Document Number:	**SASoM/METHOD/008.v5**
Title:	**Use of Trypsin for passaging cells in Cell Culture**
Version:	**v5**
Author:	**Peter Mullen**

Effective from:	01/01/2021
Valid to:	01/01/2023

SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

1.0 Purpose –
This SOP describes the current procedure for passaging 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 with passaging cells for cell culture.

3.0 Responsibilities –
All staff involved in passaging cells 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 –
Thaw and warm an aliquot of 0.05% Trypsin-EDTA (Gibco; 25300-062) to 37ºC in TC waterbath.

Remove medium from attached cells which are being passaged and wash with warmed sterile PBS.

Pour off PBS.

Add trypsin (approximately 5mL) to the TC flask / petri dish containing the attached cells, the volume required will depend of size of flask being used.

Flasks can then be returned to CO_2 incubator for a few minutes - you should check cells after a couple of minutes to see if they are detaching from each other and "rounding up". Once this starts to happen, tap flask gently and cells will detach from flask and fall into trypsin solution.

Remove trypsin solution containing cells and transfer to a centrifuge tube.

Top up centrifuge tube with medium, this will help neutralise the trypsin and stop it from damaging cells.

Spin cells at 1200PRM for five minutes..

After centrifugation, cells will have formed a pellet at bottom of centrifuge tube. Gently pour of the solution and discard.

Using a sterile pastette, add a small volume of media and break up the pellet of cells. Add more medium to cells to bring up to an appropriate volume..

Cells now ready to be used for further experiments or can be returned to TC flask if you wish to maintain growing the cell line.

5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 complete an in house induction to the tissue culture area and be trained in sterile TC techniques before starting any TC work.

8.0 Related documents –

8.1 Risk assessments COSHH/004 and RA/BIOL/001

8.2 SOP - SASoM/METHOD/002
Making up routine DMEM / RPMI Media (10% FCS) for Cell Culture

8.3 SOP - SASoM/METHOD/005
Heat inactivation of FCS

8.4 SOP - SASoM/METHOD/007
Aliquoting TC Supplements

8.5 SOP - SASoM/METHOD/011
Freeze mixture for Cell Culture

9.0 Approval and sign off –

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