Cytowish Ltd
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Your authentic partner in molecular cytogenetics

USP6(17p13) Gene Break Apart Probe Detection Kit

(CW-074)

Intended use

Based on the conventional staining, the reagent was used for in situ hybridization staining to provide auxiliary information for doctors. The test results are only for clinical reference, and should not be used as the only basis for clinical diagnosis. Clinicians should combine the patient's condition, drug indications, treatment response and other laboratory test indicators to comprehensively judge the test results.

Product composition

The kit consists of USP6 dual color probe (100μL/Tube).

Storage condition

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.

Applicable instruments

Fluorescence microscopy imaging system includes fluorescence microscope and filter sets. The kit is labeled with orange fluorescein, and the filter set compatible with the fluorescent labeled dye should be selected.

DAPI: The maximum excitation wavelength is 367nm and the maximum emission wavelength is 452nm.

Orange fluorescence: The maximum excitation wavelength is 547nm and the maximum emission wavelength is 565nm.

Green fluorescence: The maximum excitation wavelength is 495nm and the maximum emission wavelength is 517nm.

Sample requirements

Tissue samples:

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1. Applicable specimen type: surgical resection or paraffin embedded specimen of biopsy tissue.

2. The tissue should be fixed with 4% neutral formaldehyde fixative within 1 hour after the tissue is detached. After the tissue is fixed, it is often dehydrated and paraffin embedded.

Sample pretreatment and hybridization

Tissue samples:

Recommended to use Cytowish's pretreatment reagent (catalogue number CW-PT-FISH).

Denaturation and hybridization

The following operations should be performed in a darkroom.

1. Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (vortex instrument vibration is prohibited). Take 10μl l drop in the tissue hybridization area, and immediately cover the cover glass of 22mm×22mm. The probe shall be evenly expanded under the cover glass without bubbles, and edge shall be sealed with rubber glue (the edge must be completely sealed to prevent dry chips from affecting the test results in the hybridization process).
2. The tissue sections were placed on the hybridizer and denatured at 85°C for 5min (the hybridizer should be preheated to 85°C in advance), and hybridized at 42°C for 2-16h.

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 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.

Counterstaining

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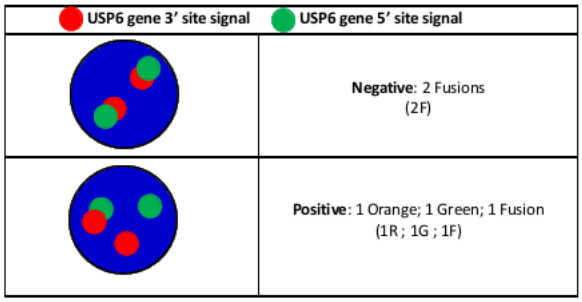
The following operations should be performed in a darkroom.

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.

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 (60x, 100x).



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.

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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: V1.0 reviewed

Approval date: