Document Number:	**SASoM/METHOD/064.v4**
Title:	**RNAscope 2.0 DAB Protocol for Perfusion-fixed Cryosectioned Tissue**
Version:	**v4**
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Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	12/09/2014	Original
v2	12/09/2016	Update
v3	12/09/2018	Update
V4	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for RNAscope 2.0 Sample Preparation, Pretreatment and HD Detection 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 RNAscope 2.0 Sample Preparation, Pretreatment and HD Detection.

3.0 Responsibilities –
All staff involved in RNAscope Sample Preparation and Pre-treatment and HD Detection (Brown) 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 –

A recognised user-friendly procedure is detailed in the User Manual section of SOPS. More detail about the procedure is in the ACD User Manuals. The RNAscope protocol procedure must be printed off before commencing this method and used as a tick sheet whilst performing the procedure. Also refer to Equipment SOP/076 for HyBEZ Hybridisation Oven.

Milli-Q water must be used at all stages of the procedure when making up the Wash Buffers contained within the ACD kit as well as the water washes.

Special care must be taken when using DAB (Brown A and Brown B in kit). Ensure any residual DAB solution is soaked onto tissue and disposed of tissue in Chemical Waste (Red bin).

Elga (green tap) water can be used in the procedure after detecting with DAB reagent. When counterstaining slides, as detailed in protocol tick sheet, the Haematoxylin is 'Ready to Use' stock solution and must be filtered just prior to use. The Ammonia water is 'Scott's Tap Water' which is already made up at the Immunohistochemistry Bench area in 248 lab.

After dehydration the slides are mounted using DPX mounting medium – located in under bench cupboard.

Day 1:

Date _____
Probes _____
Tissue _____

1. For Paraffin wax embedded sample – follow Dewaxing SOP (Method #24)
2. Immersion in PBS for 5 min (to remove OCT) ____
3. Block sections with 'Pretreat 1' for 10 min at RT ____
4. Rinse slides in ddH_20 ___
5. Permeabilize the sections by immersing in 'Pretreat 2' solution for 15 min at 100°C ____
6. Rinse briefly in H_20 container ___
7. Repeat with fresh H_20 ___
8. Wash slides in fresh 100% ETOH then air dry ____
9. Circle sections with PAP barrier pen and let dry ____

Leave slides at RT overnight or proceed to next section

Day 2:

Place AMP1-6 reagents at RT

Before use warm probes for 10 min at 40°C in water bath or incubator and mix gently.

1. Wash slides briefly in ddH_20 ___
2. Incubate slides in protease-containing Pretreat 3 for 30 min at 40°C ____
3. Wash slides briefly in ddH_20 ___
4. Add up to 4 drops of the appropriate **PROBE** to entirely cover sections.
5. Place slides into Hyb oven for 2 hr at 40°C ____
6. Wash slides in 1X Wash Buffer for 2 min at RT ___
7. Repeat wash with fresh Wash Buffer ____
8. Flick off excess buffer from each slide and place on slide rack then add up to 4drops of **AMP1** to cover the section then incubate inside oven for 30 min at 40°C____
9. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
10. Repeat wash with fresh Wash Buffer ____
11. Flick off excess buffer from each slide and place on slide rack then add up to 4 drops of **AMP2** to cover the section then incubate inside oven for 15 min at 40°C____
12. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
13. Repeat wash with fresh Wash Buffer ____
14. Flick off excess buffer from each slide and place on slide rack then add up to 4 drops of **AMP3** to cover the section then incubate inside oven for 30 min at 40°C____
15. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
16. Repeat wash with fresh Wash Buffer ____
17. Flick off excess buffer from each slide and place on slide rack then add up to 4 drops of **AMP4** to cover the section then incubate inside oven for 15 min at 40°C____

18. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
19. Repeat wash with fresh Wash Buffer ____

(Procedure all at RT from now on)

20. Flick off excess buffer from each slide and place on slide rack then add up to 4 drops of
    AMP5 to cover the section then incubate inside oven for 30 min at **RT** ____
21. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
22. Repeat wash with fresh Wash Buffer ____
23. Flick off excess buffer from each slide and place on slide rack then add up to 4 drops of **AMP6** to cover the section then incubate inside oven for 15 min at **RT** ____
24. Wash slides in 1X Wash Buffer for 2 min at RT with mixing ___
25. Repeat wash with fresh Wash Buffer ____

Detecting signal with DAB reagent

1. Mix equal volumes of Brown-A and Brown-B (make ~150 µl/slide) and mix well ___
2. Apply ~150 µl DAB substrate per slide and incubate for 10 min at RT ___
3. Wash slides in distilled water for 2 min at RT with mixing ___
4. Repeat wash with fresh distilled water ____

Counterstain the slides

1. Immerse slide rack into 50% Haematoxylin 1 for 2 minutes at RT ___
2. Immediately transfer the slide rack into distilled water and wash slides by moving slide carrier up and down 3-5 times ___
3. Repeat wash with fresh distilled water ___
4. Repeat wash with fresh distilled water until the slides clear but sections remain purple ___
5. Place slides in to 0.02% Ammonia water and move slides up and down 3-5 times ___
6. Place slides in distilled water ___

Dehydrate slides (in fumehood)

1. Immerse slide rack into 70% EtOH for 2 min with occasional mixing ___
2. Place slides into 100% EtOH for 2 min with occasional mixing ____
3. Place slides into 100% EtOH for 2 min with occasional mixing ____
4. Place slides into Xylene for 5 min with occasional mixing ____
5. Remove slides from carrier and lay flat facing up and apply 1-2 drops of cytoseal or xylene based mounting medium and carefully place a coverslip over the section ___
6. Air dry slides ____

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 water.

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

8.0 Related documents –

8.1 User Manuals Folder under SOPs –
1. RNAscope Sample Preparation and Pretreament Guide for FFPE Tissue PART1
2. RNAscope 2.0 HD Detection Kit (Brown) PART 2

8.2 SASoM-EQUIP-076-HybEZ Hybridisation System

8.3 RA/Gen/003 - Heated Equipment
RA/Gen/009 - General Lab Safety
RA/Gen/024 - Fume Cupboards

9.0 Approval and sign off –

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