English

miRNA Probe
Catalog ID: MI0020

Intended Use

Abnova **miRNA Probes** are designed to detect the miRNAs via chromogenic in situ hybridization (CISH) in formalin-fixed, paraffin-embedded (FFPE) human tissue specimens.

Principles of Procedure

miRNA in situ hybridization is a powerful technique that allows researchers to visualize and localize miRNAs within cells of FFPE tissues. It is based on the principle of hybridization, where a complementary probe specifically binds to the target miRNA, enabling its detection.

Reagent Provided

1. **miRNA Probe:** Ready-to-use, provide as 100 µl

Reagent Required but Not Provided

1. Xylene
2. Ethanol, 100%, denatured
3. FFPE miRNA ISH Pretreatment Solution (Abnova, Cat # U0574)
4. 2X SSC
5. IGEPAL CA-630 (Sigma-Aldrich, Cat #I-3021)
6. Hydrogen peroxide (H_2O_2) (Merck Millipore, Cat #1.07210.1000)
7. 1X PBS
8. miRNA CISH Blocking Solution (Abnova, Cat #U0577)
9. Rabbit anti-DIG (Abnova, Cat #U0527)
10. EnVision+ Single Reagents, HRP. Rabbit (Dako, Cat #K4003)
11. DAB+ Substrate Chromogen System (Dako Omnis, Cat #K3468)
12. Mayer's Hematoxylin (Genemed Biotechnologies, 10-0049)
13. Mounting Solution (Mutokagaku, Cat #245B032)

Users are advised to use the reagents recommended by Abnova, otherwise the expected results may not be achievable.

Recommendation on Working Condition

Use 10 µL miRNA probe in 22 mm x 22 mm tissue working area.

These are guidelines only and the users should determine their own optimal condition.

Storage and Stability

Please store at 2-8°C. Do aliquot before use. Do not mix different lot of miRNA probe into one. Storage condition other than those specified in the package insert, they must be verified by the user.

Specimen Preparation

Tissues specimens fixed in 10%(v/v) formalin are suitable for use prior to paraffin embedding and sectioning. Prepare 3-5 µm microtome sections on silane-coated or adhesion slides and fix at 50°C.

Precautions

- Keep this product under proper storage and away from heat or flammable substances.
- Verify the temperature of solution, hot plate, water baths, incubators used in the test with a calibrate thermometer.
- Protocols that other than those specified may affect the results.
- Do not mix different lots of probes into one.
- Prepare reagents freshly.
- Wear appropriate personal protective equipment during operation, such as safety goggles, gloves, or protective clothing.
- All biological samples are a potential biohazard and should be handled accordingly using universal precautions.
- Do not expose materials that cannot be properly decontaminated to potentially infectious material.
- Upon completion of the operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant.

Staining Procedure

For Professional Users

The following protocol is used in ABNOVA.

[Slide preparation]

Deparaffinization

1. Place slides in the 65°C oven or on the hot plate for 30 minutes.
2. Place slides in a xylene bath and incubate for 5 minutes. Change baths and repeat twice.
3. Tap off excess liquid and place slides in 100% Ethanol for 5 minutes and repeat once.
4. Air dry slides.

Pre-Treat Slides

1. Immerse slides in FFPE miRNA ISH Pretreatment Solution at 95℃ for 30 minutes.
2. Apply 800 µl 2X SSC wash buffer on tissues for 5 minutes and repeat once.
3. Air dry slides.

[In Situ Hybridization Procedure]

Mark Hybridization Area

Use diamond-tipped scribe to mark the areas to be hybridize.

Hybridization

1. Apply 10 µL of miRNA probe to target area and cover with 22 mm x 22 mm cover glass.
2. Seal cover glass with rubber cement.
3. Place slides in the pre-warmed humidity chamber and incubate at 50℃ overnight.

Post Hybridization Procedure

1. Gently remove the rubber cement and cover glass.
2. Immerse slides in 2X SSC wash buffer for 5 minutes.
3. Immerse slides in 50℃ 0.2X SSC/0.3% NP40 wash buffer for 1 minutes.
4. Apply 800 µl 2X SSC wash buffer on tissues for 2 minutes.
5. Remove 2X SSC wash buffer. Apply 800 µl 3% H_2O_2 in 1X PBS on tissues at room temperature for 10 minutes.
6. Remove 3% H_2O_2 in 1X PBS. Apply 800 µl 1XPBS wash buffer for 5 minutes and repeat once.
7. Remove 1XPBS wash buffer. Apply 800 µl 2.5% normal goat serum on tissues at room temperature for 20 minutes.
8. Remove 2.5% normal goat serum. Apply 100 µl Rabbit anti-DIG (Abnova, Cat #U0527) on tissues at 37℃ for 30 minutes.
9. Remove Rabbit anti-DIG. Apply 800 µl 1XPBS wash buffer for 5 minutes and repeat twice.
10. Remove 1XPBS wash buffer. Apply 100 µl HRP Labbeled Polymer Anti-Rabbit (DAKO, Cat #K4003) on tissues at 32℃ for 40 minutes.
11. Remove HRP Labbeled Polymer Anti-Rabbit. Apply 800 µl 1XPBS wash buffer on tissues for 5 minutes and repeat once.
12. Remove 1XPBS wash buffer. Apply 100 µl DAB+ substrate-chromogen solution (Dako Omnis, Cat #K3468) on tissues for 10 minutes. Immerse slides in 1X PBS wash buffer for 5 minutes.
13. Counter stain in Mayer's hematoxylin.
14. Immerse slides in 1X PBS wash buffer for 5 minutes.
15. Remove 1XPBS wash buffer. Air dry and mount slides.