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Product Catalogue Number FP-085 **For clinical diagnosis and scientific research.**

PTEN Gene Deletion Probe Detection Kit

[Product Name] PTEN Gene Deletion Probe Detection Kit (Fluorescence In Situ Hybridization Method).

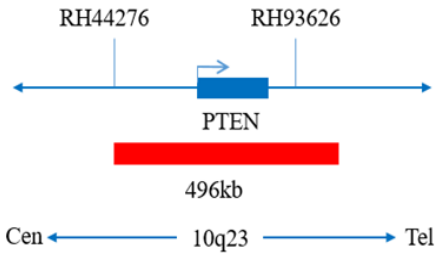
[Product Introduction]
The kit uses orange fluorescein to label PTEN probe and green fluorescein to label CEP10 probe. PTEN/CEP10 probe can be combined with the target detection site by in situ hybridization.

[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 PTEN/CEP10 dual-color probes as shown in Table 1.

Table 1: Kit composition

Component name	Cat.#	Specifications	Quantity	Main components
PTEN/CEP10 dual color probe	FP-085	100μL/Tube	1	PTEN Orange probe ; CEP10 Green probe



[Storage conditions & Validity]
This kit is shipped below 0°C. 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 specimen type: paraffin embedded specimen of surgical resection or biopsy tissue.
2. The tissue in vitro should be fixed with 4% neutral formaldehyde fixative within 1 hour. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

[Instructions]

1. Hybridization pretreatment

Recommended to use the "FISH Pretreatment Reagent" of Wuhan HealthCare Biotechnology Co., Ltd. for pretreatment.

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°C for 5 min (the hybridizer should be preheated to 85°C) and hybridized at 42°C 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 5 seconds, 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 3% NP-40/0.4 xSSC solution preheated at 68°C for 2min.

④ The sides were immersed in deionized water preheated at 37°C for 1min, and then dried naturally in the dark.

4. Counterstaining

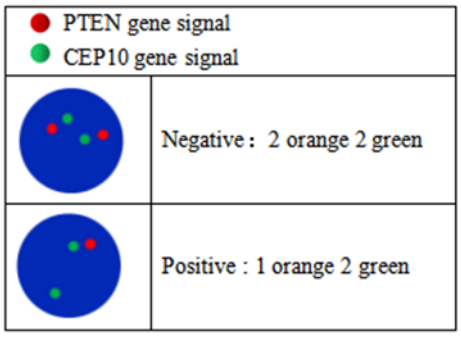
The following operations should be performed in a darkroom

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.

6. FISH results observation

Place the stained slides under a fluorescence microscope and confirm the cells area under a low magnification objective (10x). Under magnification objective (40x) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100x).

[Common Signal Type Interpretation]



PTEN: Orange-red (R) pattern; **CEP10:** 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. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals 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, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated 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 limitations of current molecular biology technology, which may lead to wrong results. The user 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 wastes and should be properly disposed of.
6. This product is for clinical diagnosis and scientific research.

[Manuscript version and approval date]
Manual version: V1.3 reviewed on May 13, 2022
Approval date: 15 August 2020