

## 11q23.3/11q24.3 Gene Deletion Probe Detection Kit

**[Product Name]** 11q23.3/11q24.3 Gene Deletion Probe Detection Kit (Fluorescence In Situ Hybridization Method).

**[Product 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.

**[Detection Principle]**

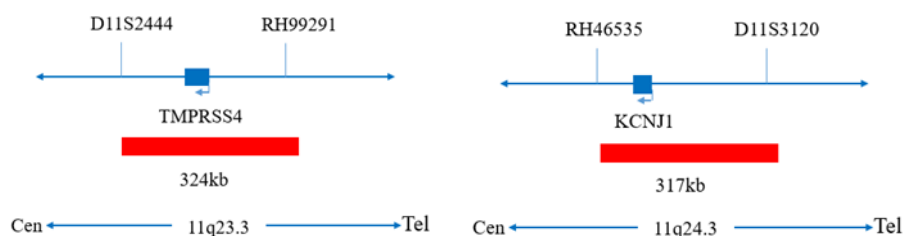
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of complementary base pairing, a specific probe is complementary to the target sequence in the cell. Because the probe is fluorescent, the hybridization probe and the target sequence can be clearly observed under a fluorescence microscope under the appropriate excitation light and the genetic status is observed.

**[Product Composition]**

The kit consists of two sets of probes: 11q23.3/CEP11 dual color probe and 11q24.3/CEP11 dual color probe as shown in Table 1.

**Table 1 Kit composition**

Component name	Specifications	Quantity	Main components
11q23.3/CEP11 dual color probe	100μL/Tube	1	11q23.3 orange probe, CEP11 green probe
11q24.3/CEP11 dual color probe	100μL/Tube	1	11q24.3 orange probe, CEP11 green probe



**[Storage conditions & Validity]**

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 systems, 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.

**[Related reagents]****① 20×SSC, pH 5.3±0.2**

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3±0.2 at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2-8°C, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**② 2×SSC, pH 7.0±0.2**

Take 100mL of the above 20×SSC, dilute with 800mL deionized water, mix, adjust the pH to 7.0±0.2 at room temperature, complete to 1L with deionized water, stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**③ Ethanol Solution: 70% ethanol, 85% ethanol**

Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**④ 0.3% NP-40/0.4×SSC solution, pH 7.0-7.5**

Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0-7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**⑤ Fixation solution (methanol: glacial acetic acid = 3:1)**

Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.

**⑥ 0.075M KCl solution**

Weigh 2.8g of potassium chloride, dissolve in 400mL of deionized water and complete to 500mL with deionized water. Stored at room temperature, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**⑦ DiamidinyI phenylindole (DAPI) counterstain**

Use commercially available anti-quenching DAPI counterstain.

**[Instructions]****1. Pretreatment**

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd. pretreatment reagent kit (Cat# CL-003).

**2. Denaturation & Hybridization**

The following operations should be performed in a 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 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 85°C) and hybridize at 42°C for 2 to 16 hours.

**3. 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 2×SSC 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-40/0.4×SSC 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.

**4. Dyeing**



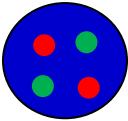
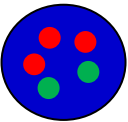
The following operations should be performed in a darkroom

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.



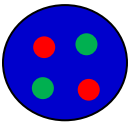
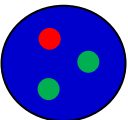
**5. FISH results observation**

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).

**[Common Signal Type Interpretation]**

 11q23.3 signal	 CEP11 signal
	<b>Negative:</b> 2 Orange & 2 Green (Negative: 2R-2G)
	<b>Positive:</b> n Orange ; 2 Green [Positive: nR-2G], n≥3

11q23.3: Orange-red (R) pattern; CEP11: Green (G) pattern

 11q24.3 signal	 CEP11 signal
	<b>Negative:</b> 2 Orange & 2 Green (Negative: 2R-2G)
	<b>Positive:</b> 1 Orange ; 2 Green [Positive: 1R-2G]

11q24.3: Orange-red (R) pattern; CEP11: Green (G) pattern

**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. This product is for in vitro diagnosis usage only.
2. Please read this manual carefully before testing. The testing personnel should undergo professional technical training. The signal counter personnel must be able to observe and distinguish the orange-red and green signals.
3. The test will not provide any results when testing clinical samples it is difficult to count the hybridization signal and the sample is not enough to repeat the test, or the amount of cells is not enough for analysis.
4. The DAPI counterstaining agent used in this experiment is potentially toxic or carcinogenic. It must be operated in a fume hood. Inhalation and direct contact should be avoided by wearing the appropriate masks and gloves.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be disposed of properly.

**[Manuscript version and approval date]**

Manual version: V1.2 reviewed on 07 December 2021

Approval date: 30 April 2020