

Product Cat. No.: GBS-016

**For Research Use Only.**

## BRAF(7q34) gene break apart probe reagent Instructions Manual

**[Product Name]** BRAF(7q34) gene break apart probe reagent.

**[Package Specifications]** 10 Tests/box.

### **[Intended use]**

In situ hybridization (ISH) was performed on the basis of routine staining to provide auxiliary information for diagnosis. The test results are only for clinical reference and should not be used as the only basis for clinical diagnosis. Clinicians should make a comprehensive judgment on the test results in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

### **[Detection principle]**

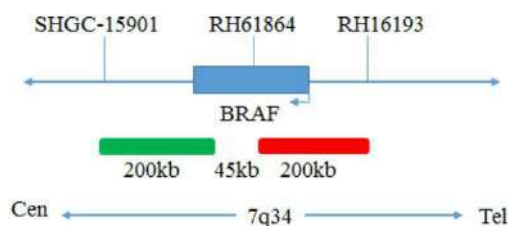
Fluorescence in situ hybridization (FISH) is a technique to directly observe specific nucleic acids in cells. According to the principle of base complementary pairing, a specific probe is complementary to the target sequence in the cell. Because the probe has fluorescence, the hybridization probe and the target sequence can be clearly observed under the fluorescence microscope under the appropriate excitation light.

### **[Product Main Components]**

The kit consists of BRAF dual color probe, as shown in Table 1.

**Table 1: Kit composition**

Component name	Specifications	Quantity	Main components
BRAF dual color probe	100μL/Tube	1	BRAF orange probe, BRAF green probe



### **[Storage conditions & Validity]**

Keep sealed away from light at -20°C±5°C. The product is valid for 12 months. Avoid unnecessary repeated freezing and thawing that should not exceed 10 times. After opening, within 24 hours for short-term preservation, keep sealed at 2~8°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light. The kit was transported below 0 °C.

### **[Applicable Instruments]**

Fluorescence microscope imaging system, including fluorescence microscope and filter set suitable for DAPI (367/452), green (495/517) and orange (547/565).

### **[Sample Requirements]**

1. Applicable specimen type: paraffin embedded specimen of surgical resection or biopsy tissue.
2. The tissue should be fixed with 4% neutral formaldehyde fixative. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

#### [Testing Method]

##### 1. Hybridization pretreatment

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.

Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying.

Permeation: Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.

Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 0.5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.

Washing: Wash with 2xSSC at room temperature for 5 minutes.

Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time.

Remove the excess of EtOH solution on the slides by air-drying.

##### 2. Denaturation and Hybridization

The following operations should be carried out in the dark room.

① Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, mix the probe well, centrifuge it briefly (do not vibrate with vortex apparatus), drop 10μl into the hybridization area of the cell drop, cover the 22mm×22mm cover glass immediately, the probe should be evenly spread under the cover glass without bubbles, and seal the edge with rubber (the edge sealing must be thorough to prevent the dry slide from affecting the test results in the hybridization process).

② Place the glass slide in the hybridization instrument, denature at 85°C for 5 min (the hybridizer should be preheated to 85°C) and hybridized at 42°C for 2-16h.

##### 3. Washing

The following operations should be carried out in a dark room.

① Carefully tear off the adhesive around the cover glass with tweezers to avoid sticking off or moving the cover glass. Immerse the cell drop into 2xSSC for about 5s, and take it out. Gently push one corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;

② The cells were placed at 2xSSC room temperature for 1min;

③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2) solution and wash for 2min.

④ The slides were immersed in deionized water preheated at 37°C for 1min, and then dried naturally in the dark.

##### 4. Counterstaining

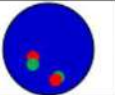
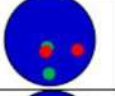
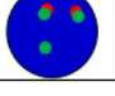
The following operations should be performed in a darkroom

10μl DAPI compound dye is dropped in the hybridization area of the glass slide and immediately covered. The suitable filter is selected for glass slide observation under the fluorescence microscope.

##### 5. FISH results observation

Place the re stained glass slide under the fluorescence microscope, first confirm the cell area under the low power objective lens (10x); turn to the 40x objective lens, find a place where the cells are evenly distributed, the nuclear boundary is complete, DAPI staining is uniform, and the nuclei do not overlap; then observe the fish results of the nuclei under the high power objective lens (100x).

#### [Interpretation of common signal types]

<div> <div><span style="color: red;">●</span> BRAF gene 5 site signal</div> <div><span style="color: green;">●</span> BRAF gene 3 site signal</div> </div>	
	Negative: 2 fusion
	Positive: 1 orange 1 green 1 fusion
	Positive: 1 green 2 fusion

#### [Precautions]

1. Please read this manual carefully before testing. Operator should undergo professional technical training. Signal counting personnel must be able to observe orange and green signals.
2. When testing clinical samples, the test will not provide any test results when the hybridization signal is difficult to count and the sample is not sufficient for repeated retests. If the amount of cells is not sufficient for analysis, the test will not provide test results.
3. The DAPI dye used in this experiment are potentially toxic or carcinogenic and should be handled in a fume hood. Wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, as well as restrictions such as enzyme digestion time, hybridization temperature and time, operating environment, and limitations of current molecular biology techniques, which may result in erroneous interpretation results. User must understand the potential errors and accuracy limitations that may exist during the testing process.
5. All chemicals are potentially dangerous. Avoid direct contact. The used kits are clinical waste and should be properly disposed off.