Document Number:	**SASoM/METHOD/093.v3**
Title:	**Preparation of RNA using the Qiagen RNeasy kit**
Version:	**v3**
Author:	**Peter Mullen**

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Valid to:	12/04/2022

SOP History		
Number	Date	Reason for Change
v1	13/04/2016	Original
v2	13/04/2018	Update
v3	13/04/2020	Biennial Update

1.0 Purpose –

This SOP describes the current procedure for preparing RNA from cell lines using the Qiagen RNeasy kit (product #74104 / instruction manual Number 2) in lab 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

The scope of this document is to describe the procedure for preparing RNA from cell lines using a commercially available Qiagen kit.

3.0 Responsibilities –

All staff involved in preparing RNA from cell lines using a commercially available Qiagen kit 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 –

RNA Extraction

1. Set up cells in 10cmØ petri dishes and allow to reach 70-80% confluence. If hypoxic cells are required, transfer cells to Hypoxystation 24hrs after initial setup and then leave until desired confluence is reached. This can be scaled down if required.
2. Discard media and wash cells in excess **PBS**.
3. Discard PBS and then place the dishes on ice at an angle to allow any residual liquid to collect in a pool at the bottom of the dish. Remove the last remaining PBS using a sterile pastette.
4. Add 10µL of β -Mercaptoethanol to 1000µL of RLT buffer. Add 600µL of **RLT** buffer (with β-Mercaptoethanol) to the dishes and then scrape the cells off the dish with a sterile cell scraper. Allow the cells to then lyse in the dish.
5. *As an alternative to point [3] above, cells can be washed in PBS, trypsinised, made up in excess culture media and then centrifuged at 1200rpm for 5mins to yield a pellet. This pellet can then be lysed in 600µL of **RLT** buffer (with β-Mercaptoethanol as above) before proceeding to the next step [6].*
6. Transfer the lysates into sterile micro-centrifuge tubes and vortex for 1min.
7. Pipette the lysate directly into a 'QIA Shredder' (Qiagen #79654) placed inside a 2mL collection tube. Centrifuge for 2min at full speed.
8. Remove the shredder column, place the lid on the tube and then store at -70°c or -80°c until ready to continue with RNA Isolation **OR** continue with RNA Isolation as described below.

RNA Isolation

1. *Make up sufficient 70% ETOH (14mL of ETOH + 6mL RNAse-free DW = 20mL).*
2. *Add ETOH to the RPE Buffer and indicate on the RPE bottle that this has been done!*
3. *Prepare RNase-Free DNase 1 (Qiagen #79254) by injecting 550uL RNAse-free water into the DNAse I vial using an RNAse-free needle and syringe. Mix gently by inverting the vial - Do not vortex. For long term storage of this solution, divide it into single-use aliquots and store at –20 for up to 9 months. Thawed aliquots can be stored at 2-8C for up to 6 weeks. Do not refreeze aliquots after thawing.*

4. Add 600µL of 70% ETOH to each lysate and mix well by pipetting up and down – DO NOT VORTEX!
5. Transfer up to 700µL of the sample to an 'RNeasy Spin Column' placed inside a 2mL collection tube. Close the lid and centrifuge at 10,000rpm for 15secs. Carefully discard the 'flow through' and then place the column back inside the same 2mL collection tube.

6. Add (pool) any remaining sample(s) to the respective column(s) before again spinning at 10,000rpm for 15secs. Carefully discard the 'flow through' and then place the column back inside the same 2mL collection tube.
7. DNAse Treatment *(Qiagen kit #79254) – This Step is Optional!*
    - Add 350µL Buffer RW1 to RNeasy column, close lid, centrifuge for 15sec at 10,000 rpm. Discard flow-through.
    - In a separate tube, add 10µL DNAse I stock solution to 70µL Buffer **RDD**. Mix by gently inverting the tube. Centrifuge briefly.
    - Add **DNAse I incubation mix** (80µL) directly to RNeasy column membrane, and leave on the benchtop (room temperature) for 15min.
    - Add 350µL **RW1** to RNeasy column, close lid, centrifuge for 15sec at 10,000 rpm. Discard flow-through.

    - **NB: If you carry out this DNAse step [7], now proceed directly to step [9] and skip step [8].**

8. Add 700µL of RW1 Buffer to the column and spin at 10,000rpm for 15secs to wash the column membrane. Carefully discard the 'flow through' and then place the column back inside the same 2mL collection tube.
9. Add 500µL of **RPE** Buffer / ETOH to the column and spin at 10,000rpm for 2mins to wash the spin column membrane. Carefully transfer the column to a fresh 2mL collection tube (discarding the old collection tube with the 'flow-through').
10. Close the lid of the (empty) collection tube and spin at full speed for 1 minute.
11. Place the RNeasy Spin Column in a new 1.5mL collection tube (Eppendorf tube).
12. Add 50µL of RNAse-free water directly to the spin column membrane. Close the lid and centrifuge at 10,000rpm for 1min to elute the RNA. Discard the filter column.
13. Store at -80°c until ready to quantify the RNA (using the Nanodrop).

5.0 Personal protection -

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

6.0 Spillages -

Always clean up and disinfect any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training -

All staff should undergo training in this technique before performing procedure.

8.0 Related documents – Qiagen RNeasy kit (product #74104) Instruction Manual.

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
Management Approval:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
QA release by:		
Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date:

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