Document Number:	**SASoM/METHOD/108.v2**
Title:	**Preparation of Protein Lysates from Adherent Cell Cultures Using New England Biolabs (NEB) Cell Lysis Buffer.**
Version:	**v2**
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Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	01/11/2018	Original
V2	11/11/2020	Updated

1.0 Purpose –

This SOP describes the current procedure for preparing Protein Lysates from Adherent Cell Cultures using New England Biolabs (NEB) Cell Lysis 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 New England Biolabs (NEB) cell lysis buffer.

3.0 Responsibilities –

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

This Cell Lysis Buffer is used to lyse cells under non-denaturing conditions. This product is stable for 24 months when stored at-20°C. Cell Lysis Buffer can be stored at 4°C for a short period of time (1-2 weeks). This product (15mL) supplies enough 10X material to make 150 mL of whole cell extract. Once made up, the lysis buffer will have the following composition:

1X Cell Lysis Buffer:
20 mM Tris-HCl (pH 7.5)
150 mM NaCl
1 mM Na_2EDTA
1 mM EGTA
1% Triton
2.5 mM sodium pyrophosphate
1 mM β-glycerophosphate
1 mM Na_3VO_4
1 µg/ml leupeptin

Materials:

- Cell Lysis Buffer 10X (New England Biolabs; #9803).
- PhosSTOP™ phosphatase inhibitor cocktail (Roche; #04906845001).
- cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Roche; #11836170001).
- AEBSF (Sigma; SBR00015).
- Elga Water.

Directions for use:

- If the buffer will be used regularly, it is recommended that the 10X buffer be kept at 4°C for 1-2 weeks. For longer periods of time (more than 1-2 weeks), buffer should be stored at -20°C. Aliquotting of 10X buffer is recommended if many small experiments are to be performed.
- Thaw 10X buffer (1mL) at 24-30°C, mixing end-over-end and transfer to a suitable container.
- Add distilled water (9mL) and then chill on ice.
- Add cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (1 tablet)
- Add PhosSTOP™ phosphatase inhibitor cocktail (100µL)
- Add AEBSF (100µL).
- Ensure that the contents are all dissolved before use. Keep on ice until ready to use.

Cell Culture:

- Grow cells in suitably sized sterile plastic dishes or flasks (e.g. 1x14cm diameter petri dish or 1x175cm cell culture flask for maximum protein yield)

until 80-90% confluent. Do not harvest lysates if cells have reached confluence.

- 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 and aliquots prepared for western blot in the usual manner.

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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