Document Number: **SASoM/METHOD/047.v4**

Title: **siRNA transfection of mammalian cells**

Version: **v4**

Author: **Paul Reynolds**

Effective from:	10/08/2019
Valid to:	09/08/2021

SOP History		
Number	Date	Reason for Change
v1	29/08/2013	Original
v2	10/08/2015	Update
v3	10/08/2017	Update
V4	10/08/2019	Update

1.0 Purpose –

This SOP describes the current procedure for siRNA transfection for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in siRNA transfection work.

3.0 Responsibilities –

All staff involved in cell culture 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 –
Perform in dedicated biosafety cabinet under sterile, tissue culture working conditions. For a 10cm plate of adherent cells, add 20μM stock of siRNA (Ambion):

Day 0:
Seed $1.5x10^6$ cells per 10cm tissue culture plate in a volume of

Day 1:
Prepare two separate tubes (A and B) for each siRNA being studied. In addition, include a control / mismatch RNA, making a minimum of four tubes:

1A. 800μL OPTImem in a tube +16μL Lipofectamine 2000 (added to liquid, do NOT touch the plastic tube)
1B. 800μL OPTImem in a tube + 10μL of 20μM siRNA duplexes

Incubate each pair of tubes separately at RT for 5 min

Gently mix together (1) and (2)

Incubate at RT for 20 min

Add the mixture to the labelled 10cm plate, drop by drop.

Day 2:
Replace the media on the plates with fresh media. Then,
Repeat Day 1 steps as above.

Day 3:
Replace media

Day 4:
Perform assays with cells.

5.0 Personal protection -
A Howie laboratory coat and lab gloves 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 100% ethanol or water.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –
8.1 Risk assessments –
RA/BIOL/04 - Culture of primary and established cell lines

9.0 Approval and sign off –

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