Product Cat. No.: FP-074 | ***For Professional Use***

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]

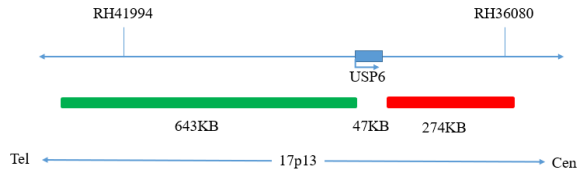
The kit is based on fluorescence in situ hybridization technology. A nucleic acid probe is labeled with fluorescein. The target gene is detected with homologous complementary to the nucleic acid probe used. Both after denaturation, annealing and renaturation, the hybrid of the target gene and the nucleic 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

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	USP6 dual color probe	100μl/Tube	1	USP6 orange probe, USP6 green probe, hybridization buffer



[Storage conditions & Validity]

Keep sealed away from light at -20ºC±5ºC, and the validity period is 20 months.

After the cover is opened, it can be sealed and stored in 2~8°C away from light within 24 hours. After the cover is opened, it should be sealed and stored in -20±5°C away from light for a long time. Transport with temperature below 0°C.

[Applicable Instruments]

Fluorescence microscopic imaging system, including fluorescence microscope and filter set suitable for DAPI, green and orange

[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 4~5μm.
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]

1. Related reagents

The following reagents are required for the experiment but not provided in this kit

① 20×SSC (sodium citrate buffer), pH 5.3±0.2

Sodium chloride	176g
Sodium citrate	88g

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 2~8ºC, 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 20xSSC, 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 2~8ºC, 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 Protease K

Protease K stock solution (20mg/mL): Weigh 0.1g of proteinase K dry powder, dissolve in 5mL 2xSSC (pH value 7.0). Gently mix the solution till completely dissolved, and store at -20°C. Shelf life is 6 months.

Protease K working solution (200μg/mL): Dissolve 0.8mL Proteinase K stock solution in 80mL 2×SSC (pH value 7.0), mix well and the solution is ready for immediate use.

⑤ 0.3% NP-40/0.4xSSC solution, pH 7.0 ~ 7.5

NP-40	0.6mL
20xSSC	4mL

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 2~8ºC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑥ For diamidino phenylindole (DAPI) counterstaining agent, please select commercial DAPI counterstaining agent containing anti quenching agent.

⑦ Xylene

2. Pretreatment

It is recommended to select specimens with known positive and negative amplification as controls.

① Sectioning: Place on clean slides tissue section fixed in neutral formalin and paraffin-embedded.

② Heating slices: Place the slices in the heating machine and heat overnight at 65°C.

③Dewaxing: Immerse the tissue sections in xylene for 10 minutes, dewax, repeat once, and then immediately immerse in 100% ethanol for 5 minutes.

④ Rehydration: At room temperature, place in 100% ethanol, 85% ethanol, and 70% ethanol for 2 minutes each the tissue sections, immediately immerse in deionized water for 3 minutes. Take out the slices and use a lint-free tissue to absorb excess water around the tissue.

⑤ Water treatment: Soak tissue sections in deionized water at 95°C for 30 to 40 minutes (deionized water is applied in a water bath to preheat).

⑥ Washing: At room temperature, soak the tissue sections in 2xSSC solution and rinse twice for 5 minutes each.

⑦ Proteinase K treatment: Soak the tissue sections in proteinase K working solution and treat at 37°C for 5-30 minutes.

⑧ Washing: At room temperature, soak the tissue sections in 2xSSC solution and rinse twice for 5 minutes each.

⑨ Dehydration: Placed the tissue sections in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes each, and then dry naturally.

3. Denaturation and Hybridization

The following operations should be performed in a darkroom.

① Take TOP2A/CEP17 dual-color probe. Mix and centrifuge briefly. 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 slides in the hybridization instrument, denature at 85°C for 5 minutes (the hybridizer should be preheated to 85ºC) and hybridize at 42°C for 2 to 16 hours.

4. 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 for 1 min.

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 37ºC for 1min, and dry naturally in the dark.

5.Counterstaining

The following operations should be performed in a darkroom.

Dip 10~15μL of DAPI counterstain into the hybridization area of the glass slide, immediately cover and place in dark for 10~20 minutes, and then use the suitable filter to observe the sections under the fluorescence microscope.

6. FISH results observation

Place the stained sections under a fluorescence microscope and the area of the breast cancer cells 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). Select randomly 50 tumor cells at least and count the orange and green signals in the nuclei.

[Positive judgment value or reference interval]

USP6 gene site 3' signal USP6 gene site 5' signal	
	Negative :2 Fusion
	Positive: 1 Orange 1 Green 1 Fusion

[Limitations of test methods]

The results of this kit will be affected by various factors of the sample itself, as well as by the limitations of hybridization temperature and time, operating environment, and limitations of current molecular biology techniques, which may lead to erroneous results.

Users must be aware of potential errors and limitations of accuracy that may exist in the detection process.

[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 of.

6. This product is only used for in vitro diagnosis.

[References]

[1]. K. E. Olsen et al. Amplification of HER2 and TOP2A and deletion ofTOP2A genes in breast cancer investigated by newFISH probes, ActaOncologica 43 (2004)

[2]. K. V. Nielsen et al. The value of TOP2A gene copy number variation asa biomarker in breast cancer: Update of DBCG trial89D, ActaOncologica, 2008; 47: 725734

[3]. Zaczek, Markiewicz, Seroczynska et al. Prognostic Significance of TOP2A Gene Dosage in HER-2-NegativeBreast Cancer, The Oncologist 2012;17:1246–1255

[4]. Järvinen TA, Tanner M, Bärlund M, et al. Characterization of topoisomerase II alpha gene amplification and deletion in breast cancer. Genes Chromosomes Cancer. 1999 Oct; 26(2):142-50.

[5]. Li Xuyuan, Li Yubing, Lin Ying Cheng breast cancer TOP2A gene and anthracycline efficacy, [J] International Journal of oncology 2011.2.38 (2)

[Basic information]

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