Wuhan HealthCare Biotechnology Co., Ltd.
Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.
For Professional Use

MET gene probe reagent Instructions Manual

[Product Name] MET gene probe reagent

[Package Specifications] 5 Tests/box 10 Tests/box

[Product Introduction]

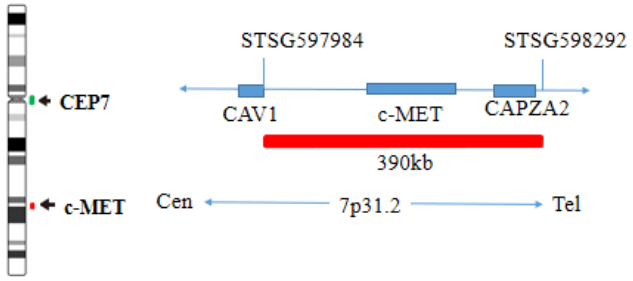
The kit uses orange fluorescein-labeled C-MET probe and green fluorescein-labeled CEP7 probe to bind C-MET/CEP7 probe to the target detection site by in situ hybridization.

[Product Composition]

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

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
5 Tests/box	C-MET/ CEP7 dual color probe	50μl/Tube	1	C-MET Orange probe, CEP7 Green probe
10 Tests/box	C-MET/ CEP7 dual color probe	100μl/Tube	1	C-MET Orange 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°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light. The kit is transported below 0°C.

[Applicable Instruments]

Fluorescence microscopy imaging system includes fluorescence microscopy and filter sets 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 should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after in vitro. After tissue fixation, it should be regularly dehydrated and embedded in paraffin.

[Related Reagents]

1. Pretreatment

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd. pretreatment reagent kit.

2. Denaturation & Hybridization

The following operations should be performed in a darkroom.

① Take the TOP2A probe at static room temperature for 5 minutes. Briefly centrifuge (1-2s) 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 5minutes (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. Counterstaining

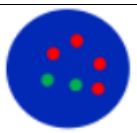
The following operations should be performed in a darkroom

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.

5. FISH results observation

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

[Common Signal Type Interpretation]

C-MET CEP7	
	**Negative**: 2 orange 2 green
	**Positive**: n Orange 2 Green (n≥4)
	

[Precautions]

① This product cannot be used for clinical diagnosis, but only for scientific research.

② 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 the limitations of current molecular biology technology, which may lead to wrong results.

③ Users must understand the potential errors and accuracy limitations that may exist in the detection process.

④ All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed of.

[Basic information]

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

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[approval date and modification date of the specification]

V1. 0 approval date: September 3, 2018

V1. 2 approval date: December 7, 2021

Manufacturer

Wuhan HealthCare Biotechnology Co., Ltd.

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