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Preparation and RNAscope-labeling of fresh mouse midbrain tissue

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ABSTRACT

Below we describe preparing and labeling coronal sections of mouse substantia nigra (SNc) and ventral tegmental area (VTA) for probes targeting the genes Slc17a6 (VGLUT2), Slc32a1 (VGAT), and Slc18a2 (VMAT2).

ATTACHMENTS

fresh frozen prep rnascope protocol.docx



PROTOCOL integer ID: 94571

Keywords: rnascope, vta, snc, mouse, fresh tissue, v1, vmat2, vgat, vglut2, sectioning

Funders Acknowledgement:

ASAP & MJFF

Grant ID: ASAP-020600

MATERIALS

Part A:

Material	Supplier and Catalog Number
RNAseZap	Invitrogen (AM9780)
Pentobarbital	Virbac
Scissors	Fine Science Tools (14058-09)
Forceps	Fine Science Tools (11152-10)
Culture tubes	Fisher Scientific (14-959-11)

Part B:

Material	Supplier and Catalog Number
Cryostat	Leica (CM3050S)
Anti-roll plate	Leica (14047742497)
Brushes	Electron Microscopy Sciences (66100-30)
OCT Medium	Sakura (4583)
Superfrost Plus Microscope Slides	Thermo Fisher Scientific (12-550-15)

Part C & D:

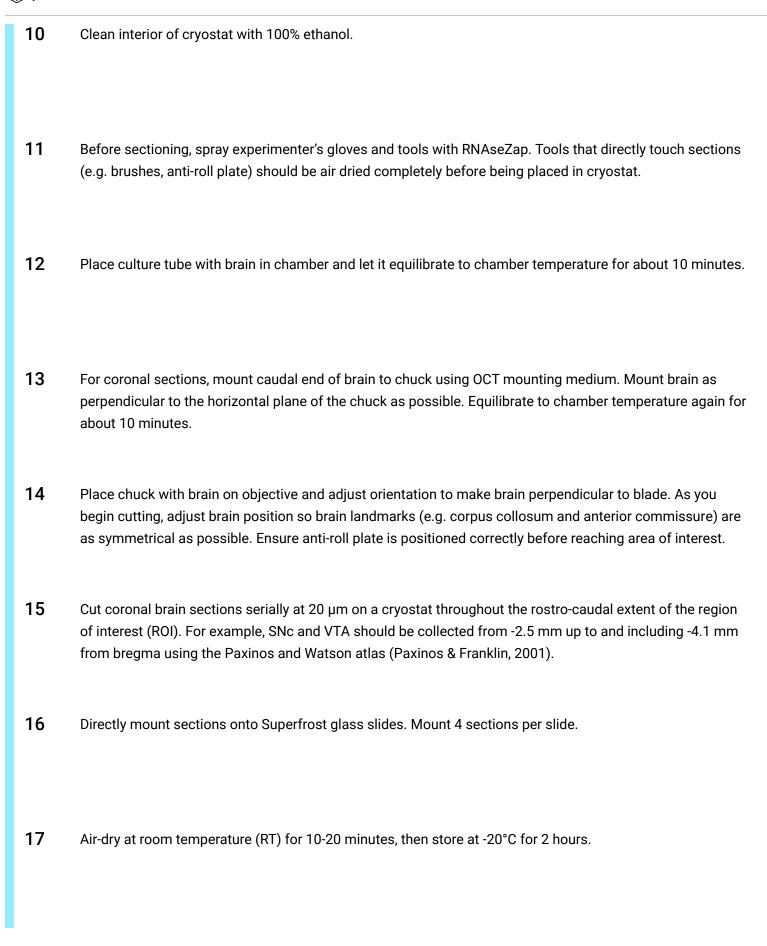
Α	В
Material	Supplier and Catalog Number

А		В
ı	Multiplex Florescent Kit	Advanced Cell Diagnostics (320851)
١	Wash Buffer	Advanced Cell Diagnostics (310091)
ŀ	HybEZ Oven	Advanced Cell Diagnostics (310010)
F	Pretreatment Kit (Protease)	Advanced Cell Diagnostics (310842)
	UltraPure DNase/RNase-Free istilled Water	Invitrogen (10977049)
\	Wash containers	Andwin Scientific (7154801)
	Safe Lock Centrifuge Tube, .5 mL	Eppendorf (0030123611)
B	mmEdge Hydrophobic arrier PAP Pen	Vector Laboratories (H-4000)
	DAPI	Roche (10236276001)
F	Fluoromount-G	Southern Biotech (0100-01)
	Coverslip	Corning (2980-225)
1 1	alcon 50 mL Conical entrifuge Tubes	Fisher Scientific (352098)

A. Mouse brain extraction

- 1 Spray experimenter's gloves and all tools with RNAseZap before starting and in between brain extractions.
- 2 Add approximately 50mL of isopentane in a beaker (cleaned with RNAseZap) and chill on dry ice.

3	Anaesthetize mouse with pentobarbital (200 mg/kg i.p.).
4	Decapitate mouse and extract brain from skull.
5	If planning on collecting coronal sections, use a razor blade to block the brain caudal of your region of interest. For example, around -6 mm from bregma for midbrain sections.
6	Immediately snap freeze brain by submerging brain for 30 to 45 seconds in isopentane chilled on dry ice.
7	Place brains in labeled culture tubes pre-chilled on dry ice.
8	Frozen brains can be moved to either an RNAse-free cryostat at -18°C or stored at -80°C.
	B. Sectioning
9	Set temperature of cryostat objective and chamber to −18°C.



