Document Number:	**SASoM/METHOD/068.v3**
Title:	**Preparation of 4X Denaturation buffer for RPPA**
Version:	**v3**
Author:	**Peter Mullen**

Effective from:	12/08/2018
Valid to:	11/08/2020

SOP History		
Number	Date	Reason for Change
v1	12/08/2014	Original
V2	12/08/2016	Biennial Review
V3	12/08/2018	Update

1.0 Purpose –
This SOP describes the current procedure for preparing 4X Denaturation buffer for RPPA 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 RPPA 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 4X denaturation buffer for RPPA serial dilutions.

The composition of the (4X) buffer is as follows:
250mM Tris, pH6.8 (final concentration when diluted will be 62.5mM)
8% SDS (final concentration when diluted will be 2%)
35% glycerol (final concentration when diluted will be 8.75%)
10% Mercaptoethanol (final concentration when diluted will be 2.5%)

1. Make up a stock 1M Tris buffer by adding 12.114g of Tris base to a beaker. Add approximately 50mL of distilled water (green Elga taps) and correct to pH6.8 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 final volume of 100mL.

1M Tris buffer, pH6.8 (stock)
moles = Molarity x Litres
= 1M x 100x10-3 Litres
= 0.1moles

grams = moles x gfw
= 0.1 x 121.14
= 12.114g
ie 12.114g in total volume of 100mL = 1M stock

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

	4X (100mL)
Tris (1M, pH6.8 stock)	25mL
SDS	8g
Glycerol	35mL
Mercapthoethanol	10mL
DW (green Elga taps)	make up to 100mL

3. Store at room temperature.

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 – RA/COSHH/028 (RPPA)

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Mary Wilson

Position: Laboratory Manager

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date: