Document Number: **SASoM/METHOD/148.v1**

Title: **RNA in-situ hybridisation using Leica Bond RX**

Version: **v1**

Author: **In Hwa Um**

Effective from:	05/11/2021
Valid to:	05/11/2023

SOP History		
Number	Date	Reason for Change
v1	05/11/2021	Original

1.0 Purpose –

This SOP describes the current procedures for performing RNA in-situ hybridisation in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing RNA in-situ hybridisation in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing RNA in-situ hybridisation 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 –

- Make up the following buffers:
  1. Hybridisation buffer;
     2xSSC – 375ul
     Formamide – 125ul
     50% Dextran sulfate – 120ul
     10xCasein – 65ul

  2. 0.3% Triton X
     Triton X –300ul
     PBS buffer – 100mL

For Immunofluorescence or Immunohistochemistry polyA for 4% PFA (paraformaldehyde) fixed cells:

1. Dilute Leica Bond RNA positive control in hybridisation buffer at 1 in 4 dilution.
2. Dilute anti-fluorescein antibody (Leica, AR0833) in Agilent antibody diluent at 1 in 6 dilution.
3. Prepare Agilent anti-mouse HRP secondary antibody (Agilent, K400111-2)
4. In Leica Bond RX (SASoM-Equip_106), select ER1 for 10min and the protocol 'polyAish_LeicaIF_cells' which has below main steps;
   0.3% Triton X for 12min
   Peroxide block for 5min
   RNA positive control for 120min at 37degree
   Anti-Fluorescein for 30min
   Anti-mouse HRP for 20min
   TSA (Cy3 or Cy5) for 20min for IF or DAB c for 10min for IHC
   Hoechst 33342 for 10min for IF or Haematoxylin for 5min for IHC
5. On completion of the run, take the slide and mount in prolong gold anti-fade mountant for IF or in DPX for IHC after dehydration and clearing.

For Immunofluorescence or Immunohistochemistry polyA for FFPE tissue sections:

1. Dilute Leica Bond RNA positive control in hybridisation buffer at 1 in 4 dilution.
2. Dilute anti-fluorescein antibody (Leica, AR0833) in Agilent antibody diluent at 1 in 6 dilution
3. Dilute pepsin from Leica Enzyme Pretreatment kit (Leica, AR9551) in the Enzyme diluent at 1 in 3000 dilution.
4. In Leica Bond RX (SASoM-Equip_106), select Bake and Dewax, ER1 for 20min and the protocol 'polyALeica_IHC_F' which has below main steps;
   Pepsin 1:3000 for 10min at 37 degree
   Peroxide block for 5min
   RNA positive control for 120min at 37degree
   Anti-Fluorescein for 30min
   Post primary for 15min
   Polymer for 15min
   TSA (Cy3 or Cy5) for 20min for IF or DAB for 10min for IHC
   Hoechst 33342 for 10min for IF or Haematoxylin for 5min for IHC

5. On completion of the run, take the slide and mount in prolong gold anti-fade mountant for IF or in DPX for IHC after dehydration and clearing.

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 –
RA22386: Use of the Leica Bond Rx Autosampler

9.0 Approval and sign off –

Author:		
Name:	In Hwa Um	
Position:	Post Doctorate	
Signature:		Date: 05/11/2021
Management Approval:		
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date: 05/11/2021
QA release by:		
Name:	Claire Sneddon	
Position:	QA Manager	
Signature:		Date:11/11/2021

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