

6q Gene Probe Detection Kit (CW-006)

Intended use

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 composition

The kit consists of 6q dual color probe (100 µl/Tube).

Storage condition

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 $2-8^{\circ}\text{C}$ 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. This kit shipment should be made 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.

Operating instructions

Pretreatment

Recommended to use the FISH pretreatment reagent of Cytowish (catalogue number CW-CT-FISH).

Denaturation and hybridization

The following operations should be performed in a darkroom.

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1. Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. Take 10 μ l 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.

Washing

The following operations should be performed in a darkroom.

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

Counterstaining

The following operations should be performed in a darkroom.

Dip 10 μ L 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.

FISH results observation

Place the counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10 \times) Confirm the cell area under the microscope; Go to 40 \times Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100 \times) The FISH results of nuclei were observed.

Common signal type interpretation

<ul style="list-style-type: none"> • 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 the below table.

Problem	Problem cause	Recommended solution
	Improper use of filter sets	Replace with suitable filter sets to reduce the background light.
	Improper hybridization conditions	Ensure that the hybridization instrument temperature is 42°C
	Low washing temperature	Ensure that the solution temperature of the washing glass slides is up to the required temperature
	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 -20°C and protected away from light, assure its effect
No signal or weak signal	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83°C, at least 10 minutes in advance is needed to preheat hybridization instrument

	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83°C, 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 -20°C and protected from light. Place the slides without hybridization at -20°C for long-term storage or at room temperature for short-term storage. Place the hybridized slides at -20°C, 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

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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 reviewed on 07 December 2021](#)

Approval date: [18 April 2019](#)