



Fluorescence In-Situ Hybridization Pretreatment Reagent Kit Manual





Wuhan HealthCare Biotech Co., Ltd.

[Product Name]: FISH Pre-treatment Reagent Kit

[Packing Specifications]: 20 tests/box.

[Intended Use]

This kit is suitable for the pretreatment of the sample to be tested, so that the sample in the test substance is released from the combined state with other substances, in order to facilitate the use of in vitro diagnostic reagent or instrument to be tested on the analyte.

[Working Principle]

Paraffin tissue samples are deparaffinized by non-toxic dewaxing agent, cells permeabilized and pepsin used for enzymatic digestion of the tissue sample. The cells permeability is further improved so as to facilitate the qualitative or quantitative detection of nucleic acids in tissue by fluorescence in situ hybridization.

[Main Components]

FISH pretreatment reagent kit components:

Components	Specifications	Quantity	Main components composition
Dewaxing agent	250ml/bottle	01	Bis-amyl (Dipentene), Limonene, mineral oil
Permeation agent	250ml/bottle	01	Distilled water, EDTA
Protease work buffer	250ml/bottle	01	Hydrochloric acid, Distilled water
10x Protease solution	250ml/bottle	01	Pepsin, Distilled water
Washing solution	250ml/bottle	01	Sodium chloride, Sodium citrate, Distilled water

Other reagents needed but not provided: Distilled water, Anhydrous ethanol.

[Storage Conditions & Expiration Date]

Store at $2 \sim 8^{\circ}$ C (10x protease solution -20±5°C), valid for 12 months; store at $2 \sim 8^{\circ}$ C during transport.

[Sample Requirements]

Applicable specimen types: Neutral formalin-fixed paraffin-embedded tissue sections.

[Usage Method]

- 1. Take already baked 4~5µm thick paraffin slices and immerse in the preheated dewaxing agent at 68°C for 15 minutes.
- 2. Remove the glass slides and immerse in 100% ethanol for 5 minutes at room temperature.
- 3. Remove the glass slides and immerse it in the preheated permeation agent at 90°C for 20 minutes.
- 4. Remove the slides and immerse it in pre-warmed distilled water at 37 $\mbox{\ensuremath{\mathbb{C}}}$ for 5 minutes.
- 5. Remove the slides and immerse it in the pre-warmed at $37 \,^{\circ}$ C protease working solution for 20-30 minutes (preparation of protease working solution: Take 10x protease solution, shake well and dilute to 1x protease solution with protease working buffer and mix thoroughly; the protease working buffer should be preheated to $37 \,^{\circ}$ C before preparation; protease working solution is ready for use and discard the solution after use).





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- 6. Remove the slides after soaking in the washing solution, rinse twice for 5 minutes each.
- 7. After, the glass slides are immersed in 70%, 85% and 100% gradient of ethanol for 2 minutes each.
- 8. Remove the glass slides to dry at room temperature for subsequent testing operation.

[Method Limitations]

This method is only applicable to the pretreatment of neutral formalin-fixed paraffin-embedded tissue sections by fluorescence in situ hybridization. Do not apply to other methods of fixed and embedded tissue sections.

[Product Performance]

- 1. The kit should be clean, clearly marked and without leakage.
- 2. After the product is processed into paraffin-embedded tissue sections, the cells are stained with DAPI counterstain and observed under a fluorescence microscope. No paraffin is coated on the surface of the tissue and is completely replaced in the tissue cells. The tissue sections are not shed, nuclei are full with clear contour and without cavity.

[Precautions]

- 1. Please read this note carefully before using this kit.
- 2. The temperature of the dewaxing agent should not be lower than 65° C and not higher than 75° C.
- 3. If the paraffin section tissue is too thick, the dewaxing agent and permeation agent processing time should be prolonged, and appropriately increase the temperature.
- 4. Protease treatment time varies according to the tissue slices.
- 5. Kits from different batches cannot be mixed.

[Symbols Significance]

	Manufacturer	
LOT	Batch Number	
IVD	In Vitro Diagnostic Medical Device	
2°C	Store at 2 °C to 8 °C	
Σ	Validity Period	
Σ	Specifications	





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[References]

[1]. Masuda N, Ohnishi T, Kawamoto S, et al. Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. Nucleic Acids Res. 1999;27:4436–4443.

[2]. McKinney MD, Moon SJ, Kulesh DA, et al. Detection of viral RNA from paraffin-embedded tissues after prolonged formalin fixation. J Clin Virol. 2009; 44:39–42.

[Basic Information]

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