Document Number:	**SASoM/METHOD/112.v1**
Title:	**Preparation of Cell Lines for HPLC analysis of GSSG and GSH**
Version:	**v1**
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SOP History		
Number	Date	Reason for Change
v1	01/12/18	Original

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 –

Reagent Preparation:

Prepare a 12 mM MTT stock solution by adding 1 mL of sterile PBS to one 5 mg vial of MTT (Component A). Mix by vortexing or sonication until dissolved. Occasionally there may be some particulate material that will not dissolve; this can be removed by filtration or centrifugation. Each 5 mg vial of MTT provides sufficient reagent for 100 tests, using 10 µL of the stock solution per well. Once prepared, the MTT solution can be stored for four weeks at 4°C protected from light.

Add 10 mL of 0.01 M HCl to one tube containing 1 gm of SDS (Component B). Mix the solution gently by inversion or sonication until the SDS dissolves. Once prepared, the solution should be used promptly. Each tube makes sufficient solution for 100 tests, using 100 µL per well.

Culturing Cells:

The culture conditions used to grow the cells can affect the results and must be taken into consideration when analysing the data. The age of the cultures, number of passages and details of the growth medium can all be important factors. Natural variation in the requirements and growth rates of different cell lines make it difficult to provide precise guidelines for preparing your cells. In general, cells seeded at densities between 5000-10,000 cells per well should reach optimal population densities within 48-72 hours. Note that the presence of phenol red in the final assay samples can seriously affect results. ***We strongly recommend that the cells be cultured in medium free of phenol red, if possible. Alternatively, the final incubation with the MTT can be performed after exchanging the cells into medium free of phenol red.***

Labeling Cells:

For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium.

Add 10 µL of the 12 mM MTT stock solution (prepared in step 1.1) to each well. Include a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone.

Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours.

Add 100 µL of the SDS-HCl solution (prepared in step 1.2) to each well and mix thoroughly using the pipette. *You can use different solvents to dissolve formasan crystals, including DMSO, sodium dodecyl sulphate, n-propanol acidified with a few drops of HCl, or ethanol acidified with few drops of HCl. The protocol may therefore need a bit more optimisation.*

An older protocol (Peter Twentyman et al, 1987) suggests carefully aspirating the MTT solution before adding 200uL of DMSO, mixing for 5mins and then reading on a platereader at 570nm.

Similarly, other protocols suggest blotting off the media on paper towels to remove residue if necessary and then resuspending the formazan (MTT metabolic product) in 200µl of DMSO. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the formazan into the solvent.) Read optical density at 560 / 570nm and subtract background at 670nm.

Incubate the microplate at 37°C for 2 hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay.

Mix each sample again using a pipette and read absorbance at 570 nm.

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 –
RA-COSHH-007-Fixation and staining of cells;
RA-COSHH-008- Immunohistochemistry-fluorescence

8.2 SOP -
SASoM-METHOD-103-Preparation, culture, fixation and immunocytochemistry of cells on Poly-D-lysine coated coverslips

9.0 Approval and sign off –

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