Product Catalogue Number MBI-FP-A046

MET Gene Probe Detection Kit

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

[Product Introduction]

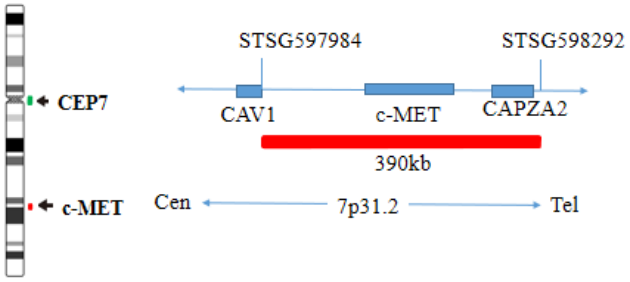
The kit uses orange-red fluorescein-labeled C-MET probe and green fluorescein-labeled CEP7 probe to bind C-MET/CEP7 probe to the target detection site by in situ hybridization.

[Product Composition]

The kit consists of C-MET/ CEP7 dual-color probes, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
C-MET/ CEP7 dual color probe	100μl/Tube	1	C-MET Orange-red probe, CEP7 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℃ 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 specimens' types: Surgical resection or paraffin-embedded biopsy specimens.
2. Isolated tissue should be fixed in vitro with 4% neutral formaldehyde fixative within 1 hour. After tissue fixation, regular dehydration and paraffin embedding should be performed.

[Instructions]

1. Pretreatment

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd. pretreatment reagent kit (Cat# CL-003).

2. Denaturation & Hybridization

The following operations should be performed in a darkroom.

① Take the probe at room temperature for 5 minutes. Briefly centrifuge after manually mixing the probe (do not use vortex/swirl or shaker instrument/oscillator). 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 slide 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 Take out the hybridized glass slides, remove the rubber on the coverslip and immediately immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.

② Place the slides in a 2×SSC at room temperature for 1 min.

③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min.

④ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

4. Dyeing

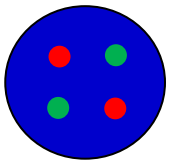
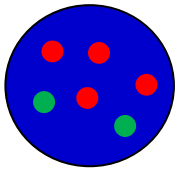
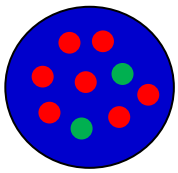
The following operations should be performed in a darkroom

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 (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).

[Common Signal Type Interpretation]

C-MET signal	CEP7 signal
	**Negative**: 2 Orange & 2 Green (Negative: 2R-2G)
	**Positive**: n Orange-red ; 2 Green [Positive: nR-2G, n>3]
	

C-MET: Orange-red (R) pattern; CEP7: 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. This product is for in vitro diagnosis usage only.
2. Please read this manual carefully before testing. The testing personnel should undergo professional technical training. The signal counter personnel must be able to observe and distinguish the orange-red and green signals.
3. The test will not provide any results when testing clinical samples it is difficult to count the hybridization signal and the sample is not enough to repeat the test, or the amount of cells is not enough for analysis.
4. The DAPI counterstaining agent used in this experiment is potentially toxic or carcinogenic. It must be operated in a fume hood. Inhalation and direct contact should be avoid by wearing the appropriate masks and gloves.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be disposed of properly.

[Manuscript version and approval date]
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