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Product Cat. No.: GBS-164 ***For Research Use Only***.

11q23.3/11q24.3 gene deletion probe reagent Instructions Manual

[Product Name] 11q23.3/11q24.3 gene deletion probe reagent.
[Package specification] 10Tests /box.

[Intended use]

This reagent 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 sole basis for clinical diagnosis. Clinicians should make a comprehensive judgment of the test results by combining the patient's condition, drug indications, therapeutic response and other laboratory test indicators and other factors.

[Detection principle]

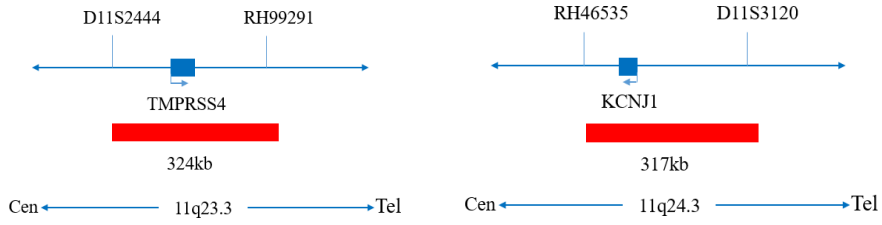
Fluorescence in situ hybridization is a technique to directly observe specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, a specific probe binds complementarily to the target sequence in the cell, and since the probe is fluorescent, under the irradiation of a suitable excitation light, the hybridization probe and the target sequence are able to be clearly observed under a fluorescent microscope to visualize the genetic state.

[Product Main Components]

The kit consists of 11q23.3/CEP11、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
11q24.3/CEP11 dual color probe	100μL/Tube	1	11q24.3 orange probe ; CEP11 green probe



[Storage conditions & Validity]

This kit is transported below 0℃. -20℃±5℃ sealed and stored away from light, the validity period is 12 months, the number of freezing and thawing is not more than 10 times does not affect the performance of the product, but unnecessary repeated freezing and thawing should be avoided. After opening the lid, it can be sealed and stored at 2-8℃ away from light for 24 hours, long-term storage should be stored at -20±5℃ away from light and sealed.

[Applicable Instruments]

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

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[Sample Requirements]

1. Applicable specimen type: paraffin-embedded specimen of surgically excised or biopsied tissue.
2. The tissue should be fixed with 4% neutral formaldehyde fixative within 1 hour after dissection, and the tissue should be fixed after routine dehydration and paraffin embedding.

[Test method]

1. Pretreatment

① Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.
② Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).
③ Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.
④ Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying.
⑤ Permeation: Immerse the slides in deionized water at 100oC and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.
⑥ Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 0.5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.
⑦ Washing: Wash with 2xSSC at room temperature for 5 minutes.
⑧ Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time. Remove the excess of EtOH solution on the slides by air-drying.

2. Denaturing hybridization

The following operations should be carried out in the dark room.
① Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, mix the probe well, centrifuge it briefly (do not vibrate with vortex apparatus), drop 10μl into the hybridization area of the cell drop, cover the 22mm×22mm cover glass immediately, the probe should be evenly spread under the cover glass without bubbles, and seal the edge with rubber (the edge sealing must be thorough to prevent the dry slide from affecting the test results in the hybridization process).
②Place the glass slide in the hybridization instrument, denature at 85℃for 5 min (the hybridizer should be preheated to 85℃) and hybridized at 42℃for 2-16h.

3. Washing

The following operations should be carried out in a dark room.
① Carefully tear off the adhesive around the cover glass with tweezers to avoid sticking off or moving the cover glass. Immerse the cell drop into 2xSSC for about 5s, and take it out. Gently push one corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;
② The cells were placed at 2xSSC room temperature for 1min;
③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2) solution and wash for 2min.
④ The sides were immersed in deionized water preheated at 37℃ for 1min, and then dried naturally in the dark.

4. 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.

5. 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 (100x).

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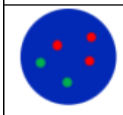
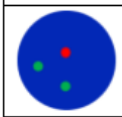
[Interpretation of common signal types]

11q23.3 gene signal CEP11 gene signal	
	Negative： 2 orange 2 green
	Positive : n orange 2 green (n≥3)

11q24.3 gene signal CEP11 gene signal	
	Negative： 2 orange 2 green
	Positive : 1 orange 2 green

[Limitations of test methods]

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 the limitations of current molecular biology technology, which may lead to wrong results.

② Users must understand the potential errors and accuracy limitations that may exist in the detection process.

[Precautions]

1. This product is only used for in vitro diagnosis.
2. Please read this manual carefully before testing. The testing personnel shall receive professional technical training, and the signal counting personnel must be able to observe and distinguish orange and green signals.
3. When testing clinical samples, when the hybridization signal counting is difficult and the sample is not enough to repeat the retest, or the cell volume is not enough for analysis, the test will not provide the test results.
4. DAPI counterstaining agent used in this experiment has potential toxicity or carcinogenicity, so it is necessary to operate in the fume hood, wear masks and gloves to avoid direct contact.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed of.

[Manual Approval date & Revision date]

V1. 0: Approval date: April 30, 2020.

V1. 3: Revision date: May 13, 2022.