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Product Catalogue Number FP-006 **For clinical diagnosis and scientific research.**

6q Gene Probe Detection Kit

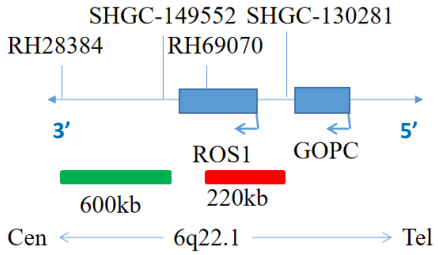
[Product Name] 6q Gene Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Product introduction]
The kit adopts 6q probes labeled with orange fluorescein and green fluorescein. The gene rearrangement of 6q can be detected by in situ hybridization.

[Product Main Components]
The kit consists of 6q dual color probe, as shown in Table 1.

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	6q dual color probe	100μL/Tube	1	(5'Tel) 6q Green probe ; (3'Cen) 6q Orange 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 is transported under 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.

[Operating instructions]
1. Pretreatment
Recommended to use the FISH pretreatment reagent of Wuhan HealthCare Biotechnology Co., Ltd.

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 Place the slides in a 2×SSC at room temperature for 1 min.
② Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min.
③ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

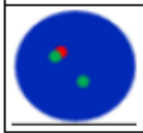
4. Counterstaining

The following operations should be performed in a darkroom.
Dip 10μL of DAPI counterstain into the hybridization area of the glass slides, immediately cover with a lid and place in dark for 10-20min, then use the appropriate filter to observe the sections under the fluorescence microscope.

5. FISH results observation

Place the counterstained film under the fluorescence microscope, and first put it 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]

6q 6q	
	Negative :2 Fusion
	Positive: 1 Orange 1 Green 1 Fusion
	Positive: 1 Green 1 Fusion

[Troubleshooting]

The common factors influencing test results and the handling methods are shown in Table 2:

Table 2: Frequent problems and handling methods

Problem	Probable cause	Recommended solution
Strong background of slides	Inadequate wash of glass slide before preparation of specimens	Wash the glass slide using the absolute ethyl alcohol.
	Inadequate wash after hybridization	Assure that the wash buffer is prepared in line with Instruction For Use, assure the correct pH value and temperature of wash buffer, remove the coverslip and repeat the washing steps.

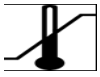
	Improper use of filter sets	Replace with suitable filter sets to reduce the background light.
	Improper hybridization condition	Assure the temperature of hybridization instrument is set as 42ºC.
	The temperature is too low when washing	Assure that the wash buffer reaches to the required temperature when washing the slides.
	The washing intensity of wash buffer is too low	Assure the wash buffer is prepared in line with Instruction For Use. (low SSC concentration or high NP-40 concentration would help improving the washing intensity of wash buffer).
Weak counterstaining	Weak counterstaining	Remove coverslip, at room temperature, immerse the slides in the wash buffer containing 2 × SSC/ 0.1%NP-40 for 5 minutes. And then sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining.
	The counter stain has been kept under long-term storage or excessive light	Assure the counter stain is stored at -20ºC and protected away from light, assure its effect.
No signal or weak signals	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83ºC, at least 10 minutes in advance is needed to preheat hybridization instrument.
	The probe mixture and hybridization buffer were not mixed sufficiently before use	Blow the probe mixture and mix the probe sufficiently, centrifuge for a short time.
	The probe mixture on tissue slides dries too fast	After dropping probe mixture the target area should be covered by coverslip immediately, when washing the slides you can only remove one coverslip at a time, and dip it into wash buffer immediately before removing next coverslip.
	Air bubbles formed under coverslip during hybridization	The coverslip should cover the probe mixture in order to gently squeeze out air bubbles.
	Inappropriate hybridization condition	Ensure to comply with the time and temperature required by hybridization and do not leave gaps when sealing the slides with rubber cement. The hybridization time should be adjusted according to the situation.
	Improper wash buffer or incorrect washing conditions	Be sure to follow the requirements of Instruction for Use to formulate the wash buffer. Ensure that the temperature of wash buffer reaches to the temperature predetermined in washing step. The thermometer and pH meter should be accurately calibrated. Remove coverslip before immersing the slide into wash buffer.
	Inappropriate storage of probe or specimens slides	Make sure that the probe mixture is stored at -20ºC and protected from light. Place the slides without hybridization at -20ºC for long-term storage or at room temperature for short-term storage. Place the hybridized slides at -20ºC, away from light, and store for less than 6 months.
	Incorrect use of DAPI counter stain, excessively high brightness of counter stain	Remove the coverslip, immerse the slides in 2 × SSC/ 0.1%NP-40 for 5 minutes at room temperature. Sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining after air drying the slides.
	Inappropriate filter sets were selected for observation	Use correct filter sets to observe the probe fluorescence. For the detailed information, please consult the technical service department of Wuhan HealthCare Biotechnology Co., Ltd.

If there are other problems, please contact our technical support at: cs@healthcare-bio.com

[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.

[Explanation of Symbols]

	Use by		In vitro diagnostic medical device
	Batch code		CE marking
	Manufacturer		Warning sign
	Catalogue number		European Authorised Representative
	Temperature limitation		Consult Instructions For Use
	Contains sufficient for <n> tests		

[Manuscript version and approval date]
Manual version: V1.2 reviewed on 07 December 2021
Approval date: 18 April 2019