p53 Gene Detection Kit（FISH）
Instruction For Use

[Product Name]

p53 Gene Detection Kit（FISH）

[Specification]

5 tests/kit, 10 tests/kit, 20 tests/kit.

[Intended Use]

This kit is used to detect the deletion of p53 gene in blood, bone marrow, urine and other tissue samples to aid in diagnosis and treatment.

[Test Principle]

Based on the principle of complementary base pairing, marked the fluorescent stain in DNA fragments (probe) matching with the target DNA.The probe and the corresponding DNA fragments in material waiting for test specificaly combined under certain conditions (hybrid), to form double-stranded nucleic acid, by means of fluorescence microscope to observe and record the hybrid double chain type, quantity, to judge target DNA in inspected samples normal or not.

The samples were fixed by 10% neutral formalin, paraffin-embedded and sliced, then placed on a slide. The denatured DNA formed a single strand state and hybridized with the probe. After hybridization, unbound probes are washed away. Use DAPI (4,6- diimiyl -2- diphenylindoles) to restain the nucleus. DAPI is a nucleic acid stain used as a specific DNA stain. The kit's probe hybridization signals can be observed by fluorescence microscopy equipped with corresponding filters (blue, red and green fluorescent signals).Fluorescence signals in the nucleus were observed under a microscope.

Normal cell signal pattern: Each cell shows two green signals and two red signals (2G2R).

Abnormal cell signal pattern: There are two green signals and less than two red signals in the cell.

[Main Components]

The kit is mainly composed of p53/CEP17 probe and DAPI counterstain solution.

The reagent does not contain but necessary for the test: FISH Sample Pretreatment Kit.

Other reagents not provided: positive contrast photo; Xylene; Ethanol (anhydrous, 85%, 70%);Purified water; Sealing gum.

[Storage and Validity]

Stored at -20℃±3℃ in dark, sealed conditions, valid for 12 months.

[Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: It is recommended to use 100 times achromatic objective lens of immersion type, which can obtain satisfactory results.

Immersion Oil: Low level self-fluorescence Immersion Oil exclusive use for fluorescence microscopy.

Optical Filters: It is recommended to select filters suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 490nm , and emission wavelength was 516nm.

Orangered fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm.

[Sample Requirements]

Collect specimens: peripheral blood (bone marrow). Samples should be collected by a pathology professional according to standard laboratory procedures. After collection, it should be stored at 2℃ ~ 8℃ within 72h.

[Test Method]

Specimen collection:

1. Take out 2~3ml peripheral blood or bone marrow (heparin sodium anticoagulation) and centrifuge at 2000rpm for 5 minutes, discard the supernatant.
2. Add 10 mL hypotonic buffer to the cell suspension, mix well and stand for 2 minutes.
3. Constant temperature water bath at 37℃ for 20 minutes.
4. Add 1ml stationary liquid (glacial acetic acid: Methanol 1:3), and fix for 10min at room temperature.
5. Centrifuge for 5 minutes, discard the supernatant
6. After precipitation, add 10ml stationary liquid, mix well, and precipitate at -20℃ for 30min.
7. Centrifuge at 2000rpm for 5 minutes, discard the supernatant.
8. The above washing steps can be repeated until the cell precipitation is washed white and clean (repeated washing does not need to stand for 30min).

Slide preparation:

1. Take out a -20℃ glass slide
2. After resuspending cells, take 3μl, drop it to a glass slide, and dry it at room temperature.
3. Observe the cell density under a phase contrast microscope with a 10× objective lens. It is required that the cells do not overlap, and the appropriate number of cells is 100-200 in a single field of view.
4. Bake the prepared samples at about 65°C for more than 3 hours.

Note: At least one additional slide should be prepared for each case. The remaining cell suspension can be stored at 2-8℃ for one month.

Slide Pretreatment (optional step):

1. Soak in the 1×PBS buffer solution at 37℃ for 5min.

2. Remove the slide and then put it into 37℃ pepsin solution (Add 1 tube pepsin for in situ hybridization per 50 mL pepsin solution) to digest for 1-2min (enzyme efficacy can be determined by preliminary experiment)

Sample Dehydration

1. After digestion, remove the slide, wash in the 1 × PBS buffer solution for 2-3min at room temperature

2.5 Remove the slide, fix them in 1% paraformaldehyde/PBS for 10 minutes at room temperature

2.6 Remove the slide, wash in the 1 × PBS buffer solution for 2-3min at room temperature

2.7 Remove the slide, the slides were successively treated in 70%, 85%, and anhydrous ethanol solution for 2 min. Remove the slide, dried at room temperature.

Denaturation and hybridization (avoid light):

1. Remove the probe,mix and centrifuge. Take 10ul probe and drop it on the target area. Cover the coverslip and avoid air bubbles.

2. Seal it with the sealing gum. Note that the coverslips are completely prevented from volatilizing the hybridization solution.

3. Put the slides into a hybridization instrument to denature and hybridize.

Washing after hybridization:

1. 30 minutes before washing. Put the washing buffer I into the water bath and heat it to 72 ± 1℃.

2. Gently remove the sealing gum from the slide with tweezers. Soak the glass slides in the washing buffer II solution, placed for 5 minutes, and remove the coverslip.

3. Put the slide into washing buffer I at 72 ± 1℃, placed for 2 minutes

4. Remove the slide, soak in the washing buffer II solution at room temperature, placed for 2min

5. Remove the slide in 70% and 85% ethanol solution for 2min in turn

6. Add 10ul DAPI to the target area of the slide and cover with a coverslip. The slides were placed in -20℃ condition for 20 minutes and then taken out for observation and analysis under a fluorescence microscope.

[Attentions in result analysis]

1. Tumor cells should be randomly counted in the samples.

2. Counting cells must have clear and recognizable signals in each channel.

3. Do not analyze the heterogeneous regions.

4. Do not analyze nucleus with unclear outline or overlap.

5. Do not analyze the area where the background is so deep that affects the signal judgment.

6. Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.

7. When analyzing paraffin tissue sections, attention should be paid to areas where tumor cells are concentrated (to be identified by a pathologist).

8. If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.

9. If signals are present in more than 10% of the cytoplasm, the region should not be analyzed.

[Method Limitations]

This kit is only suitable for detection of p53 gene deletion in patients by fluorescence in situ hybridization, not for detection of other gene mutation modes.

[Cautions]

1. This kit is only used for in vitro diagnosis and must be used by strictly trained professionals.

2. In order to obtain ideal results, it is necessary to ensure that the reagents are correctly prepared and stored in accordance with the instructions.

[Symbols]

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Avoid overexposure to the sun		Date of manufacture

[Basic Information]

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