Document Number:	**SASoM/METHOD136.v1**
Title:	**Metabolite Extraction from cell lines for Mass Spectrometry Analysis**
Version:	**v1**
Author:	**Jen Bre**

Effective from:	19/04/2021
Valid to:	19/04/2023

SOP History		
Number	Date	Reason for Change
V1	19/04/2021	Original Document

Controlled

1.0 Purpose –

This SOP describes the current procedure for preparing metabolite samples from cell lines (for subsequent mass spectrometry) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM involved in preparing metabolite samples from cell lines (for subsequent mass spectrometry) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff involved in preparing such metabolite samples from cell lines 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 1: Ice-cold methanol (80%) is provided by the Mass Spec team (Czekster Lab; School of Biology) in order to ensure that the correct (LC-MS) grade of reagents have been used. Under no circumstances should 80% methanol be made up using methanol / water kept in the School of Medicine as this will cause damage to the Mass Spectrometer instrument. Methanol (80%) should be stored in a spark-proof freezer at -20C prior to use. All methanol will be (i) supplied, and (ii) disposed of by the School of Biology (Czekster Lab).

NOTE 2: Methanol (80%) is used in very small quantities (0.5mL/sample)

Method –

1. Follow **SASoM/METHOD/008 'Use of Trypsin for passaging cells in cell culture'** up to the step where the trypsin is neutralised with media and cells have been spun at 1200 rpm for 5min.
2. Remove the media, resuspend cells in 1mL PBS and transfer to 1.5mL Eppendorf tubes.
3. Spin at maximum speed (14,800 rpm) in a small benchtop centrifuge for 5min.
4. **All remaining steps should be carried out on ice . . .**
5. Remove 900uL PBS with a P1000, discard
6. Remove any residual PBS with a P100 without disturbing the pellet.
7. Resuspend / break up the pellet in 500uL of 80% ice-cold methanol (by passing it up and down the pipette) and then vortex thoroughly.
8. Leave the samples at -80C for 20min.
9. Spin at maximum speed (14,800 rpm) in a small benchtop centrifuge for 5min at 4° Celsius.
10. Transfer supernatant into fresh Eppendorf tubes and store at -80C prior to Mass Spec Analysis.
11. Take samples to School of Biology for subsequent analysis / disposal.
12. Store the pellets at -20C for normalisation with Qubit (protein or DNA concentration).

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5.0 Personal protection -

A Howie laboratory coat and lab gloves 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 a paper towel, disinfected with Virkon or Haztabs, and finally wiped with 70% ethanol.

7.0 Training -

All personnel using this method must have received cell-culture and sterile lab-practice training.

8.1 Risk assessments –

RA22963_Preparation of Metabolites for Mass Spec Analysis.

8.2 Standard Operating Procedures –

SASoM/METHOD/008 Use of Trypsin for passaging cells in cell culture

9.0 Approval and sign off –

Author:

Name: Jen Bre

Position: Postdoc

Signature: Jen Bre Date: 19/4/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Peter Mullen Date: 19/4/2021

QA release by:

Name: Peter Mullen

Position: Research Fellow

Signature: Peter Mullen Date: 19/04/2021

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
Jen Bre	Post doc	JB	19/04/21

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