Document Number: **SASoM/METHOD/141.v1**

Title: **DNA & RNA hydrolysis for analysis by mass spectrometry.**

Version: **v1**

Author: **Jennifer Bre**

Effective from:	24/08/2021
Valid to:	24/08/2023

SOP History		
Number	Date	Reason for Change
v1	24/08/21	Original

1.0 Purpose –

This SOP describes the current procedures for performing DNA & RNA hydrolysis for analysis by mass spectrometry in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing DNA & RNA hydrolysis for analysis by mass spectrometry in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing DNA & RNA hydrolysis for analysis by mass spectrometry 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 –

Reagents & buffers
Alkaline phosphatase #18011015 - Thermo
DNAse I # 4716728001 - Roche
Venom phosphodiesterase # P3134 - Sigma
S1 nuclease # M5761 - Promega
Calf intestinal phosphatase #M0525 - NEB
LC-MS water: provided by CMC group (biology)

DNA buffer

Tris HCl 20 mM pH7.9	240mg
NaCl 100 mM	584 mg
MgCl2 20 mM	190.4 mg

in 100 mL dH2O

RNA buffer

100 mM Tris HCl	1.2 g
50 mM NaCl	292 mg
10 mg MgCl2	95.2 mg

In 100 mL dH20
Store both on bench

For DNA hydrolysis
3.75 mL DNA buffer + 1 µL alkaline phosphatase + 25 µL DNAse I + 1.5 µL venom
Keep leftover in freezer for next batch of samples

For RNA hydrolysis
Per sample: 142 µL RNA buffer + 3.2 µL S1 nuclease + 5 µL CIP

Protocol

1. DNA and RNA to be collected according to the relevant SOPs as detailed in (i) SASoM-METHOD-129-DNA extraction using the QIAmp DNA Kit and (ii) SASoM-METHOD-093-Preparation of RNA using the Qiagen RNeasy kit.
2. Nanodrop for quantification

N.B *Amounts required vary based on what target you are looking for (i.e 3 µg DNA for dFdC but 10 µg for FdUr) - talk with Alison and Jen.*

3. Add the nucleic acid in a total volume of 10 µL in LC-MS water (it is possible to go up to 50 µL if the starting concentration is low to start with)
4. Add 150 µL hydrolysis buffer
5. Briefly vortex
6. Incubate at 37°C overnight (16h min) in a thermocycler
7. Prepare samples spreadsheet for mass spec analysis

5.0 Personal protection –
A Howie coat 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 detergent.

7.0 Training –
All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –
8.1 Risk assessments –
RA12771: DNA & RNA hydrolysis for analysis by mass spectrometry

9.0 Approval and sign off –

Author:

Name: Jennifer Bre

Position: Post Doctorate

Signature:  Date: 06/09/21

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 06/09/21

QA release by:

Name: John O'Connor

Position: QA Manager

Signature:  Date: 07/09/2021

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