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MANUSCRIPT CITATION:

Adapted from ACD Standard Protocol/Cheadle/Otero-Garcia Protocols

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Protocol status: Working We use this protocol and it's working

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2023

Oual In Situ Hybridization/Immunofluorescence

In 1 collection

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ABSTRACT

This protocol details about the Dual In Situ Hybridization/Immunofluorescence in tissue.

ATTACHMENTS

338-741.pdf

GUIDELINES

ACD protocol notes that tissues should be fixed in 10% NBF for 16-32 hours, and embedded in paraffin. Then, sectioned and dried overnight at RT. They suggest sectioned tissue be used in less than a year (4°C) or less than 3 months at room temperature.

PROTOCOL integer ID: 56572

Keywords: Dual In Situ Hybridization, Immunofluorescence, RNAscope Multiplex Fluorescent v2 Assay, ASAPCRN

MATERIALS

Solutions

A	В	С
Needed (mL)	Stock Solution	Final Concentration
5 L	dH2O	
485 g	Tris base	0.5 M
240 mL	Concentrated HCI	
pH to 7.6		
To 8L	dH2O	

0.5 M Tris (8 L)

Reagents

A	В	С	D	E
Vendor	Catalog #	Qty	Unit Price	Description
RNAscope® Multiplex Fluorescent Reagent Kit V2	323100		1330	Contains H2O2, protease reagents, target retrieval reagent, wash buffer, HRP reagents
RNAscope® 3- plex	320861		100	Polr2a (C1 channel) and PPIB (C2 channel), UBC (C3 channel)
Positive Control Human Sigma	199664- 25G	1	66.6	Sudan Black B
Vector Laboratories	H-4000	1	120	ImmEdge Hydrophobic Barrier Pen
Southern Biotech	0100-01	1	45.1 4	DAPI Fluoromount-G

- Sudan black B Sigma
 Aldrich Catalog #199664
- ImmEdge hydrophobic barrier pap pen **Vector**Laboratories Catalog #H-4000

Preparing Tissue (Day 1): Prepare Tissue

1h 19m

- 1 Bake slides in a dry oven for 01:00:00 at 60 °C. Use slides within a week. 2 De-paraffinize slides in fresh xylenes, then in 100% ethanol. 2.1 De-paraffinize slides for 00:05:00 in fresh xylenes. (1/4) 2.2 5m De-paraffinize slides for 00:05:00 in fresh xylenes. (2/4) 2.3 2m De-paraffinize slides for 00:02:00 in 100% ethanol. (3/4) 2.4 De-paraffinize slides for 00:02:00 in 100% ethanol. (4/4) 2m 3 Place slides on absorbent paper and dry in the oven from 00:05:00 at 60 °C or until dry.
- Preparing Tissue (Day 1): Hydrogen Peroxide Treatment

 Place slide horizontally in an incubation tray. Add ~5-8 drops of RNAscope Hydrogen Peroxide to cover each section. Incubate for 00:10:00 at Room temperature.

5 Dab solution off and move to a rack in distilled water. Move up and down 5 times. Repeat with a fresh boat of distilled water.

