Sample Pretreatment Kit（FISH）
Instruction For Use

[Product Name]

Sample Pretreatment Kit（FISH）

[Specification]

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

[Intended Use]

This kit is used for pretreatment of paraffin tissue samples for fluorescence in situ hybridization (FISH).

[Test Principle]

The tissue samples are enzymatically digested by pepsin to improve the permeability of tissue cells and facilitate the hybridization of FISH probes with target DNA. In addition, the samples are washed with pretreatment and washing solutions to remove non-specific binders and unhybridized probes.

[Main Components]

The kit is mainly composed of pretreatment buffer, washing solution I, washing solution II, pepsin solution and pepsin powder.

[Storage and Validity]

Stored at 2-8℃, valid for 12 months. Transport condition: at room temperature.

[Applicable Instrument]

Fluorescence microscope

[Sample Requirements]

Fresh biopsy or surgical sample tissue fixed with 10% neutral buffer formalin for 8 ~ 24h. According to the requirements of pathological technical specifications, sampling, dehydration, paraffin embedding into paraffin block. The tissue sections with a thickness of 3 ~ 5 μm were spread on sticky slides. Remove the excess water in the tissue sections by gently patting on the slide stand and absorbing with hygroscopic paper. The sections were then placed in a drying oven at 56-60℃ for 60min. Remove it, cool down at room temperature.

[Test Method]

Reagent Preparation:

Pepsin digestive solution: Add 1 tube pepsin powder (0.1g) per 50 mL pepsin solution. Placed in a 37±1℃ water bath to keep warm for later use.

Test procedures:

1. Deparaffinization: Bake the tissue sections at 60℃ for 3-5 hours to age the glass slides; Under at room temperature, sections were placed in fresh xylene and soaked three times, 5-10 min/time.
2. Incubate the slices twice at room temperature in anhydrous ethanol for 5 min. Then incubate the slices in 85% and 70% ethanol for 3 min at room temperature, in sequence. Wash with deionized water 3 times, 2 min/time.

3. Retrieval: Add an appropriate amount of pretreatment buffer to the pressure cooker, add glass slides, repair at high temperature and high pressure for 3-5 minutes, and then wash with deionized water for 3 times, 2 minutes/time.

4. Enzymolysis: Put the slices directly into the preheated pepsin solution containing pepsin powder, digest for 15±10min, and wash twice with deionized water, 2min/time.

Note: Digestion times depending on a number of factors such as conditions and procedures of slide fixation, slide thickness, and characteristics of tissues/cells. We recommend: Digest 5-25 min for tissue section samples, 3-10 min for cell samples, wash twice with deionized water, 2 min/time.

5. Gradient dehydration: 70% ethanol immersion for 2min; Immerse in 85% ethanol for 2min; Soak in 95% ethanol for 2min, in sequence. Dry sections.

6. Denaturation and hybridization: After adding the probes, the slides were put into the hybridization system for reaction, denatured at 83℃ for 6 min, and hybridized at 40℃ overnight.

7. Washing: Gently remove the sealing gum. The sections were placed in wash buffer II, incubated for 10 min, and the coverslips were removed. Place the slices in washing buffer I (heated to 72±1℃ in a water bath 30 minutes in advance), pull the slices up and down for 1-3 s, and incubate for 2 minutes.

8. Gradient dehydration: Immerse in 70% ethanol for 2min; Soak in 85% ethanol for 2min, in sequence.

9. DAPI staining and sealing: Add 10ul DAPI to the target area of the slide and cover with a coverslip. Avoid bubbles, cover with a cover slip and incubate at -20℃ for 30min in the dark.

10. The slides were taken out for observation and analysis under a fluorescence microscope and placed in -20℃ storage.

[Reference Value]

This kit is a pretreatment reagent for fluorescence in situ hybridization test, and there is no specific reference value.

[Results Interpretation]

Any interpretation of fluorescence signal should be made by the pathologist in combination with morphology.

[Limitations of detection Methods]

This kit is only for pretreatment of paraffin tissue samples in fluorescence in situ hybridization test.

[Product Performance]

Appearance: The appearance of the reagent should be neat and the text symbols should be clearly identified.

Completely packaged and no damage

Liquid reagents are clear, free of precipitation, suspended solids and flocs.

Packing volumes: The filling quantity shall not be less than the marked quantity.

pH value: Pretreatment buffer pH: 6.0±0.05; pepsin solution pH: 2.0±0.05

washing solution I pH: 7.5±0.05; washing solution II pH: 7.0±0.05

[Cautions]

1. When storing reagents, try to avoid high temperature environment or direct sunlight.
2. This antibody must be used by highly trained professionals.
3. Read the instruction manual carefully before use, use it within the validity period, and take personal hygiene protection.
4. After use, the waste should be disposed according to the requirements of the hospital or environmental protection department.

[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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