

**English****miRNA Probe****Catalog ID: MI0003****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<sub>2</sub>O<sub>2</sub>) (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.

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**Pre-Treat Slides**

1. Immerse slides in FFPE miRNA ISH Pretreatment Solution at 95°C 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°C 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°C 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<sub>2</sub>O<sub>2</sub> in 1X PBS on tissues at room temperature for 10 minutes.
6. Remove 3% H<sub>2</sub>O<sub>2</sub> 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°C 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 Labeled Polymer Anti-Rabbit (DAKO, Cat #K4003) on tissues at 32°C for 40 minutes.
11. Remove HRP Labeled 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.