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RNAscope for FFPE Mouse Tissue

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We use this protocol and it's working

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Abstract

This protocol details the RNAscope for FFPE Mouse Tissue.

Materials

RNAscope Multiplex Fluorescent Reagent Kit v2 (Cat. #323100).

RNAscope® Multiplex Fluorescent Reagent Kit v2 **Advanced Cell Diagnostics** **Catalog #**323100

Day 1:

- Fresh Xylene
- Absolute alcohol
- Hydrogen peroxide & Protease plus from same kit
- Absorbent paper
- 10X Target retrieval
- C1, C2, C3 probes
- RNAscope™ 3-plex Negative Control Probe **Advanced Cell Diagnostics** **Catalog #**320871
- RNAscope™ 3-plex Positive Control Probe- Mm **Advanced Cell Diagnostics** **Catalog #**320881
- 1X wash buffer

Day 2:

- AMP1, AMP2, AMP3
- HRP-C# (need C1-C3 if developing all 3 channels)
- TSA buffer
- HRP-Blocker
- Opal 570 Reagent Pack **Perkin Elmer** **Catalog #**FP1488001KT
- Opal 690 Reagent Pack **Perkin Elmer** **Catalog #**FP1497001KT
- TSA-DIG & Opal 780 (Cat. #FP1501001KT)
- 1X Antibody Diluent (Cat. #ARD1001EA)
- DAPI
- TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO **Biotium** **Catalog #**23014
- ProLong™ Gold Antifade Mountant **Invitrogen - Thermo Fisher** **Catalog #**P36930
- Cover Slip
- Tween 20
- PBS

Solutions

1X Wash Buffer

- 40 mL 50X WashBuffer + 1960 mL RNase-free water

Target Retrieval

- 25 mL 10X Target Retrieval + 225 mL RNase-free water

5x SSC

- 50 mL 20X SSC + 150 mL RNase-freewater

TSA-DIG: for 8 samples

- 2 µL TSA-DIG + 1000 µL TSA buffer

Opal 780 Dye: for 8 samples

- 8 µL opal 780 + 1000 µL 1X Antibody Diluent

1X TrueBlack Plus: for 8 samples

- 25 µL 40X TrueBlack Plus + 1000 µL 1X PBS → **VORTEX**

probe channel	Channel 1 (C1)	Channel 2 (C2)	Channel 3 (C3)
channel sensitivity	highest	weakest	high
cell type analysis of target gene expression	gene of interest	cell type marker 1 (e.g. vGLUT1/2)	cell type marker 2 (e.g. GAD1/2)

DAY 1 - Bake/Adhere

1h

1 Spray RNase away on bench top, slide holder, metal container.

2 Warm oven and slide holder up to 60 °C .

3 Label the lower part of the slide with a pencil.

- Can place slides on bench (since sprayed with RNase away).

4 Put slides into slide holder and place into oven → bake for 01:00:00 @ 60 °C . 1h

- **Meanwhile..**

4.1 Turn plate warmer on to 60 °C .

4.2 Make Target Retrieval solution.

4.3 Fill Xylene and Ethanol containers.

> Note
>
> **After Baking: Possible stopping point: Store @** Room temperature **, good for ~** 168:00:00 **.**

5 Set hyb oven temp to 40 °C .

5.1 Wet humidifying paper with nanopure water (does not need to be dripping).

5.2 Place paper on bottom of slide tray and put into the oven for 00:30:00 @ 40 °C . 30m

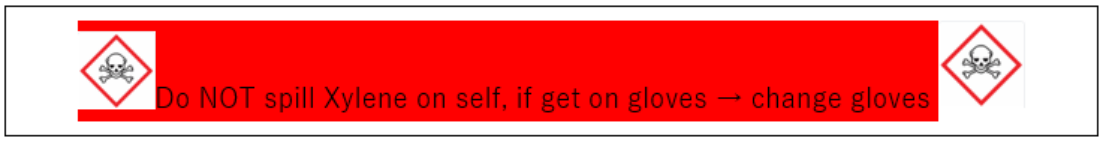
6 Place slides into tissue tek container and transport to fume hood.

DAY 1 - Deparaffinize

5m

7

5m



Xylene container 1 for 00:05:00 .

7.1 **Meanwhile..**Turn on vegetable steamer.

7.2 When transferring to container 2 → shake off excess.

8 Xylene container 1 for 00:05:00 .

5m

8.1 When transferring to ethanol → shake off excess.

9 Absolute ethanol container 1 for 00:02:00 .

2m

9.1 When transferring to container 2 → shake off excess.

10 Absolute ethanol container 2 for 00:02:00 .

2m

11 Dry on slide warmer for ~ 00:05:00 @ 60 °C until dry.

5m

11.1 **Meanwhile...** clean bench with RNase away.

DAY 1 - Hydrogen Peroxide

10m

12 Place slides on bench top and cover tissue completely with Hydrogen Peroxide (~5-8 drops).

13 Incubate for 00:10:00 @ Room temperature . 10m

- **Meanwhile..**

1. Boil Target Retrieval solution and a container of Nano pure water in microwave until boiling.

13.1 *Microwave for 00:01:00 at a time* 1m

13.2 When reaches a boil → place containers in warm steamer to ensure solution is at least 99 °C .

14 Remove solution using absorbent paper by tapping long side on paper.

- Place in slide holder.

15 Wash slides in slide holder with 200 mL of Nano pure water ~3-5 dunks.

16 Take slides out and repeat step - **1x**.

DAY 1 - Target Retrieval Step

10s

17 Keep in water and transport to steamer.

17.1 Dunk a couple times and soak for 00:00:10 in nanopure water (steamer). 10s

18 Place in target retrieval solution in the steamer for 00:30:00 (for brain samples) **or** 00:15:00 (for other tissue). 45m

19 Rinse slides in fresh 200 mL of Nano pure water → 00:00:15 . 15s

20 Transfer slides to the 2nd container of absolute ethanol in the fume hood → 00:03:00 . 3m

21 Dry slides in slide warmer - ~ 00:05:00 @ 60 °C . 5m

- **Meanwhile..**

21.1 Rinse slide holder in DI water in sink and let dry on paper towel.

21.2 Spray bench with RNase away.

DAY 1 - Barrier/Protease Plus 30m

22 Put dry slides on bench and square off with hydrophobic pen.

22.1 Leave a little extra room at one side of square to allow space to aspirate later.

23 Apply ~5 drops of protease plus to tissue until sample is completely covered.

24 Incubate in oven for 00:30:00 @ 40 °C . 30m

- **Meanwhile..**

24.1 Warm probes @ 40 °C **for ~** 00:10:00 **.** 10m

24.2 Create probe solution.

Note

C1 probe is at 1X → assign to low expresser gene
C2 & C3 probes are at 50 X → dilute w/ C1 probe if using C1 or with probe diluent to 1X

25 Wash with DI water in wash tray - **2X.**

26 Aspirate with 200 µL pipette tip in the fume hood.

26.1 Wipe bottom of wash tray with big kimwipe.

DAY 1 - Hybridize Probe

30m

27 Add ~ 120 µL of probe mix to respective samples → cover sample completely.

28 Incubate for 02:00:00 @ 40 °C .

2h

29 Wash in 1X wash buffer for 2 minutes **2X**.

Note

Store till day 2 in 5X SSC @ Room temperature **.**

- **Pour ethanol back into reagent bottle if not doing RNAscope within the next week so doesn't evaporate.**

29.1 Wash in 1X wash buffer for 00:02:00 (1/2).

2m

29.2 Wash in 1X wash buffer for 00:02:00 (2/2).

2m

DAY 2

2m

30 Spray RNase away on bench top, slide holder, metal container.

31 Hydrate Paper and turn on oven to 40 °C .

32 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**.

32.1 Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2). 2m

32.2 Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2). 2m

33 Aspirate.

34 Hybridize AMP1.

34.1 4-5 drops covering the sample with AMP1.

34.2 Incubate for 00:30:00 @ 40 °C . 30m

34.3 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

34.4 Aspirate.

35 Hybridize AMP2.

35.1 4-5 drops covering the ample with AMP2.

35.2 Incubate for 00:30:00 @ 40 °C . 30m

35.3 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

35.4 Aspirate.

36 Hybridize AMP3.

36.1 4-5 drops covering the sample with AMP3.

36.2 Incubate for 00:15:00 @ 40 °C . 15m

36.3 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

36.4 Aspirate.

Develop Channels (C1-C3) 1h 6m

37

Note

- Don't need to do all 3 channels for each sample just for respective channels, do one channel at a time/slide.
- PC and NC need all 3 Channels.
- Develop 780 channel Last!!

Develop HRP-C2 (570 or 690) signal.

37.1 Add 4-5 drops covering the sample with HRP-C2 (or respective HRP-C#) and incubate for 00:15:00 @ 40 °C . 15m

- Meanwhile.. Dilute Opal dyes → **KEEP IN DARK**

  1 µL Dye (570 or 690) + 1000 µL TSA buffer

37.2 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

37.3 Add 1st Opal dye and Incubate for 00:30:00 @ 40 °C . 30m

37.4 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

- Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
- Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

37.5 Add 4-6 drops HRP blocker and incubate for 00:15:00 @ 40 °C . 15m

37.6 Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**. 4m

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

38 Repeat using HRP-C3.

39 For 780 Channel

39.1 Add HRP-C1 and incubate for 00:15:00 @ 40 °C . 19m

- Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**.

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

39.2 Add ~ 120 µL of TSA-DIG per section and incubate for 00:30:00 @ Room temperature . 34m

- Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**.

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

39.3 Add HRP Blocker and incubate for 00:15:00 @ 40 °C . 19m

- Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**.

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

39.4 Add ~ 120 µL of Opal 780 per section and incubate for 00:30:00 @ Room temperature . 34m

- Wash slides with 1X wash buffer for 2 minutes with slight agitation **2X**.

1. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (1/2).
2. Wash slides with 1X wash buffer for 00:02:00 with slight agitation (2/2).

- Aspirate.

Quench w/ TrueBlack Plus

10m

40 Wash w/ PBS **3X**.

- Aspirate.

41 Apply ~ 120 µL of 1X TrueBlack Plus for 00:10:00 @ Room temperature . 10m

42 Wash w/ PBS **3X**.

- Aspirate.

DAPI and Mount

30s

43 Add 4-5 drops of DAPI to completely cover section.

44 Incubate for 00:00:30 @ Room temperature . 30s

45 Remove DAPI with absorbent paper → tap long side gently to paper.

46 Add 3-4 drops of Prolong Gold Mountant.

47 Add Coverslip.

47.1 Use a forceps to attach coverslip.

47.2 Gently press down.

47.3 Kim wipe the bottom of the slide and the bench in between samples.

48 Store in dark Overnight on absorbent paper (in a drawer). 30s

- Dry slides in the dark overnight and then Store slides at 4 °C the next day.

Protocol references

Refer to ACD RNAscope Multiplex Fluorescent v2 Assay for reference (doc #323100).