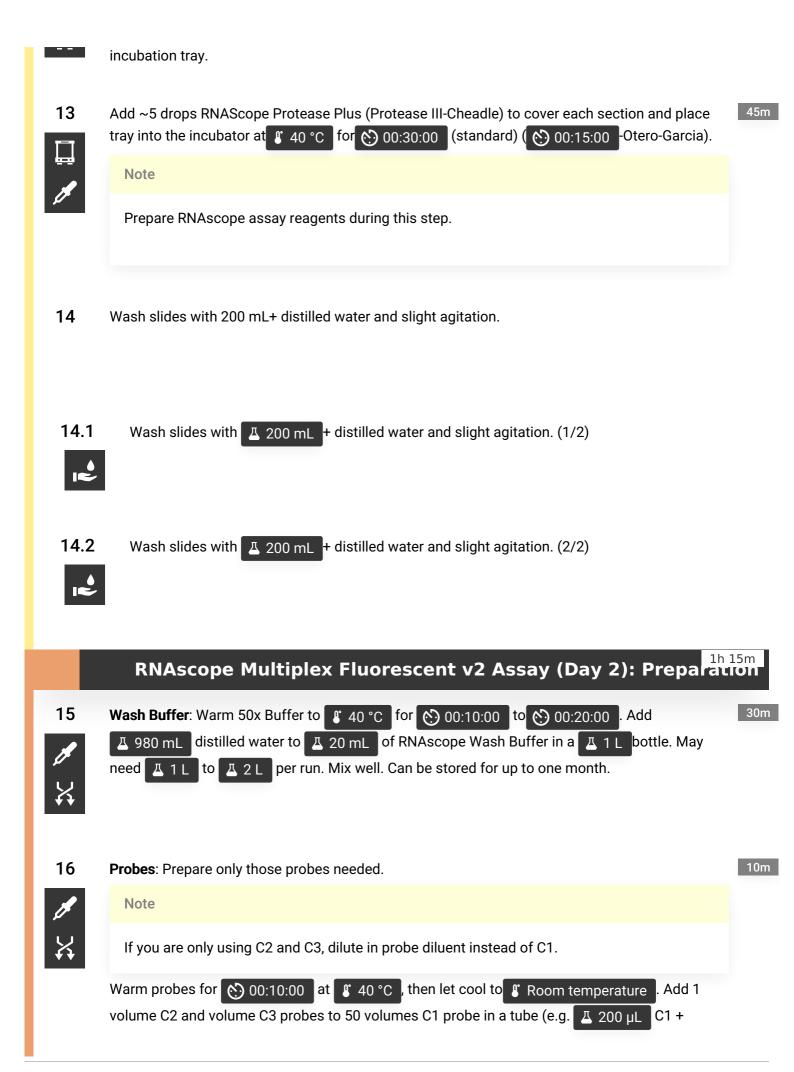
1h 19m Preparing Tissue (Day 1): Target Retrieval Dilute Target Retrieval Regents (RNAscope) 1:10 in dH₂O (△ 25 mL / △ 225 mL dH2O/boat). Mix well. 7 Place in microwave for 00:15:00 at \$\circ\$ 95 °C 15m 8 15s Transfer slides to a slide boat with A 200 mL distilled water for 00:00:15 9 3m Transfer the slides to 100% ethanol for 00:03:00 10 5m Dry the slides in a \$\ 60 \circ\$ incubator (or \$\ \text{Room temperature} \) for \(\chrc{\chi}{\chi}\) 00:05:00 11 10m Draw a hydrophobic barrier onto slides with ImmEdge pen. Do NOT due for fluorescent slides. Let the barrier dry for 00:05:00 . OPTIONAL PAUSE POINT Overnight at Second Research Research Room temperature

RNAscope Multiplex Fluorescent v2 Assay (Day 2): Protease

Place a wet Humidifying Paper in an incubation tray and warm for 00:30:00 at (TC incubator). Keep the tray in the incubator when not in use. Insert the slides into the

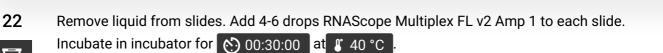
12

30m





RNAscope Multiplex Fluorescent v2 Assay (Day 2): Hybridize A.



30m



23 Wash slides with Wash Buffer.

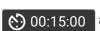
23.1 Wash slides with Wash Buffer 00:02:00 at Room temperature . (1/2) 2m



23.2 Wash slides with Wash Buffer 00:02:00 at Room temperature . (2/2) 2m



24 Repeat steps 22 and 23 for Amp 2 and Amp 3. Amp 3 only requires 00:15:00 at 40 °C



15m

25 During this incubation, dilute necessary Opal Dye fluorophores in TSA Buffer (1:1500 standard).

RNAscope Multiplex Fluorescent v2 Assay (Day 2): Develo

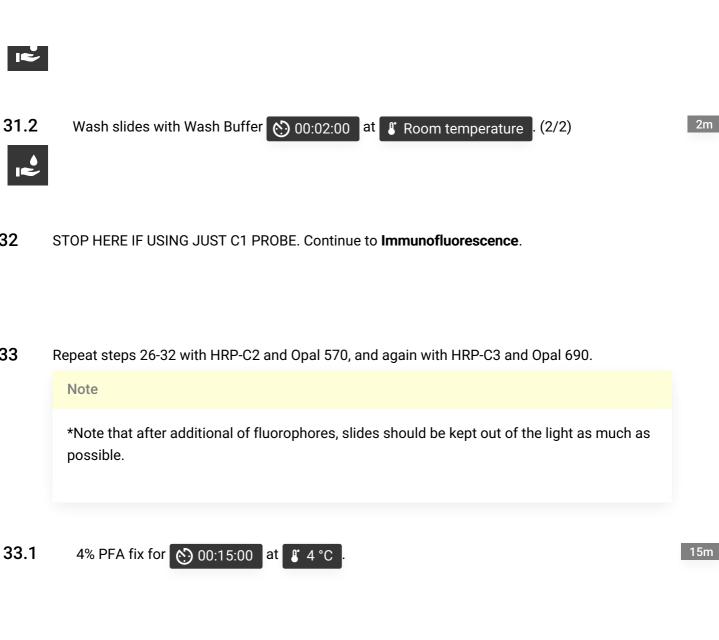
26 Remove liquid from slides. Add 4-6 drops RNAScope Multiplex FL v2 HRP-C1 to each slide.



