

Product Cat. No.: GBS-342

For Research Use Only.

FishBIO KIT gene probe reagent Instructions Manual

[Product Name] KIT gene probe reagent.

[Package specification] 10Tests /box.

[Product introduction]

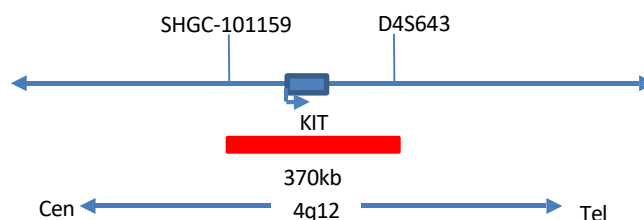
This kit uses orange fluorescein to label KIT orange probe and use green fluorescein to label CEP4 green probe. KIT probe can be combined with the target detection site by in situ hybridization.

[Product Main Components]

The kit consists of KIT/CEP4 dual color probe, as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
KIT/CEP4 dual color probe	100μL/Tube	1	KIT orange probe ; CEP4 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 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.
- 3.

[Instructions]

1. 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 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

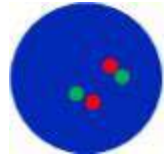

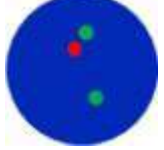
The following operations should be performed in a darkroom

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 stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10×); under magnification objective (40×) 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 (100×).

[Interpretation of common signal types]

<div> <div></div> KIT gene signal <div></div> CEP4 gene signal </div>	
	Negative: 2 orange 2 green
	Positive : n orange 2 green (n≥3)
	Positive : n orange 2 green (n≤1)

[Precautions]

- 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 understand the potential errors and accuracy limitations that may exist in the detection process.
- ③ All chemicals are potentially dangerous. Avoid direct contact. Used kits are waste and should be properly disposed off.

[Manual Approval date & Revision date]

V1. 0: Approval date: March 15, 2022.

V1. 1: Revision date: September 21, 2022.