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

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BaseScope In Situ Hybridization

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ABSTRACT

BaseScope *in situ* hybridization on mouse brain sections

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Day 1

1 Use 35µM floating brain sections

2 Wash sections 3 times (5 minutes) in PBS to remove cryoprotectant solution

3 Mount tissue onto Superfrost plus slides

4 Dry slides at room temp and then dip in mQ H_2O to remove salt

5 Dry slides at 60°C for 2 hours

6 Let tissue dry at room temp overnight – make sure tissue is completely stuck to slides

Day 2

7 Dry slides at 60°C for 30 minutes

8 Dehydrate slides in **50%, 70%, 100% EtOH**, for 5 min each at room temp

> Note
>
> Use sterile distilled H_2O for the rest of the protocol

8.1 Let slides air dry for 5 min at room temp.

9 Make 200mL Target Retrieval Reagent (1:10 180mL H_2O + 20mL 10X Target Retrieval Reagent)

10 On the bench – add 5-8 drops of Hydrogen Peroxide to tissue sections

10.1 Incubate at room temp for 10 min

11 Rinse slides 2 times in H_2O

11.1 Submerge in dish of H_2O and move slides up and down 3-5 times for each wash

12 In the steamer heat one dish of H_2O and one dish of Target Retrieval Reagent

12.1 Check temp of Target retrieval reagent (must be at least 95°C)

12.2 After preheating – place slides into H_2O for 10 sec to acclimate.

12.3 Move slides to Target Retrieval Reagent and steam for 5 min

13 Remove the water container and the container with slides from the steamer and place on the bench.

13.1 Let slides cool on the bench for 20 -30 minutes (wait until temperature of water container has dropped to 30°C)

14 Wash slides in H_2O for 15 sec at room temp

15 Wash slides in 100% EtOH for 3 min at room temp

16 Dry slides at 60°C for ≥5 min

17 Use an Immedge hydrophobic barrier pen to draw a barrier around your tissue section(s)

17.1 Let the barrier dry ≥ 1 min or overnight at room temp (you can take a break at this point)

18 Turn on RNA Scope oven – set to 40°C

18.1 Wet humidifying paper (filter paper) completely with H_2O and place in the Humidity Control Tray

18.2 Warm the covered tray in the oven for 30 minutes before use

19 Place slides in slide rack and add ~5 drops of Protease IV

19.1 Incubate in pre-warmed RNAscope oven in the humidity tray at 40°C for 30 min

20 Wash slides in H_2O at room temp

21 Prepare 1L of wash buffer (20mL of 50X Wash buffer + 980mL H_2O)

21.1 *Heat 50X wash buffer to 40°C for 10-20 min before making 1X wash buffer*

22 Equilibrate each BaseScope reagent and probe to room temp for 30 min before use

Important: *Do not let sections dry out between incubation steps*

All incubation steps are in the slide rack – remove slides from slide rack to wash between incubations

For the incubations in the HybEZ oven – cover the slide rack with the lid and make sure to lock the oven so that the water in the humidity chamber does not evaporate.

23 Add ~4 drops of BaseScope probe to completely cover tissue

23.1 Incubate in the oven at 40°C for **2 hours**

23.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

24 Add ~4 drops of **AMP1** to completely cover tissue

24.1 Incubate in the oven at 40°C for **30 min**

24.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

25 Add ~4 drops of **AMP2** to completely cover tissue

25.1 Incubate in the oven at 40°C for **30 min**

25.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

26 Add ~4 drops of **AMP3** to completely cover tissue

26.1 Incubate in the oven at 40°C for **15 min**

26.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

27 Add ~4 drops of **AMP4** to completely cover tissue

27.1 Incubate in the oven at 40°C for **30 min**

27.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

28 Add ~4 drops of **AMP5** to completely cover tissue

28.1 Incubate in the oven at 40°C for **30 min**

28.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

29 Add ~4 drops of **AMP6** to completely cover tissue

29.1 Incubate in the oven at 40°C for **15 min**

29.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

29.3 *We are done with the oven – but keep using the slide rack for incubations at room temp*

30 Add ~4 drops of **AMP7** to completely cover tissue

30.1 Incubate at **room temp** for **15 min**

30.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

30.3 *Staining intensity can be modified by adjusting the AMP7 incubation time*

31 Add ~4 drops of **AMP8** to completely cover tissue

31.1 Incubate on the bench at **room temp** for **15 min**

31.2 Wash 2 times in Wash Buffer at room temp - 2 min each wash

32 Prepare BaseScope Fast RED working solution (1:60 ratio)

32.1 Spin down BaseScope Fast RED-B before using

32.2 2uL RED-B + 120uL RED-A : mix well

32.3 Use the Fast Red solution **within 5 minutes** – do not expose to direct sunlight or UV light

33 Add ~120µL Fast Red solution to each slide / section

33.1 Cover sections on tray to protect from light

33.2 Incubate at room temp for **10 min**

33.3 Rinse 2 times in H_2O at room temp

34 Dry slides at 60°C for ≥ 15 min (until slides are completely dry)

34.1 *The red substrate is alcohol sensitive. DO NOT dehydrate the slides in alcohol, and make sure your reagents are not contaminated with alcohol.*

35 Place 1-2 drops of VectaMount or Ecomount on the slide and coverslip the tissue sections.

36 Dry slides at room temp for ≥ 5 min