Document Number: **SASoM/METHOD/052.v5**

Title: **Agarose Gels**

Version: **v5**

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Effective from:	19/04/2021
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SOP History		
Number	Date	Reason for Change
v1	29/08/2013	Original
v2	01/06/2015	Update
v3	01/06/2017	Update
V4	01/06/2019	Update
V5	19/04/2021	Update

1.0 Purpose –

This SOP describes the current procedure for preparing Agarose Gels for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in Laboratory 248 at the St Andrews School of Medicine (SASoM) involved in running Agarose Gels.

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 –

Preparation of Reagents:

0.5M EDTA (pH 8.0) - Add 46.525g of EDTA to a large glass beaker and add 150ml of deionized water. Mix with a magnetic stirrer before adjusting to pH8 by adding concentrated NaOH until the solution becomes clear. Adjust the final volume to 250mL with deionized water. (NB Make sure that you have safety glasses on when using concentrated NaOH).

TBE Buffer (x5) - Dissolve 54g of Tris base (mw = 121.14) and 27.5g of boric acid (mw = 61.83) in approximately 800mL of deionized water. Add 20mL of 0.5 M EDTA (pH 8.0) and adjust final volume to 1L. Once prepared, dilute 1:10 (0.5X concentration) prior to use. TBE is stable at 4°C for 3 months.

For a 1% Agarose gel 1% (w/v):

1. Add 1g of agarose to 100 mL of 1X TBE Buffer.
2. Microwave for 3 minutes on 80% power and leave to cool until reasonably hot to touch. CAUTION! agarose contents can become very hot and boil over when shaken or vigorously swirled.
3. Add 5uL of 'SYBR Safe' DNA gel stain (Invitrogen; P0N S33102) and mix carefully by gentle swirling.
4. Pour into a large, horizontal gel chamber containing a well-comb and leave to set for 20 min at RT.
5. Fill the tank with 1xTBE buffer (to the maximum fill line) and remove the well-comb.
6. Prepare DNA samples by adding an appropriate amount of Bromophenol Blue 6x DNA sample loading buffer (NEB, Hitchin, UK) (11 mM EDTA, 3.3 mM Tris-HCl, 0.017% SDS, 0.015% Bromophenol Blue, pH 8.0).
7. Load 5-20µl of each sample into each well.
8. In a separate lane, add 5µl Hyperladder I (Bioline) as a molecular weight marker.
9. Separate DNA bands at 60V for 30-45 min and visualized under UV light using a Bio-Rad Molecular Imager Gel Doc XR System (Bio-Rad, Hertforshire, UK) in lab 249.
10. Discard aqueous waste to appropriate container and solid waste to red bin.

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 water.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments – RA20918 – Mycoplasma Testing
Gen – 006 - Microwave Ovens
Gen – 009 - General Laboratory Safety
Gen – 017 - Balances & pH Meters
Gen – 037 - Electrophoresis Equipment

8.2 SOP - Equip – 022 - Gilson Pipettes
Equip – 032 – Biorad Power Pacs
Equip – 072 – Sartorius BL610 Balance

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 19/04/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 19/04/2021

QA release by:

Name: Peter Mullen

Position: QA Manager

Signature:  Date: 19/04/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE