FISH Pretreatment Kit

Nº cat. MAD-FISH-PTK

(40 tests)

DESCRIPTION

The **FISH Pretreatment kit** has been specifically designed for manual use and includes reagents to perform the Pre-treatment of the histological preparations for the fluorescent in situ hybridization technique (FISH) on buffered formalin-fixed and paraffin-embedded tissue sections.

REAGENTS INCLUDED IN THE KIT (40 tests):

Name	Reference	Volume
ISH Solution concentrated 10X	MAD-004070R/D1	150 ml
ISH Enzyme (pepsin) RTU	MAD-024084R1	12 ml
Wash Buffer concentrated 10X	MAD-004077R1	500 ml

NECESSARY EQUIPMENT AND MATERIAL NOT PROVIDED IN THE KIT

Coplin jars of 50 ml.
Specific slides for immunostaining techniques. (MAD-15-188-55/100)
Coverslips of 18x18 mm^2.
Thermostatic bath (90º C).
Xylene, ethanol battery (pure ethanol, 80%, 70%).
Rubber Cement Fixogum (Ref: MAD-011530Q-125).
Hybridizer.
Thermal plate.
Fluorescence Contrast Agent-DAPI+Antifade (Ref: MAD-FS0106-1).
Fluorescence microscope with appropriate filters and digital camera.
Specific FISH probe labelled with fluorescence.

WARNINGS AND PRECAUTIONS:

-Avoid the contact of the reagents with the eyes and the mucous membranes. If the reagents come into contact with sensitive areas, wash them with plenty of water.
-Check with the local or state authorities about the recommended disposal method.
-Avoid the microbial contamination of the reagents.

SAFETY RECOMMENDATIONS:

The product is only intended for professional laboratory purposes, and it is not intended for pharmacological, home or any other type of use. The current version of the material safety data sheet of this product can be downloaded by searching its reference at www.vitro.bio or can be requested at regulatory@vitro.bio

STORAGE:

Keep refrigerated between 2 and 8°C until the expiration date of the product. **DO NOT FREEZE.**

For "in vitro" diagnostic use

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TECHNICAL PROTOCOL FOR FISH ON PARAFFIN EMBEDDED TISSUES

1. SAMPLES PREPARATION

1.1. Cut the tissue sections with a thickness of between 4-5µm (Optimal thickness to perform the FISH technique);
1.2. Place the tissue sections in the water bath so that the section expands and take them onto slides treated for IHC.
1.3. Place the slides with the tissue sections a minimum of 60 minutes in a heater at 60ºC to melt the paraffin and allow the tissue to perfectly adhere to the slide.

2. DEWAXING

2.1. Xylene 2x 10 min
2.2. Absolute ethanol 2 x 5 min
2.3. Ethanol 80% 5 min
2.4. 70% Ethanol 5 min
2.5. Distilled water (5-10 ')

3. HEAT PRE-TREATMENT

Before starting:
- Dilute **ISH solution concentrated 10x** in distilled water: example, 10 ml of solution in 90 ml of water.
- Dilute **Wash Buffer concentrated 10x** in distilled water: example, 100 ml of solution in 900 ml of water.
- Store both solutions at room temperature, preferably protected from light.

3.1. Place 50 ml of **diluted ISH Solution** in a "Coplin" jar and preheat in a water bath at 90 ° C (for at least 20 min).
3.2. Place the slides in **diluted ISH Solution** at 90 ° C for 30 min.
3.3. Wash the slides in a "Coplin" jar with **diluted Wash Buffer** for 3 min at room temperature.
3.4. Wash the slides with **Distilled Water** for 3 min at room temperature.
3.5. Let the preparations air dry.

4. ENZYMATIC DIGESTION

4.1. Apply a few drops of **ISH Enzyme (Pepsin) RTU** making sure to cover the tissue sections well.
4.2. Incubate at **37 ° C for 10-15 min** in a thermal plate or hybridizer. The incubation time will depend on the tissue specimen and on the fixation conditions.
4.3. Wash with water for 1 min and immediately with **diluted Wash Buffer** 5 min at room temperature.
4.4. Wash the slides with **Distilled Water** for 3 min at room temperature.
4.5. Let the preparations air dry.

5. DESNATURALIZATION / HYBRIDIZATION

5.1. Apply **10 µl of probe** on the slide (check the specific instructions included with each probe).
5.2. Place a coverslip and seal with fixogum.
5.3. Denature the preparation in a hybridizer or thermal plate for **5 min at 80º C** (or at the recommended conditions for each .
5.4. Incubate in a hybridizer or humid chamber, **16 hours at 43º C** (or at the recommended conditions for each specific probe).

Note: As of this step, preparations should be handled, as well as fluorescent probes, in the dark, avoiding prolonged exposure to ambient light.

6. POST-HYBRIDIZATION WASHING

6.1. Place **50 ml** of **diluted Wash Buffer** in a "Coplin" jar and preheat in a water bath at **75ºC** (for at least 20 min).
6.2. Remove the coverslip and Wash the slides in **diluted Wash Buffer** for **5 min** at room temperature.
6.3. Wash the slides in preheated **diluted Wash Buffer**, **5 min** at **75º C** (it is recommended not to wash more than 5-6 microscope slides/jar simultaneously). It is recommended to always use a fresh solution or use it at most twice.
6.4. Wash with some distilled water and air-dry, preferably in **darkness** or dehydrate in growing alcohols (70º, 80º and pure alcohol) for 30 seconds each and air-dry, preferably in **darkness**.
6.5. Mount with one drop of **Fluorescent Contrast Agent -DAPI+AntiFade (MAD-FS0106-1).**
6.6. Store the slides at **4º C** in **darkness**.
6.7. Visualize with the fluorescence microscope to analyze the results.

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LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

IVD	Health product for in vitro diagnosis.		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions of use		Sufficient content for <n> assays
	Material safety data sheet		

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