Document Number:	**SASoM/METHOD/102.v2**
Title:	**Preparation of Protein Lysates from Adherent Cell Cultures Using 'RIPA' buffer**
Version:	**v2**
Author:	**Peter Mullen**

Effective from:	03/04/2020
Valid to:	02/04/2022

SOP History		
Number	Date	Reason for Change
v1	01/01/2018	Original
V2	03/04/2020	Update

1.0 Purpose –

This SOP describes the current procedure for preparing Protein Lysates from Adherent Cell Cultures using 'RIPA' buffer 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 using the 'RIPA' buffer.

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 (100mL each) –

5M NaCl - add 29.2g Sodium Chloride (NaCl) to a beaker and make up to a final volume of 100ml with distilled water.

0.5M EDTA, pH8.0 - add 14.6g of ethylenediaminetetraacetic acid (EDTA) to a beaker with approximately 60ml of distilled water and then adjust to pH8.0 (first with 10M Sodium Hydroxide (NaOH), and then with 1M NaOH). Only after NaOH has been added will the EDTA go into solution! Make up to a final volume of 100ml with distilled water.

1M Tris, pH8.0 - add 12.1g Tris Base to a beaker, add approximately 60ml of distilled water and then adjust to pH8.0. Make up to a final volume of 100ml with distilled water.

10% (w/v) Sodium deoxycholate – add 10g Sodium deoxycholate to a beaker and add distilled water to a final volume of 100mL.

10% (w/v) SDS – Carefully weigh out 50g of Sodium Dodecyl Sulfate (wearing a face mask) into a 500mL bottle and then make up to a final volume of 500mL with distilled water.

Make up stock 'RIPA *Lysis Buffer*' by combining 15ml of 5M NaCl; 5mL of 0.5M EDTA, pH 8.0; 25ml of 1M Tris, pH 8.0; 5mL of NP-40; 25mL of 10% sodium deoxycholate and 5mL of 10% SDS. Make up to 500mL with distilled water and mix thoroughly. This can be summarised in the table below:

Stock	Volume	Final Concentration
5M NaCl	15mL	150mM
0.5M EDTA, pH 8.0	5mL	5mM
1M Tris, pH 8.0	25mL	50mM
NP-40 (IGEPAL CA-630)	5mL	1%
10% sodium deoxycholate	25mL	0.5%
10% SDS	5mL	0.1%
Distilled Water	420mL	

This stock buffer should be stored at 4˚ prior to use.

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!

Remove one aliquot (10ml) of stock RIPA Lysis Buffer (made up as described previously) 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).
- 100 µl Triton-X-100 (Sigma, X100-100mL: stored at room temp.)

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

Decant media from the petri dish / flask.

Wash the cells twice 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 10-15mins 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.

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 undergo 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 –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

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