

Product Cat. No.: GBS-074

For Research Use Only.

## USP6(17p13) gene break apart probe reagent Instructions Manual

[Product Name] USP6(17p13) gene break apart probe reagent.

[Package Specifications] 10 Tests/box.

### [Intended Usage]

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

### [Detection Principle]

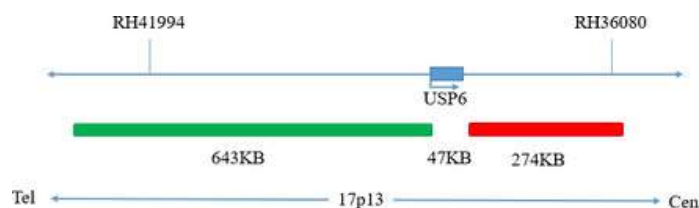
The kit is based on fluorescence in situ hybridization technology. A nucleic acid probe is labeled with fluorescein. The target gene is detected with homologous complementary to the nucleic acid probe used. Both after denaturation, annealing and renaturation, the hybrid of the target gene and the nucleic acid probe can be formed, and the qualitative, quantitative or relative positioning analysis of the gene to be measured under the microscope can be performed by the fluorescence detection system.

### [Product Main Components]

The kit consists of USP6 dual-color probes, as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
USP6 dual color probe	100μL/Tube	1	USP6 orange probe ; USP6 green probe



### [Storage conditions & Validity]

Keep sealed away from light at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ , and the validity period is 12 months. After the cover is opened, it can be sealed and stored in  $2 \sim 8^{\circ}\text{C}$  away from light within 24 hours. After the cover is opened, it should be sealed and stored in  $-20 \pm 5^{\circ}\text{C}$  away from light for a long time. Transport with temperature below  $0^{\circ}\text{C}$ .

### [Applicable Instruments]

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



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#### [Sample Requirements]

1. Applicable specimen types: Paraffin-embedded specimens from surgical excision or biopsy.
2. The tissue should be fixed with 4% neutral formaldehyde solution within 1 hour after isolation. After tissue fixation, it is routinely dehydrated and embedded in paraffin.
3. Paraffin section thickness could affect the experimental results. The recommended slice thickness is 4~5 $\mu$ m.
4. Paraffin-embedded tissue samples from breast cancer should be selected from representative tumor tissue wax blocks and confirmed by HE staining.
5. It is recommended to select paraffin-embedded tissue specimens within 5 years preservation period.

#### [Testing Method]

##### [Instructions]

##### 1. Hybridization pretreatment

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.

Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying.

Permeation: Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.

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 5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.

Washing: Wash with 2xSSC at room temperature for 5 minutes.

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. Denaturation and Hybridization

The following operations should be performed in a darkroom.

- ① Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. Take 10 $\mu$ 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 slides 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 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 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 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.


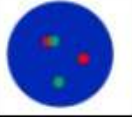
##### 4. Counterstaining

The following operations should be performed in a darkroom.

Dip 10 $\mu$ L of DAPI counterstain into the hybridization area of the glass slides, immediately cover with a lid and place in dark for 10-20min, then use the appropriate filter to observe the sections under the fluorescence microscope.

5. FISH results observation Place the counterstained film under the fluorescence microscope, and first put it 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- power objective (100x) the FISH results of nuclei were observed.

[Positive judgment value or reference interval]

<div> <div>● USP6 gene site 3' signal</div> <div>● USP6 gene site 5' signal</div> </div>	
	Negative :2 Fusion
	Positive: 1 Orange 1 Green 1 Fusion

[Product performance index]

1. Fluorescence signal intensity: after the probe is effectively hybridized with the karyotype sample, it shall send out a fluorescence signal that can be recognized by the naked eye under the fluorescence microscope.
2. Sensitivity: detect karyotype samples and analyze the chromosomes of 50 cells in metaphase division phase. At least 98 chromosomes 17 show an orange fluorescence signal and a green fluorescence signal.
3. Specificity: the chromosomes of 50 cells in metaphase division phase were analyzed. At least 98 chromosomes 17 showed a specific orange fluorescence signal in the target region and a specific green fluorescence signal in the centromere region.

[Precautions]

1. 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.
2. When testing clinical samples, when it is difficult to count the hybridization signal 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, the test will not provide test results.
3. Formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity. It is necessary to operate 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 the limitations of current molecular biology technology, which may lead to wrong interpretation results. Users 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 waste and should be properly disposed off.



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#### [\[References\]](#)

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