BASEscope-RED Protocol (Quick version EA)
To use with kit 323910

Notes: this protocol works for both retinal tissue (E14-P11 tested) and embryonic spinal cords under PFA fixation conditions.

Acronyms and shorthands:

PFA Paraformaldehyde
PBS Phosphate-buffered saline
DEPC Diethyl Pyrocarbonate
RT Room temperature
O/N Over night

Recipes:

4% PFA in 1xPBS: 10mL 16% PFA + 4mL 10x PBS, up to 40mL total volume with DEPC H_2O

Day1

Preparing

1. Bring HybEZ Oven to **40°C**. Wet the bottom filter paper with water and pre-warm tray for at least **20 MIN**
2. Equilibrate the slides @ RT for **30 MIN** before processing the sections.
3. Prepare 3 L of 1X wash buffer by adding 2.94 L distilled water to 1 bottle (60 mL) 50X wash buffer (If precipitation occurs, **warm it up @ 40°C for 10–20 MIN** before making 1X wash buffer)
4. Equilibrate probes and AMP 1 reagent at **RT.** Equilibrate AMP 2-8 reagents at **RT** on the step prior to use
5. Prepare 200mL of fresh RNAscope 1X Target Retrieval Reagents by adding 180mL distilled water to 20mL 10X Target Retrieval Reagents. Mix well.
6. Create a barrier with hydrophobic pen
7. Wash the slides with 200 mL 1X PBS in a Tissue-Tek slide rack for **5 MIN** to remove the NEG-50.
8. Postfix with 4% PFA for **10min** at **RT**
9. Rinse twice with DEPC-PBS

Hydrogen Peroxide treatment

1. Lay slides on the bench and add ~5-8 drops of RNAsope Hydrogen Peroxide to cover the entire section.
2. Incubate slides for **10min** at **RT**.
3. Remove Hydrogen Peroxide from one slide at a time by tapping the slide on absorbent paper. Immediately submerge in distilled water.
4. Wash slides 3-5 times by moving rack up and down in the distilled water.
5. Repeat with fresh distilled water.

Protease treatment

1. Apply RNAscope Protease III (3-4 drops) to permeabilize the tissue.
2. Incubate for **10 MIN @ 40°C.** (varies with tissue type, optimize).
3. Briefly submerge in 200ml of distilled water to remove excess protease.
4. Repeat washes 3-5 times.

(**1:15h**)

Probe Hybridization

1. Add **~4 drops** of appropriate probe to each slide. Incubate for **2 hours @ 40°C**.
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT**.
3. Repeat with fresh 1x wash buffer.

(**2h**)

Signal Amplification: AMP 1

1. **Add ~4 drops of AMP 1** to cover each section. Incubate for **30 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 2

1. **Add ~4 drops of AMP 2** to cover each section. Incubate for **30 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 3

1. **Add ~4 drops of AMP 3** to cover each section. Incubate for **15 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 4

1. **Add ~4 drops of AMP 4** to cover each section. Incubate for **30 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 5

1. **Add ~4 drops of AMP 5** to cover each section. Incubate for **30 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 6

1. **Add ~4 drops of AMP 6** to cover each section. Incubate for **15 MIN @ 40°C**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 7

1. **Add ~4 drops of AMP 7** to cover each section. Incubate for **30 MIN @ RT.** Intensity can be modified by adjusting time
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Signal Amplification: AMP 8

1. **Add ~4 drops of AMP 8** to cover each section. Incubate for **15 MIN @ RT**
2. Submerge the slides in 1x wash buffer for **2 MIN @ RT** with occasional agitation.
3. Repeat with fresh 1x wash buffer.

Detect the signal

1. Briefly spin down BASEscope Fast RED-B tube
2. Prepare enough RED working solution by using 1:60 ratio of Fast RED-B to Fast RED-A (for 0.75”x0.75” add 2µL of RED B to 120µL of RED A. Mix well. (Use Fast RED-B solution within 5min. do not expose to sunlight or UV light)

3. Pipette ~120µL RED solution onto each tissue section
4. Seal tray and incubate for **10min** at **RT**.
5. Tilt slides to remove and discard into waste container.
6. Submerge the slides in tap water.
7. Repeat with fresh tap water.
8. Wash with PBS (for further IF, do not counterstain with HE)

(**4h**)

OPTIONAL: RNAscope + IHC (Immunostaining)

1. After the HRP blocker and the final wash, rinse the slides briefly with 1x PBS.
2. Block the slides in blocking buffer 0.5% Triton X-100, 5% normal Donkey serum in PBS **@ RT for 1 hour**.
3. Primary antibody incubation @ **4°C O/N**

(**1:30h**)

Day 2

4. Wash 5x with 1X PBS, **5 min** each time at **RT**
5. Secondary antibody incubation @ **RT** for **1-2 hours**
6. Wash 5x with 1X PBS, **5 min** each time
7. Mount slides with DAPI-fluoromount-G.

(**3h**)