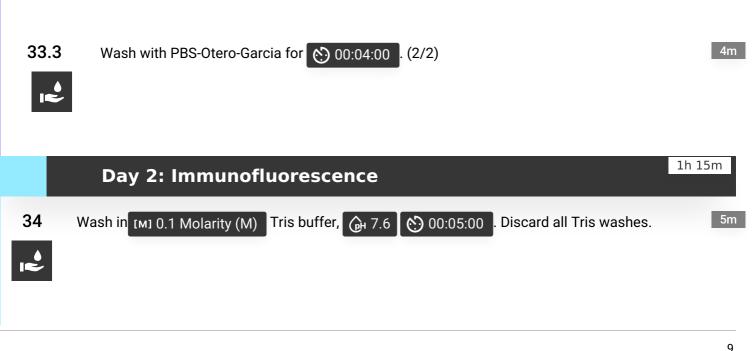
Incubate in incubator for 00:15:00 at 40 °C.

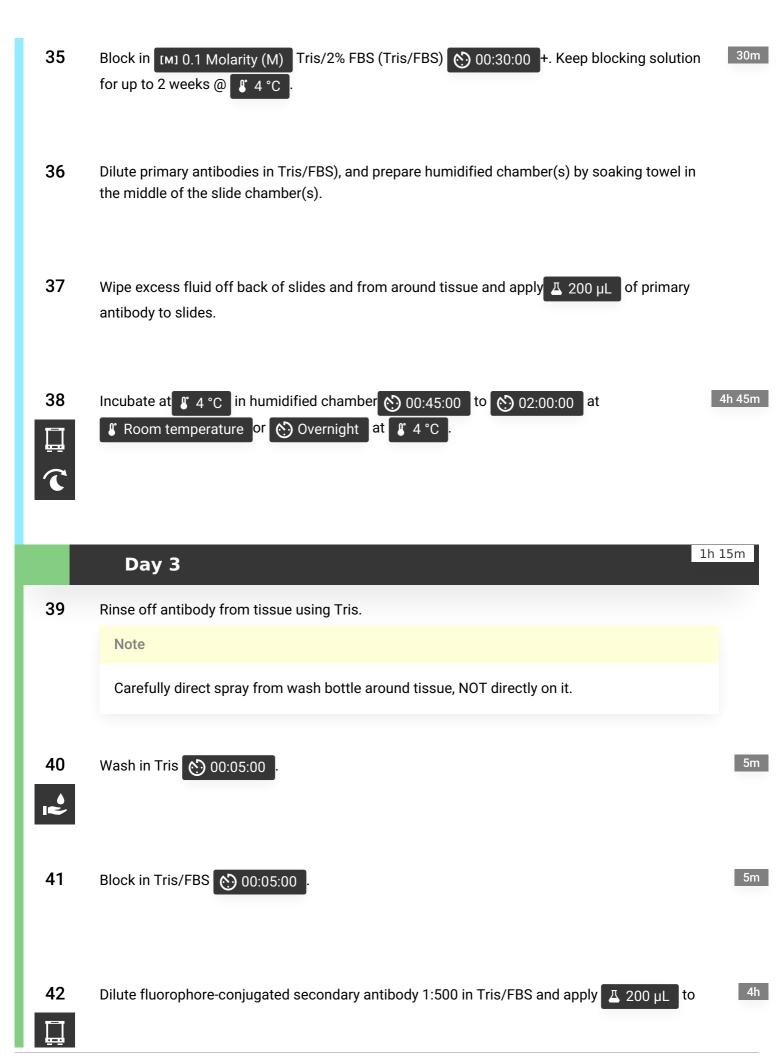
27 Wash slides with Wash Buffer.

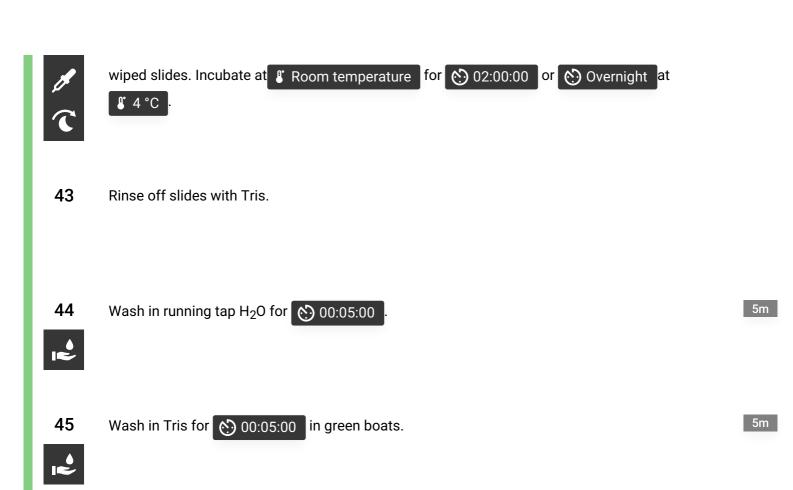












Coverslip using non-photobleaching reagent (Prolong Gold with DAPI or FluorMount with DAPI).

Allow to dry completely before imaging on scanner.