Cytowish Ltd
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Your authentic partner in molecular cytogenetics

1p/19q Gene Deletion Probe Detection Kit

(CW-045)

Intended use

This kit uses orange fluorescent probes 1p36 and 19q13, green fluorescent probes 1q25 and 19p13 to bind 1p/19q probe to the target detection site by in situ hybridization.

Product composition

The kit consists of 1p36/1q25 dual-color probe and 19q13/19p13 dual-color probe 100μl/tube.

Storage condition

Keep sealed away from light at -20oC±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 -20oC±5°C away from light. The kit should be shipped below 0°C.

Applicable instruments

Fluorescence microscopy imaging system including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

Sample requirements

1. Applicable specimen types: Surgical resection or biopsy paraffin-embedded specimens.

2. The tissue should be fixed with 4% neutral formaldehyde fixative solution within one hour after tissue isolation, tissue dehydration and paraffin embedding.

Pre-hybridization treatment

It is recommended to use the Cytowish's Pretreatment Reagent (catalogue number CW-CT-FISH).

Denaturation and hybridization

The following operations need to be carried out in the darkroom.

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1. Take the probe at static room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). 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 slide in the hybridization instrument, denature at 85°C for 5 minutes (the hybridizer should be preheated to 85oC) and hybridize at 42°C for 2 to 16 hours.

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.

Dyeing

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 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 are observed.

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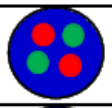
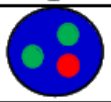
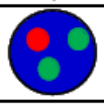
1p36 and 19q13 signal	1q25 and 19p13 signal
	**Negative**: 2 Orange-red (2R) ; 2 Green (2G)
	**Positive**: 1 Orange-red (1R) ; 2 Green (2G) ----1p36/1q25 point-out, 1p36 missing.
	**Positive**: 1 Orange-red (1R) ; 2 Green (2G) ----- 19p13/19q13 point-out, 19q13 missing.

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

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