Document Number:	**SASoM/METHOD/115.v1**
Title:	**Total RNA Isolation using TRI Reagent**
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 Total RNA Isolation using TRI Reagent 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 Total RNA Isolation using TRI Reagent in in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff performing Total RNA Isolation using TRI Reagent 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 –

Seeding and treating cells will be carried out as per individual experiments. Sufficient cells (6 x T175 flasks per condition) should be grown until they reach 80% confluence.

Total RNA Isolation: (TRI Reagent AM9738):

More detailed information can be found in the TRI Reagent whci accompanied the product.

1. Cells will be trypsinised, counted, pooled and made as 2 pellets (control and treated).
2. Add 3ml of TRI reagent (1ml for per 5-10 million cells) to each pellet and vortex immediately.
3. Incubate for 5 min at room temp.
4. (optional) centrifuge at 12000 xg for 10min at 4°C and transfer the supernatant to a fresh tube.
5. Add 300µl of BCP (100µL per 1ml of TRI sol.), vortex and incubate for 5-15 min at room temp.
6. Centrifuge at 12000 xg for 10-15 min at 4°C and transfer the aqueous phase to a (LoBind) Eppendorf tube.
7. Combine with an equal volume of isopropanol (or 500 µl of isopropanol per 1 ml of TRI)
8. Vortex at moderate speed for 5-10 sec and incubate at room temp. for 5-10 min.
9. Centrifuge at 12000 xg for 8 min at 4°C and discard the supernatant.
10. Wash RNA pellet by adding 750µl of 80% ethanol (Add 1mL of 75% ethanol per 1mL TRI Reagent solution) and then centrifuge for 5min at 12,000g (4 °C).
11. Remove the supernatant without disturbing the pellet. Remove all residual ethanol by centrifuging again briefly and removing the ethanol that collects with a fine tip pipette.
12. Air-dry the pellet for 10 min (3-5 min), resuspend in nuclease-free water (100µl final volume) by passing the solution a few times through a pipette tip or by vigorous vortexing (The resuspension volume is determined by the size of the RNA pellet. 3–5 mm pellets typically require 300–500µL. If necessary, increase the resuspension volume or incubate at 55–60°C to completely dissolve the pellet.
13. quantify and either store at –80 °C

[The total RNA isolated with this procedure should have an A260/A280 ratio of 1.8–2.2.]

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 (RNA Isolation using TRI Reagent)

8.2 SOPs –

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