Cytowish Ltd
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Your authentic partner in molecular cytogenetics

JAZF1(7p15) Gene Break Apart Probe Detection Kit

(CW-226)

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 JAZF1 dual color probe (100 μL/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 -20oC±5°C away from light. The kit is transported 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 specimens' types: Surgical resection or paraffin-embedded biopsy specimens.
2. Isolated tissue should be fixed in vitro with 4% neutral formaldehyde fixative within 1 hour. After tissue fixation, regular dehydration and paraffin embedding should be performed.

Pretreatment

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

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Denaturation and hybridization

The following operations need to be carried out in the 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 (no vortex instrument vibration). Take 10μl drop in the cell hybridization area, and immediately cover the cover glass of 22mm×22mm. The probe should be evenly expanded under the cover glass without bubbles, and then seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).
2. Put the tissue sections on the hybridizer, and denature at 85℃ for 5min (the hybridizer should be preheated to 85℃ in advance), and hybridize at 42℃ 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 place the slides into 2xSSC for 5 seconds, and gently remove the coverslip.
2. Place the glass slides in 2xSSC at room temperature.
3. Remove and immerse the slides in a 0.3% NP-40/0.4×SSC solution preheated at 68°C for 2 min.
4. Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

Counterstaining

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 (10×); under magnification objective (40×) 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).

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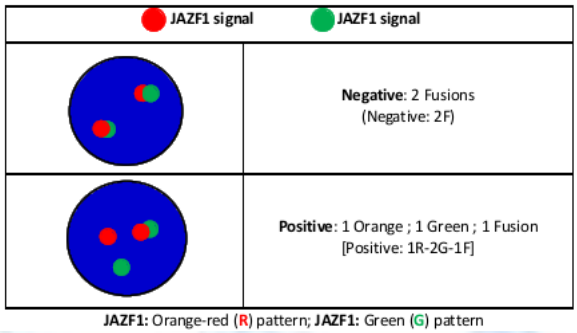

JAZF1: Orange-red (R) pattern; **JAZF1:** Green (G) pattern

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: V1.1 reviewed on 18 September 2020

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