Document Number:	**SASoM/METHOD/022.v5**
Title:	**Protein Extraction from Human Tissue and Primary Xenografts**
Version:	**v5**
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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 extracting protein from human tissue samples and primary xenograft samples 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 protein extraction from human tissue and primary xenografts.

3.0 Responsibilities –

All staff involved in protein extraction from human tissue and primary xenografts 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 –

Buffer Preparation

Make up stock 500mM Tris, pH7.5 by adding 6.05g Tris Base to a beaker, adding approximately 60ml of distilled water and then adjusting to pH7.5. Make up to a final volume of 100ml with distilled water.

Make up stock 50mM EGTA (ethylene glycol tetra-acetic acid), pH8.5 by adding 1.902g of EGTA to a beaker, adding approximately 60ml of distilled water and then adjusting to pH8.5 (first with 10M 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.

Make up stock 500mM NaCl by adding 2.922g NaCl to a beaker and make up to a final volume of 100ml with distilled water.

Make up stock 'Lysis Buffer' by combining 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).

Sample 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 pre-cool on ice before adding the remaining components. DO NOT include Triton X100 at this stage as it will froth and make homogenization impossible. This should be sufficient for eight samples.

To the buffer add the following:-

- One 'Complete Protease Inhibitor Tablet' (Roche, 11836153001; stored at 4ºC).
- 100µL of Phosphatase Inhibitor Cocktail A (Sigma, P2850; stored at 4ºC).
- 100µL of Phosphatase Inhibitor Cocktail B (Sigma, P5726; stored at 4ºC).
- 50 µL of Aprotinin (stored at 4ºC).

Weigh out 100 - 250mg (ideally 150mg) of tissue and transfer to a 50x12mm soda glass homogenization tube on ice (Fisher: TUL-460-031N).

Add 0.3ml ice-cold homogenisation buffer and place the glass tube inside a small beaker of ice. Homogenisation should be carried out with the sample held on ice to avoid excessive heat build up.

Homogenise at full power with Silverson homogeniser for 2 x 15sec (with 1min interval between bursts to allow sample to cool down).

Transfer homogenate to a pre-cooled 2ml microcentrifuge tube.

Add a further 0.3ml of fresh buffer to the original glass tube and homogenise briefly to wash the barrel. Transfer homogenate to the same microcentrifuge tube (total volume = 0.6ml).

Add a further 0.3ml of fresh buffer to the original glass tube and homogenise briefly to wash the barrel. Transfer homogenate to the same microcentrifuge tube (total volume = 0.9ml).

Add 1% Triton X-100 (9µl / 0.9ml).

Vortex briefly but gently.

Incubate for 10min on ice, mixing the samples periodically.

Spin at 13,000g for 30min at 4ºC (Sorval Biofuge Fresco).

Remove supernatant and transfer to a fresh microcentrifuge tube.

Label and store samples at -70ºC until ready to perform protein assay.

Calculate protein concentration 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 under go training in this technique before performing procedure

8.0 Related documents –

8.1 Risk assessments COSHH/011
RA/BIOL/003 and 004
RA/GEN/020

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

8.3 SOP SASoM/METHOD/048
Use and Maintenance of the Homogenisers

9.0 Approval and sign off –

Author:		
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