FullRNAlook kit/ 20 RNA FISH experiments

USER MANUAL & PROTOCOL

V 0.7, 11/2021

NOTICE! **FOR MOLECULAR BIOLOGY APPLICATIONS, NOT INTENDED FOR DIAGNOSIS**

USER MANUAL

Storage

- The FullRNAlook kit's shelf life is 6 months from date of delivery.
- It is recommended to store Hybridization solution, Mounting solution and fluorescent probes at -20 °C upon arrival.
- It is recommended to store DAPI stock solution at 2-8 °C upon arrival.

Sample slides

- For convenience, the kit includes 5 slides with the paraffin-embedded cell pellet, good for 5 RNA FISH experiments.
- The cell line is BT474 – AMSBIO (https://www.amsbio.com/bt474-ffpe-cell-pellet-block-3010-0510). Each unstained section (thickness 5 µm) is mounted on Superfrost™ Plus slide.

Fluorescent probes and stains

- 28S rRNA-ATTO-488
- polyT-ATTO-550
- DAPI

Preparation of the solutions required for the assay

- **95%, 70%** and **50% ethanol solution**: add 95 ml of 100% ethanol to 5 ml of MilliQ water, 35 ml of 100% ethanol to 15 ml of MilliQ water and 25 ml of 100% ethanol to 25 ml of MilliQ water, respectively.
- **Hybridization solution:** add 7 ml of formamide to 7 ml of Hybridization buffer.
- **Probes:** resuspend 28S rRNA-ATTO-488 and polyT-ATTO-550 in 50 µl of MilliQ water each
- **Wash buffer 1** (for 100 ml working solution): combine 10 ml of **10X Wash buffer 1** with 65 ml of MilliQ water and 25 ml of formamide.
- **Wash buffer 2** (for 50 ml working solution): combine 2.5 ml of **20X Wash buffer 2** with 47.5 ml of MilliQ water.
- **Wash buffer 3** (for 250 ml working solution): combine 12.5 ml of **20X Wash buffer 3** with 237.5 ml of MilliQ water.
- **DAPI working solution:** dilute DAPI stock solution 1:1000 in Wash buffer 3

User supplied material

In addition to all included reagents, you will need:

- **Deparaffinization solution** (Histo-Clear II, xylenes, etc.), **histology grade ethanol, MilliQ water, formamide and Coplin jar**.

PROTOCOL

Baking sections

1. Bake slides at 56 °C, 1 h. After baking slides can be safely stored at 4 °C for at least one year.

Deparaffinization

2. Incubate the slides in 50 ml of Histo-Clear II **twice** for 5 min at room temperature (RT).
3. Incubate the slides in 50 ml of 100% ethanol for 2 min at RT.
4. Incubate the slides in 50 ml of 95% ethanol **twice** for 1 min at RT.
5. Incubate the slides in 50 ml of 70% ethanol for 1 min at RT.
6. Incubate the slides in 50 ml of 50% ethanol for 1 min at RT.
7. Wash the slides **twice** with 50 ml of **Wash buffer 3** for 3 min at RT.

Pre-hybridization

8. Place the slides with the tissue section/cell pellet side up on a slide rack in a humidified chamber.
9. Add 300 µl of **Hybridization solution** to each slide. Make sure that the tissue section/ cell pellet is fully covered. Incubate slides for 30 min at RT.
10. Remove **Hybridization solution** by tilting the slide and decanting the solution.

CAUTION!

FROM NOW ON KEEP THE SLIDES IN THE DARK TO PREVENT PHOTO-BLEACHING!

Preparing the probes and hybridization

11. Add 2 µl of 28S rRNA probe and 2 µl of polyT probe to 300 µl Hybridization solution, vortex and add the whole mixture to each slide.

12. Incubate the slides at RT in a sealed humidified chamber for 90 min.

Post-hybridization washes

13. Wash the slides two times in 50 ml of **Wash buffer 1** for 5 min at RT.
14. Wash the slides in 50 ml of **Wash buffer 2** for 5 min at RT.
15. Rinse the slides in 50 ml of **Wash buffer 3** for 2 min at RT.

Mounting slides for microscopy

16. Place the slides horizontally, face up in a humidified slide rack and add 300 µl of **DAPI working solution** to each slide. Incubate for 10 min at RT.
17. Wash the slides **two times** in 50 ml of **Wash buffer 3** at RT.
18. Place the slides horizontally, face up in a humidified slide rack and add 40 µl of **Mounting solution** on the tissue sections/ cell pellets.
19. Carefully place a glass coverslip over the tissue sections/cell pellets. Avoid creating air bubbles.
20. Air dry for 10 min in the dark.
21. Clean coverslip with ethanol and image the stained tissue section/cell pellet.

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