Document Number:	**SASoM/METHOD/114.v1**
Title:	**rtPCR using Qiagen QuantiTect reverse transcription kit**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	10/04/2020
Valid to:	09/04/2022

SOP History		
Number	Date	Reason for Change
v1	10/04/20	Original

1.0 Purpose –

This SOP describes the current procedure for carrying out rtPCR using the Qiagen QuantiTect reverse transcription kit in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM carrying out rtPCR using the Qiagen QuantiTect reverse transcription kit in in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing rtPCR using the Qiagen QuantiTect reverse transcription 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 –

RNA extraction using the Qiagen RNeasy kit (product #74104):
Cultured cells are washed in ice-cold PBS, lysed in 600uL of Qiagen RLT buffer (containing 6uL of beta-Mercaptoethanol) and then passed through a QIA Shredder (Qiagen #79654).

After brief centrifugation, 70% ETOH (600uL) is added to each lysate and mixed by pipetting up and down before transferring to an RNeasy Spin Column placed inside a 2mL collection tube. Flow though is discarded after centrifugation at which point DNAse treatment can be performed if required (separate Qiagen kit #79254).

The column is then washed with RW1 buffer, then RPE Buffer / ETOH, and the sample finally eluted in 50uL of RNAse free water. [This product contains RLT Buffer (guanidinium thiocyanate), RPE Buffer and RW1 Buffer (guanidinium thiocyanate, ethanol)].

Reverse Transcription using the Qiagen QuantiTect reverse transcription kit (No.205311):
Template RNA is thawed on ice whilst all kit reagents are brought to room temp. Genomic DNA is removed with a gDNA wipeout step by combining up to 1ug of template RNA with 2uL of gDNA wipeout buffer in a final volume of 14uL of RNAse-free water.

After heating at 42 degrees C for 2 minutes, reverse transcription is carried out on ice by combining 1uL RT primer mix, 1 uL QuantiTect reverse transcriptase, 4uL QuantiTect RT buffer and then heating at 42 degrees C for 15 minutes.

The reaction is stopped by moving samples to a heat block at 95 degrees C for 3 minutes after which you can proceed with qPCR or store the cDNA samples at -20 degrees C until ready for use. [This product contains RT Primer Mix, RT Buffer 1, Reverse Transcriptase and gDNA Wipeout Buffer (Deoxyribonuclease)].

qPCR using Qiagen QuantiNova SYBR Green PCR kit (No.208054):
Template cDNA and kit reagents are thawed at room temp before making up 20uL of PCR reaction mix (QuantiNova SYBR Green PCR Master Mix / Forward Primer / Reverse Primer / Template cDNA made DNAse free water) in either (i) 0.2 ml tubes or (ii) Qiagen strip-tubes depending on which rotor you are using in the instrument. Analysis is performed by measuring real time (rt) green fluorescence using the Rotor-Gene Q / Rotor-Gene Q software. [This product contains SYBR Green (ethoxylated nonylphenol, sucrose, glycerol].

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 –
RA20163 (rtPCR using Qiagen Kits)

8.2 SOPs –
SASoM-METHOD-093-Preparation of RNA using the Qiagen RNeasy k

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

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