

## USP6(17p13) Gene Break Apart Probe Detection Kit

**[Product Name]** USP6(17p13) Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

**[Intended use]**

Based on the conventional staining, the reagent was used for in situ hybridization staining to provide auxiliary information for doctors. The test results are only for clinical reference, and should not be used as the only basis for clinical diagnosis. Clinicians should combine the patient's condition, drug indications, treatment response and other laboratory test indicators to comprehensively judge the test results.

**[Detection principle]**

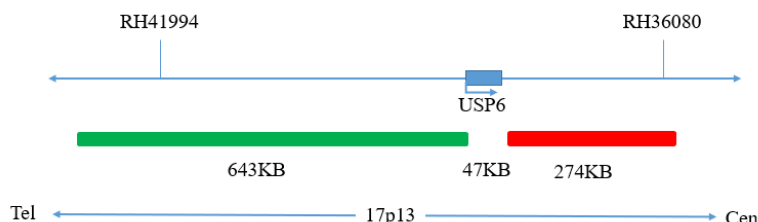
Fluorescence in situ hybridization (FISH) is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Because the probe has fluorescence, the hybridization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

**[Product Main Components]**

The kit consists of USP6 dual color probe 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°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]**

1. Fluorescence microscopy imaging system includes fluorescence microscope and filter sets. The kit is labeled with orange fluorescein, and the filter set compatible with the fluorescent labeled dye should be selected.

DAPI: The maximum excitation wavelength is 367nm and the maximum emission wavelength is 452nm.

Orange fluorescence: The maximum excitation wavelength is 547nm and the maximum emission wavelength is 565nm.

Green fluorescence: The maximum excitation wavelength is 495nm and the maximum emission wavelength is 517nm.

**[Sample requirements]**

**Tissue samples:**

1. Applicable specimen type: surgical resection or paraffin embedded specimen of biopsy tissue.
2. The tissue should be fixed with 4% neutral formaldehyde fixative within 1 hour after the tissue is detached. After the tissue is fixed, it is often dehydrated and paraffin embedded.

### [Test method]

#### 1. Sample pretreatment of hybridization

##### *Tissue samples:*

Recommended to use the FISH pretreatment reagent of Wuhan HealthCare Biotechnology Co., Ltd. for pretreatment.

#### 2. Denaturing hybridization

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

##### *Tissue samples:*

- 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 (vortex instrument vibration is prohibited). Take 10μl drop in the tissue hybridization area, and immediately cover the cover glass of 22mm×22mm. The probe shall be evenly expanded under the cover glass without bubbles, and edge shall be sealed with rubber glue (the edge must be completely sealed to prevent dry chips from affecting the test results in the hybridization process).
- ② The tissue sections were placed on the hybridizer and denatured at 85°C for 5min (the hybridizer should be preheated to 85°C in advance), and hybridized at 42°C for 2-16h.

#### 3. Washing

The following operations need to be carried out in the 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 2×SSC 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 2×SSC room temperature for 1 min.
- ③ Take out the sample and immerse it in 0.3%NP-40/0.4×SSC 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.

#### 4. Complex dyeing



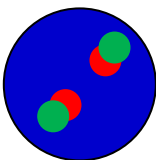
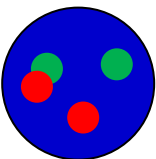
The following operations should be performed in a darkroom

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 (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 (60x, 100x).

### [Common Signal Type Interpretation]

 USP6 gene 3' site signal	 USP6 gene 5' site signal
	<b>Negative:</b> 2 Fusions (2F)
	<b>Positive:</b> 1 Orange; 1 Green; 1 Fusion (1R ; 1G ; 1F)

**[Precautions]**

1. The results of this kit will be affected by various factors of the sample itself, as well as restrictions such as enzyme digestion time, hybridization temperature and time, operating environment, and limitations of current molecular biology techniques, which may result in erroneous interpretation results.
2. User must understand the potential errors and accuracy limitations that may exist during the testing process.