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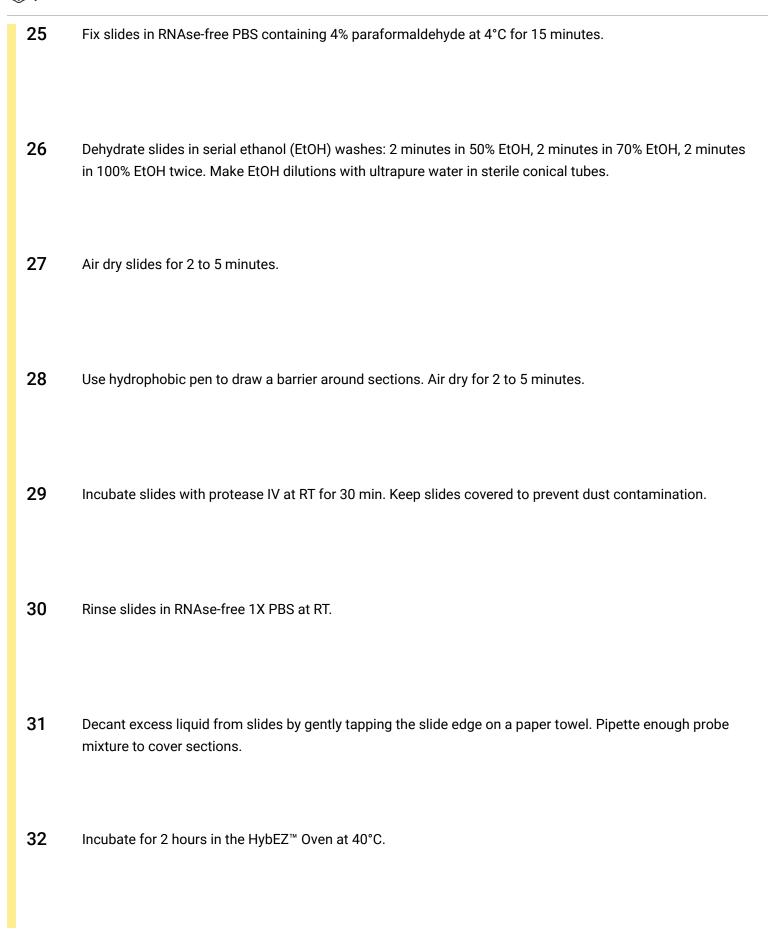
Slides can be used for RNAscope assay or stored at -80°C in a slide box within an airtight container (e.g. Ziploc bag).

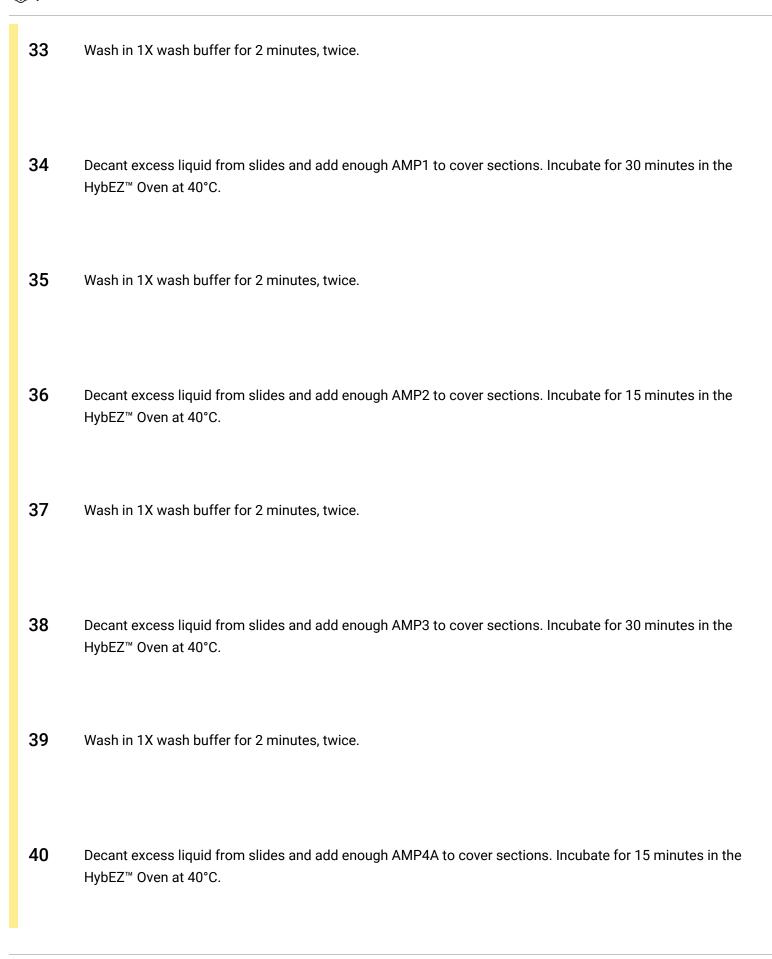
C. DAPI-containing Fluoromount-G preparation

- 19 Pipet DAPI to Fluoromount-G to reach a concentration of 0.5 μg/mL.
- Pipet up and down several times to mix, then vortex for at least 30 seconds.
- Wait at least 24 hours before use. Container should always be covered in foil and stored at 4°C.

D. RNAscope assay

- **22** Turn on HybEZ[™] Oven (ACD) and set to 40°C.
- Warm probes in heat-bath for 10 minutes at 40°C, then cool to RT.
- Make probe mixture in autoclaved Eppendorf tube by pipetting (with autoclaved pipette tips) 50 parts C1 probe or diluent, 1 part C2 probe (optional) and 1 part C3 probe (optional). Prepare about 150 μ L per slide, assuming each slide contains 4 coronal mouse sections.





- 41 Decant excess liquid from slides, add DAPI-containing Fluoromount-G, and coverslip.
- 42 Store slides in the dark at 4°C.