Document Number:	**SASoM/METHOD/066.v4**
Title:	**Preparation of 5X Loading buffer for Western Blots**
Version:	**v4**
Author:	**Peter Mullen**

Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	15/07/2014	Original
V2	15/07/2016	Biennial Review
V3	15/07/2018	Update
V4	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for preparing 5x Loading buffer for western blots in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in western blot work in Lab 248.

3.0 Responsibilities –
All staff involved in cell culture 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 SOP details how to make up denaturing / loading buffer for western blots. The final composition of the buffer is 50mM Tris base, pH6.8, 1% SDS, 10% glycerol, 5% Mercaptoethanol and 0.01% bromophenol blue.

2M Tris buffer, pH6.8 (stock)

moles = Molarity x Litres
= 2 (2M) x 100x10^{-3} L(100mL)
= 0.2moles

Grams = moles x gfw
= 0.2 x 121.14
= 24.228g

ie 24.228g in total volume of 100mL = 2M

1. Prepare 2M Tris buffer by adding 24.228g of Tris base to a beaker. Add approximately 50mL of distilled water (green Elga taps) and correct to pH8.6 by adding HCl. As a result of the HCL being added, the temperature (and pH) of the buffer may increase. Allow the buffer to cool down before re-adjusting if necessary. Make up to a total volume of 100mL.

2. Make up Loading Buffer (in the fume cabinet if necessary) as follows:

	3X (100mL)	**5X (100mL)**
Tris (2M, pH6.8 stock)	7.5mL	12.5mL
SDS	3g	5g
Glycerol	30mL	50mL
Mercapthoethanol	15mL	25mL
Bromophenol Blue (saturated)	1.5mL	2.5mL
DW	to 100mL	to 100mL

3. Aliquot into 50 x 2mL aliquots (cryovials) and freeze at -20°C.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

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 warm water.

7.0 Training -

8.0 Related documents –

8.1 Risk assessments – RA18265 (SDS PAGE Western Blotting)

9.0 Approval and sign off –

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