

SRD(1p36) Gene Deletion Probe Detection Kit (CW-050)

Intended use

This kit performs in situ hybridization staining on the basis of conventional staining to provide physicians with auxiliary information for diagnosis. The test results are for clinical reference only and should not be used as the only basis for clinical diagnosis. Clinicians should make comprehensive judgment on the test results based on factors such as the patient's condition, drug indications, treatment response and other laboratory test indicators.

Product composition

The kit consists of SRD/PBX1 dual color probe 100 μ l/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.

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 tissue paraffin-embedded specimen.
2. The tissue should be fixed within 1 hour with 4% neutral formaldehyde fixative solution, and the tissue should be routinely dehydrated and embedded in paraffin.

Pretreatment

It is recommended to use Cytowish's pretreatment reagent kit (catalogue number CW-CT-FISH).

Denaturation and hybridization

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

1. Take the probe at room temperature for 5 minutes. Briefly centrifuge after manually mixing the probe (do not use vortex/swirl or shaker instrument/oscillator). 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 slide 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. Carefully remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers.
2. Place the sample at 2xSSC room temperature for 1 min.
3. Take out the sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68°C for 2min.
4. Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark place.

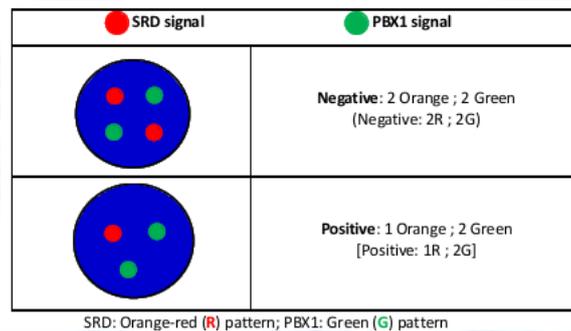
Counterstaining

The following operations should be performed in a darkroom.

10 μ l 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

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



Test Method Limitations

1. The results of this kit will be affected by various factors of the sample itself, but also limited by hybridization temperature and time, operating environment, and limitations of current molecular biology technology, which may lead to erroneous results.
2. The user must understand the potential errors and accuracy limitations that may exist in the detection process.

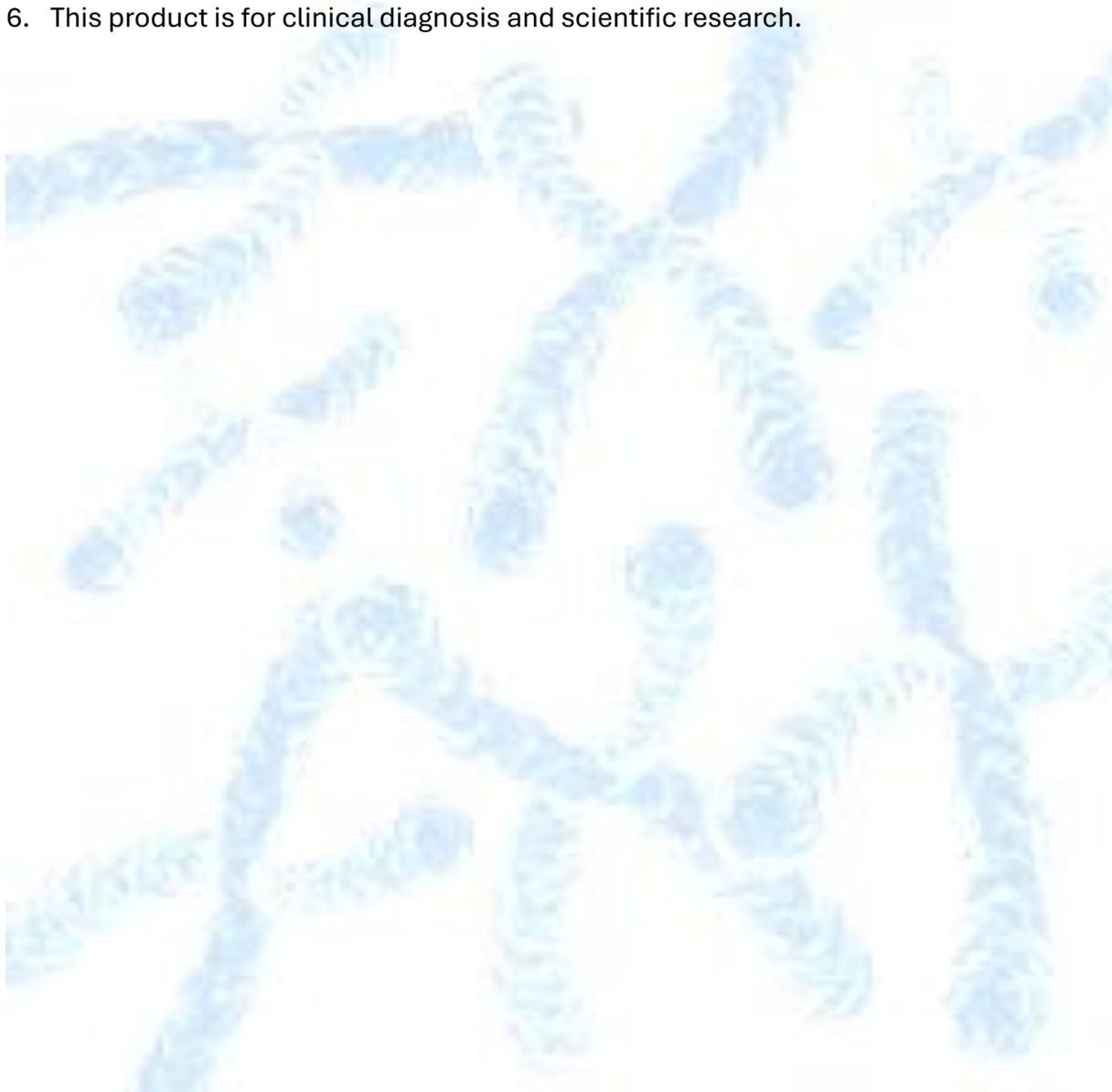
Precautions

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

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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]

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