Document Number:	**SASoM/METHOD/006.v5**
Title:	**Preparation of Protein Lysates from Adherent Cell Cultures**
Version:	**v5**
Author:	**Peter Mullen**

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

SOP History		
Number	Date	Reason for Change
v1	01/01/13	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 preparing Protein Lysates from Adherent Cell Cultures 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 the preparation of protein lysates from adherent cell cultures.

3.0 Responsibilities –
All staff involved in the preparation of protein lysates from adherent cell cultures 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 –

Lysis Buffer Preparation

Make up the following stock solutions–

500mM Tris, pH7.5 - add 6.05g Tris Base to a beaker, add approximately 60ml of distilled water and then adjust to pH7.5. Make up to a final volume of 100ml with distilled water.

50mM EGTA, pH8.5 - add 1.902g of ethylene glycol tetraacetic acid (EGTA) to a beaker with approximately 60ml of distilled water and then adjust to pH8.5 (first with 10M Sodium Hydroxide (NaOH), and then with 1M NaOH). Only after NaOH has been added will the EGTA go into solution! Make up to a final volume of 100ml with distilled water.

500mM NaCl - add 2.922g Sodium Chloride (NaCl) to a beaker and make up to a final volume of 100ml with distilled water.

To make up stock '*Lysis Buffer*' combine 30ml of 500mM Tris (pH7.5), 30ml of 50mM EGTA (pH8.5) and 90ml of 500mM NaCl. Add a further 150ml of distilled water and mix thoroughly. Aliquot out into 30 x 10ml aliquots and label as 'Lysis Buffer' (final concentrations are 50mM Tris pH7.5; 5mM EGTA pH8.5; 150mM NaCl).

Cell Culture

Grow cells in a sterile 1x14cm diameter petri dish for maximum protein yield (or 1x175cm cell culture flask) until 80-90% confluent. Do not harvest lysates if cells have reached confluence.

Lysis Preparation

It is imperative to keep all samples, buffers and inhibitors on ice at all times throughout the procedure!

Thaw out one aliquot (10ml) of Lysis Buffer (50mM Tris pH7.5; 5mM EGTA pH8.5; 150mM NaCl) and leave on ice before adding the remaining components.

- One 'Complete Protease Inhibitor Tablet' (Roche, 11836153001; stored at 4ºc).
- 100µl of Phosphatase Inhibitor Cocktail 2 (Sigma, P5726-1mL; stored at 4ºC).
- 100µl of Phosphatase Inhibitor Cocktail 3 (Sigma, P0044-1mL; stored at 4ºC).
- 50 µl of Aprotinin (A6279; stored at 4ºC).
- 100µl 1% Triton X-100.

Vortex and return to ice, use within 1-2hrs.

Decant media from the petri dish / flask.

Wash the cells with 25 ml ice-cold PBS.

Decant PBS and remove ALL residual liquid with a pipette.

Add 400µl of fully-supplemented Lysis Buffer as detailed above.

Scrape all the cells off the plastic using a 'Cell Lifter' (eg Costar; 3008) and then sit the petri dish on ice at an angle so that the cell suspension collects at the bottom of the dish. Leave for 5-10mins for cells to lyse.

Transfer lysate to a pre-cooled microcentrifuge tube and spin at 13,000g for 6 min at 4ºC (Hereaus Fresco microcentrifuge).

Remove the supernatant and transfer to a fresh microcentrifuge tube. Label and store samples at -80ºC until ready to perform protein assay.

Protein concentration can then be calculated by BCA protein assay.

For RPPA analysis we aim to spot samples in serial dilutions starting from 2mg/ml. As a guideline, lysates must therefore have a protein concentration of greater than 2666 µg/ml if this is to be achieved (which should not be a problem with cell lines).

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 under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/011 and RA/BIOL/004

8.2 SOP - SASoM/METHOD/023
Protein Determination by the Bicinchoninic Acid (BCA) Assay.

9.0 Approval and sign off –

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