Document Number: **SASoM/METHOD/140.v1**

Title: **DNA extraction using the QIAmp DNA kit.**

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 extractions using the QIAmp DNA kit in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing DNA extractions using the QIAmp DNA kit in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing DNA extractions using the QIAmp DNA kit 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 –
DNA extraction with QIAamp DNA kit (Qiagen; #51304):

1. Trypsinize cells
2. After cells have detached from the dish, collect them in medium, and transfer the cells to a 1.5ml RNAse-free microcentrifuge tube. Centrifuge for 5 min at 300 x g. Remove the supernatant completely.
3. Resuspend cell pellet in PBS to a final volume of 200ul.
4. Add 20uL proteinase K and 10uL of RNAse A.
5. Add 200uL Buffer AL to the sample. Mix by pulse-vortexing for 15s. Incubate at 56°C for 10 min.
6. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
7. Add 200uL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
8. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
9. Add 500uL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
10. Add 500uL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
11. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
12. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Add 30 or 50uL Buffer AE or distilled water (depending on how concentration of DNA required). Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

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 –
RA23770: DNA extraction using the QIAmp DNA kit.

9.0 Approval and sign off –

Author:

Name: Jennifer Bre

Position: Post Doctorate

Signature:  Date: 06/09/21

Management Approval:

Name: Peter Mullen

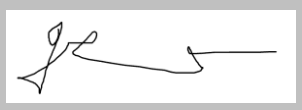
Position: SOP Administrator

Signature:  Date: 06/09/21

QA release by:

Name: John O'Connor

Position: QA Manager

Signature:  Date: 07/09/21

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