

Product Cat. No.: GBS-076

For Research Use Only.

TFEB (6p21) gene break apart probe reagent Instructions Manual

[Product Name] TFEB (6p21) gene break apart probe reagent.

[Package specification] 10Tests /box.

[Intended use]

This reagent is used for in situ hybridization staining on the basis of routine staining to provide doctors with 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 comprehensively judge the test results in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

[Detection principle]

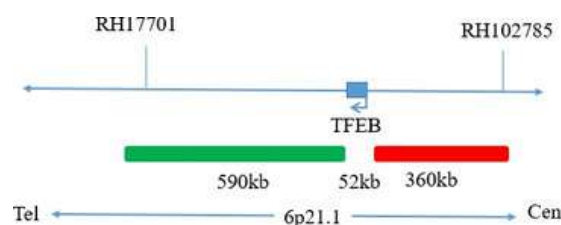
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of the hybrid 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 TFEB dual color probe, as shown in Table 1.

Table 1: Kit composition

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



[Storage conditions & Validity]

Keep sealed away from light at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. 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 $2 \sim 8^{\circ}\text{C}$ in dark. For long-term preservation after opening, keep the lid sealed at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 solution. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

[Instruction]

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. Denaturing 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 remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;
- ② Place the sample at 2xSSC room temperature for 1 min;
- 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 Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark place.

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 stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10x); under magnification objective (40x) a uniform cells distribution is observed; then the nucleus size uniformity, nuclear boundary integrity, DAPI staining uniformity, no nuclei overlapping, cells clear signal are observed in the high magnification objective (100x).

[Interpretation of common signal types]

● TFEB gene site 5 signal ● TFEB gene site 3 signal	
	Negative : 2 fusion
	Positive:1 orange 1 green 1 fusion

[Precautions]

1. This product is only used for in vitro diagnosis.
2. Please read this manual carefully before testing. The testing personnel shall receive professional technical training, and the signal counting personnel must be able to observe and distinguish orange and green signals.
3. When testing clinical samples, when the hybridization signal counting is difficult and the sample is not enough to repeat the retest, or the cell volume is not enough for analysis, the test will not provide the test results.
4. DAPI counterstaining agent used in this experiment has potential toxicity or carcinogenicity, so it is necessary to operate in the fume hood, wear masks and gloves to avoid direct contact.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed off.