in the incubator for 4-6 hours.
6. Remove media and cell suspension from each well and transfer to suitably sized tubes. Centrifuge the tubes (x rpm for y mins) and then discard the supernatant (containing GolgiStop) to a waste bucket containing a HazTab bleach tablet. Leave for 30mins before discarding down the sink with excess cold running water. Remaining cells should be free of GolgiStop.
7. Proceed with standard fixation and permeabilization protocols for intracellular antigen labelling and detection by flow cytometry as detailed in SASoM-METHOD-128-Antibody Detection by Flow Cytometry.
8. Transfer samples to 96-well trays for final analysis by flow cytometry.
9. All samples remaining in the 96-well tray after flow cytometric analysis should be neutralised using Haztabs before the tray is autoclaved / discarded. Sample waste from the flow cytometer should similarly be discarded after exposure to HazTab solution.

Note 1: it is recommended that GolgiStop is not left in cell culture for more than 12 hours as blocking golgi transport for extended time periods is toxic to cells.

Note 2: 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). Other gloves are available, but they MUST meet this standard of protection.

Note 3: All tubes / aliquots of GolgiStop should be clearly labelled with the name of the compound and placed inside a suitable container with appropriate risk labels. Hazard warning labels are available on request.

5.0 Personal protection –
A Howie coat must be worn at all times. Since GolgiStop Protein Transport Inhibitor (BD #10716676) contains monensin, all users must use disposable gloves with higher personal protection as specified in the appropriate Risk Assessment.

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 –
CHARM_RA23758_Use of GolgiStop Protein Transport Inhibitor (BD #10716676)

9.0 Approval and sign off –

Author:

Name: Dr 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