Wuhan HealthCare Biotechnology Co., Ltd.
Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

Product Cat. No.: FP-016 | ***For Professional Use***

BRAF gene break apart probe reagent Instructions Manual

[Product Name] BRAF 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]

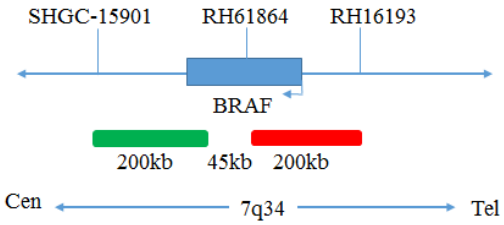
Fluorescence in situ hybridization (FISH) is a technique to directly observe specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, a specific probe is complementary to the target sequence in the cell. Because the probe has fluorescence, the hybridization 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, hybridization buffer



[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 in vitro should be fixed with 4% neutral formaldehyde fixative within 1 hour. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

[Testing Method]

1. Hybridization pretreatment

Recommended to use the "FISH Pretreatment Reagent" of Wuhan HealthCare Biotechnology Co., Ltd. for pretreatment.

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).

②Place the glass slide in the hybridization instrument, denature at 85℃for 5 min (the hybridizer should be preheated to 85℃) and hybridized at 42℃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 3% NP-40/0.4 xSSC solution preheated at 68℃for 2min;

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

4. Counterstaining

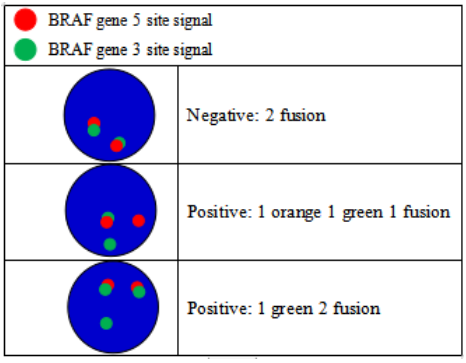
The following operations should be performed in a darkroom

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 (10 ×); turn to the 40 × 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 (100 ×).

[Interpretation of common signal types]



[Limitations of test methods]

① The results of this kit will be affected by various factors of the sample itself, but also limited by hybridization temperature and time, operating environment and the limitations of current molecular biology technology, which may lead to wrong results.

② Users must be aware of the potential errors that may exist in the detection process and the limitations of accuracy.

[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.

[Basic information]

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

Address: Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

Contact: 027-87570662

Name of after-sales service unit: Wuhan HealthCare Biotechnology Co., Ltd.

Contact: 18140559890

Production address: Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

Production license No.: Hubei Food and Drug Supervision Equipment Production Xu No. 20170738

[Medical device Filing Certificate No. / product technical requirement No.]

Medical device Filing Certificate No.: ehxb No. 20190977

[approval date and modification date of the specification]

V1. 0 approval date: November 4, 2019

V1. 2 approval date: December 7, 2021