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Product Catalogue Number FP-086 **For clinical diagnosis and scientific research.**

FGFR1 Gene Amplification Probe Detection Kit

[Product Name] FGFR1 Gene Amplification Probe Detection Kit.

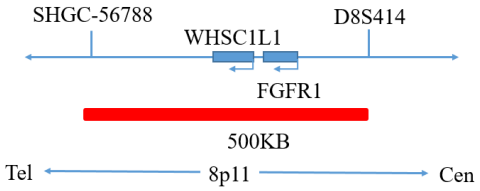
[Package Specifications] 10 Tests/box.

[Product Introduction]
The kit uses orange fluorescein-labeled FGFR1 probe and green fluorescein-labeled CEP8 probe to bind FGFR1/CEP8 probe to the target detection site by in situ hybridization.

[Product Composition]
The kit consists of FGFR1/CEP8 dual color probe as shown in Table 1.

Table 1: Kit composition

Package Specifications	Component name	Volume	Quantity	Main components
10 Tests/box	FGFR1/CEP8 dual color probe	100μL/Tube	1	FGFR1 Orange probe CEP8 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. This kit is shipped below 0°C.

[Applicable Instruments]
Fluorescence microscopy imaging systems, including fluorescence microscopy and filter sets 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 fixation solution within 1 hour after in vitro. After tissue fixation, it should be regularly dehydrated and embedded in paraffin.

[Instructions]
1.Sample collection and slides preparation
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 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 performed in a darkroom.
① Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. Take 10μl droplet in the cell and drop in the hybridization zone, immediately cover 22mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slide covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).
② Place the glass slides in the hybridization instrument, denature at 85°C for 5 minutes (the hybridizer should be preheated to 85°C) and hybridize at 42°C for 2 to 16 hours.

3. Washing

The following operations should be performed in a darkroom.
① Take out the hybridized glass slides, remove the rubber on the coverslip and immediately immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.
② Place the slides in a 2×SSC at 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 Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

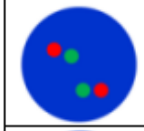
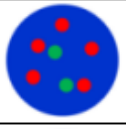
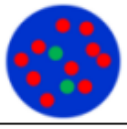
4. Counterstaining

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

Put the counterstained cell drops under the fluorescence microscope, first under the low-power objective lens (10×） Confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100×) the FISH results of nuclei were observed.

[Common Signal Type Interpretation]

FGFR1 gene signal CEP8 gene signal	
	Negative: Normal amplification
	Positive : suggests FGFR1 gene low level amplification
	Positive : suggests FGFR1 gene high level amplification

[Precautions]

1. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals and the samples are not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated in the fume hood and wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, but also limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong results. The user must understand the potential errors and accuracy limitations that may exist in the detection process.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be properly disposed of.
6. This product is for clinical diagnosis and scientific research.

[Manuscript version and approval date]
Manual version: V1.2 Revised on May 13, 2022.
Approval date: April 01, 2020.