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Your authentic partner in molecular cytogenetics

Fluorescence In Situ Hybridization (FISH) Pretreatment Reagent Kit – Instruction manual

Product name

FISH Pretreatment Reagent Kit.

Packing specification

20 test per box.

Intended use

This kit is suitable for the pretreatment of the sample to be tested, so that the analyte in the sample 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.

Principle

Paraffin tissue samples are deparaffinized by non-toxic dewaxing agent, the permeabilizing agent permeabilizes the cells, and the pepsin enzymatically decomposes the tissue sample to further improve the cell permeability so as to facilitate the qualitative or quantitative detection of nucleic acids in tissue by fluorescence in situ hybridization.

Main components

Components	Volume	Quantity	Main components	Size
Dewaxing agent[1]	250mL/bottle	01	Limonene 100% (pure)	20 tests
Permeation agent (Solution B)	250mL/bottle	01	Distilled water, EDTA	
Protease working buffer[2]	250mL/bottle	01	Hydrochloric acid, Distilled water	
10x Protease solution	250mL/bottle	01	Pepsin, Distilled water	
Washing solution (Solution D)	250mL/bottle	01	Sodium chloride, Sodium citrate, Distilled water	

[1] Limonene: Limonene, reference CAS #138-86-3.

[2] Protease working buffer preparation:

The Protease working buffer (HCl + Distilled water) preparation, the final concentration should be 50mM or 0.05M or 0.05mol/L (as 1mM = 0.001M). The concentration calculation will be based on the concentration of the Hydrochloric Acid (HCl) solution purchased and the reference is CAS #7647-01-0.

Some info on HCl:

The molar concentration (mol/L) is the ratio Number of moles of the solute/Volume of the solution generally 1L, hence C= n/V expressed in mol/L or M.

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Hydrochloric Acid molecular weight is HCl, M = 36.5g/mol.

HCl: It takes 36.5g of HCl to have 1M, so for 0.05M HCl, you need 0.05 x 36.5 = 1.825g.

Storage conditions and expiration date

Store at 2-8°C±5°C (10x protease solution -20°C±5°C), valid for 12 months; store at room during transportation.

Sample requirements

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

Instructions

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°C for 5 minutes.
5. Remove the slides and immerse it in the pre-warmed at 37°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°C before preparation; protease working solution is ready for use and discard the solution after one time usage).
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 ethanol for 2 minutes each.
8. Remove the glass slides to dry at room temperature for subsequent testing operation.

Method limits

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.

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Product performance index

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.

References

- 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.
- 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.

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