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Combined RNAscope and immunohistochemistry labeling of fresh mouse midbrain tissue

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We use this protocol and it's working

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Abstract

Here we describe labeling coronal sections of mouse substantia nigra (SNc) and ventral tegmental area (VTA) for the gene *Slc17a6* (VGLUT2) and virally-expressed histone-tagged GFP.

Attachments

DOCX

rnascope + ihc proto...

23KB

Materials

Material	Supplier/Catalog Number
Multiplex Florescent Kit	Advanced Cell Diagnostics (320851)
Wash Buffer	Advanced Cell Diagnostics (310091)
HybEZ Oven	Advanced Cell Diagnostics (310010)
Pretreatment Kit (Protease)	Advanced Cell Diagnostics (310842)
UltraPure DNase/RNase-Free Distilled Water	Invitrogen (10977049)
Wash containers	Andwin Scientific (7154801)
Safe Lock Centrifuge Tube, 1.5 mL	Eppendorf (0030123611)
ImmEdge Hydrophobic Barrier PAP Pen	Vector Laboratories (H-4000)
DAPI	Roche (10236276001)
Fluoromount-G	Southern Biotech (0100-01)
Falcon 50 mL Conical Centrifuge Tubes	Fisher Scientific (352098)

Section A materials

Normal donkey serum	Fisher Scientific NC9624464
Triton X-100	Sigma X100-1L
Primary antibody: chicken anti-GFP	Invitrogen (AB_2534023)
Secondary antibody: donkey anti-chicken (488)	Jackson ImmunoResearch (AB_2340376)
Wash containers	Andwin Scientific (7154801)

Wash Buffer	Advanced Cell Diagnostics (310091)
Coverslip	Corning (2980-225)

Section B

Before start

Tissue should be processed as described previously (dx.doi.org/10.17504/protocols.io.eq2lyjynelx9/v1)

DAPI-containing Fluoromount-G for the final step does not need to be made fresh. However, if it is out of stock it should be made at least one day prior to RNAscope + IHC assay:

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

RNAscope assay

1 Turn on HybEZ™ Oven (ACD) and set to 40°C.

2 Warm probe in heat-bath for 10 minutes at 40°C, then cool to RT.

3 Drop C1 probe into autoclaved Eppendorf tube. Prepare about 150 µL per slide, assuming each slide contains 4 coronal mouse sections.

4 Fix slides in RNAse-free PBS containing 4% paraformaldehyde at 4°C for 15 minutes.

5 Dehydrate slides in serial ethanol (EtOH) washes: 2 minutes in 50% EtOH, 2 minutes in 70% EtOH, 2 minutes in 100% EtOH twice. Make EtOH dilutions with ultrapure water in sterile conical tubes.

6 Air dry slides for 2 to 5 minutes.

7 Use hydrophobic pen to draw a barrier around sections. Air dry for 2 to 5 minutes.

8 Incubate slides with protease IV at RT for 30 min. Keep slides covered to prevent dust contamination.

9 Rinse slides in RNAse-free 1X PBS at RT.

10 Decant excess liquid from slides by gently tapping the slide edge on a paper towel. Pipette enough probe mixture to cover sections.

11 Incubate for 2 hours in the HybEZ™ Oven at 40°C.

12 Wash in 1X wash buffer for 2 minutes, twice.

13 Decant excess liquid from slides and add enough AMP1 to cover sections. Incubate for 30 minutes in the HybEZ™ Oven at 40°C.

14 Wash in 1X wash buffer for 2 minutes, twice.

15 Decant excess liquid from slides and add enough AMP2 to cover sections. Incubate for 30 minutes in the HybEZ™ Oven at 40°C.

16 Wash in 1X wash buffer for 2 minutes, twice.

17 Decant excess liquid from slides and add enough AMP3 to cover sections. Incubate for 30 minutes in the HybEZ™ Oven at 40°C.

18 Wash in 1X wash buffer for 2 minutes, twice.

19 Decant excess liquid from slides and add enough AMP4A to cover sections. Incubate for 30 minutes in the HybEZ™ Oven at 40°C.

20 Decant excess liquid from slides.

21 Wash in 1X PBS at RT, twice.

Immunohistochemistry (IHC)

22 Incubate each slide with approximately 150 µL of blocking buffer (4% normal donkey serum in 1X PBS with 0.2% Triton X-100) for 1 hour at RT, in the dark.

23 Decant excess liquid from slides, then incubate each slide with approximately 150 µL of primary antibody mixture (chicken anti-GFP diluted 1:10,000 in 4% normal donkey serum in 1X PBS with 0.2% Triton X-100) overnight at 4°C, in the dark.

24 Wash slides in PBS for 5 minutes at RT, three times.

25 Decant excess liquid from slides, then incubate each slide with approximately 150 µL of secondary antibody mixture (donkey anti-Chicken 488 diluted 1:400 in 4% normal donkey serum in 1X PBS) for 2 hours at RT, in the dark.

26 Wash slides in PBS for 15 minutes at RT, three times.

27 Briefly rinse slides in wash buffer.

28 Decant excess liquid from slides, add DAPI-containing Fluoromount-G, and coverslip.

29 Store slides in the dark at 4°C.

Protocol references

Manufacture's guide to RNAscope assay

Tissue preparation protocol