Cytowish Ltd
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Your authentic partner in molecular cytogenetics

MDM2 Gene Amplification Probe Detection Kit

(CW-054)

Intended use

This kit uses Orange fluorescein labeled MDM2 probe and Green fluorescein labeled CEP12, to combine MDM2/CEP12 genes with the target site by in situ hybridization.

Product composition

The kit consists of MDM2/CEP12 dual color probe 100μl/tube.

Storage condition

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 system including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

Sample requirements

1. Applicable specimen types: Paraffin-embedded specimens for surgical resection or biopsy.

2. Tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after ex vivo, and the tissue should be fixed by conventional dehydration and paraffin embedding.

Pretreatment

It is recommended to use Cytowish's pretreatment reagent kit (catalogue number CW-CT-FISH).

Denaturation and hybridization

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

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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 (no vortex instrument vibration). Take 10μL of it and drop it into the cell drop hybridization area, immediately cover the cover glass of 22mm × 22mm. The probe should be evenly expanded under the cover glass without bubbles, and seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).
2. The cell drops were placed on the hybridizer and denatured at 85°C for 5 min (the hybridizer should be preheated to 85°C) and hybridized at 42°C for 2-16 hours.

Washing

The following operations should be performed in a 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 2xSSC 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 2xSSC room temperature for 1 min.
3. Take out the sample and immerse it in 0.3%NP-40/0.4xSSC 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.

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.

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).

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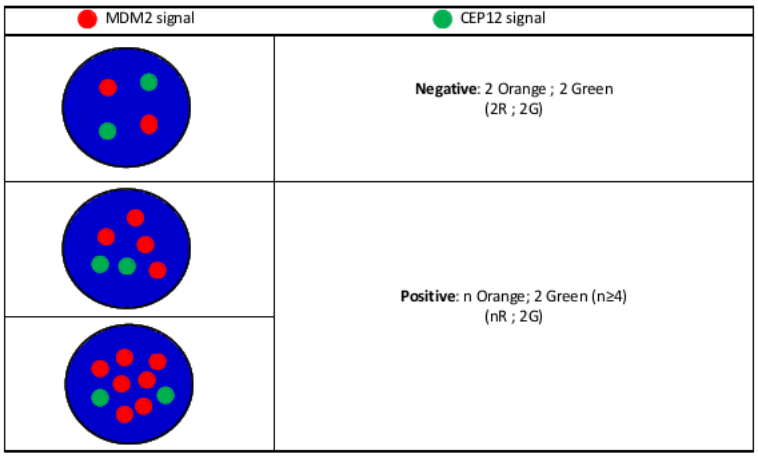

Troubleshooting

The common factors influencing test results and the handling methods are shown in the below table.

Frequent problems and solutions

Problem	Possible cause	Recommended solution
Strong background of slides	Inadequate wash of glass slide before preparation of specimens	Wash the glass slide using the absolute ethyl alcohol
	Inadequate wash after hybridization	Assure that the wash buffer is prepared in line with Instruction For Use, assure the correct pH value and temperature of wash buffer, remove the coverslip and repeat the washing steps
	Improper use of filter sets	Replace with suitable filter sets to reduce the background light
	Improper hybridization condition	Assure the temperature of hybridization instrument is set as 42°C
	The temperature is too low when washing	Assure that the wash buffer reaches to the required temperature when washing the slides
	The washing intensity of wash buffer is too low	Assure the wash buffer is prepared in line with Instruction For Use. (low SSC concentration or high NP-40 concentration would help improving the washing intensity of wash buffer)
Weak counterstaining	Weak counterstaining	Remove coverslip, at room temperature, immerse the slides in the wash buffer containing 2 × SSC/ 0.1%NP-40 for 5 minutes. And then sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining
	The counter stain has been kept under long-term storage or excessive light	Assure the counter stain is stored at -20°C and protected away from light, assure its effect
No signal or weak sivgnals	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83°C, at least 10

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		minutes in advance is needed to preheat hybridization instrument
	The probe mixture and hybridization buffer were not mixed sufficiently before use	Blow the probe mixture and mix the probe sufficiently, centrifuge for a short time
	The probe mixture on tissue slides dries too fast	After dropping probe mixture the target area should be covered by coverslip immediately, when washing the slides you can only remove one coverslip at a time, and dip it into wash buffer immediately before removing next coverslip
	Air bubbles formed under coverslip during hybridization	The coverslip should cover the probe mixture in order to gently squeeze out air bubbles
	Inappropriate hybridization condition	Ensure to comply with the time and temperature required by hybridization and do not leave gaps when sealing the slides with rubber cement. The hybridization time should be adjusted according to the situation.
	Improper wash buffer or incorrect washing conditions	Be sure to follow the requirements of Instruction for Use to formulate the wash buffer. Ensure that the temperature of wash buffer reaches to the temperature predetermined in washing step. The thermometer and pH meter should be accurately calibrated. Remove coverslip before immersing the slide into wash buffer
	Inappropriate storage of probe or specimens slides	Make sure that the probe mixture is stored at -20°C and protected from light. Place the slides without hybridization at -20°C for long-term storage or at room temperature for short-term storage. Place the hybridized slides at -20°C, away from light, and store for less than 6 months
	Incorrect use of DAPI counter stain, excessively high brightness of counter stain	Remove the coverslip, immerse the slides in 2 × SSC/ 0.1%NP-40 for 5 minutes at room temperature. Sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining after air drying the slides
	Inappropriate filter sets were selected for observation	Use correct filter sets to observe the probe fluorescence

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

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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: V1.1 reviewed on 20 November 2021

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