

Chromosome 7 Centromeric Probe Detection Kit (CW-108)

Intended use

This kit uses Green fluorescein labeled CEP7 probe to bind to the target site by in situ hybridization.

Product composition

The kit consists of CEP7 green mono-color probe (100 μ L/Tube).

Storage condition

The kit is transported below 0°C. Keep sealed away from light at -20°C \pm 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 \pm 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

Tissue sample

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 in vitro, and the tissue should be fixed by conventional dehydration and paraffin embedding.

Cell sample

1. Applicable specimen type: unfixed fresh bone marrow specimen (stored at 2-8°C for no more than 24 hours).
2. Sample collection: Take 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.

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3. **Sample storage:** After fixation, the cell suspension can be stored at $-20\pm 5^{\circ}\text{C}$ for no more than 12 months. The prepared cell slides can be stored at $-20\pm 5^{\circ}\text{C}$ for no more than 1 month. When the storage temperature of the specimen is too high or too low, or when the cell suspension is excessively volatile or contaminated during storage, the sample should not be used for testing.

Testing method

Related reagents

1. 20×SSC, pH 5.3±0.2

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 $2-8^{\circ}\text{C}$, 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 $2-8^{\circ}\text{C}$, 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. 0.3% NP-40/0.4xSSC solution, pH 7.0-7.5

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 $2-8^{\circ}\text{C}$, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

5. Fixation solution (methanol: glacial acetic acid = 3:1)

Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.

6. 0.075M KCl solution

Weigh 2.8g of potassium chloride, dissolve in 400mL of deionized water and complete to 500mL with deionized water. Stored at room temperature, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

7. Diamidinyl phenylindole (DAPI) counterstain

Use commercially available anti-queching DAPI counterstain.

Sample pretreatment

Tissue sample

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

Cell sample

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.

Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying. **Permeation:** Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.

Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 0.5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.

Washing: Wash with 2xSSC at room temperature for 5 minutes.

Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time. Remove the excess of EtOH solution on the slides by air-drying.

Denaturation and hybridization

Tissue sample

The following operations should be performed in a darkroom.

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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 10 μ l into the hybridization area of the cell drop, cover the 22mm \times 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).
2. Place the glass slide in the hybridization instrument, denature at 85 $^{\circ}$ C for 5 minutes (the hybridizer should be preheated to 85 $^{\circ}$ C) and hybridize at 42 $^{\circ}$ C for 2 to 16 hours.

Cell sample

1. Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). Take 10 μ l droplet in the cell and drop in the hybridization zone, immediately cover 22mm \times 22mm 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 88 $^{\circ}$ C for 2 minutes (the hybridizer should be preheated to 88 $^{\circ}$ C) and hybridize at 45 $^{\circ}$ C for 2 to 16 hours.

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 \times SSC solution preheated at 68 $^{\circ}$ C for 2 min.
4. Immerse the glass slides in deionized water at 37 $^{\circ}$ C for 1min, and dry naturally in the dark.

Counterstaining

The following operations should be performed in a darkroom.

10 μ L 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 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 (100×).

Test method limitation

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]

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