Zyto*Fast*

Pepsin Solution

8 ml

For use in *in situ* hybridization procedures

FOR RESEARCH USE ONLY

Product No.: T-1051

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1. Scope of Application

The Pepsin Solution is designed to be used for enzyme digestion of formalin-fixed, paraffin-embedded tissue or cell samples prior to *in situ* hybridization (ISH)

This product is designed for research purposes only and not for use in diagnostic applications.

2. Safety Precautions and Disposal

- ✓ Read the operating instructions prior to use!
- ✓ Do not use the reagents after the expiry date has been reached!
- ✓ Avoid any cross-contamination and micro-bacterial contamination of the reagents!
- ✓ Some of the system components contain substances (in low concentrations and volumes) that are harmful to health (pepsin). Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses and lab garments)!
- ✓ If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
- ✓ Never pipet solutions with your mouth!
- ✓ The disposal of reagents must be carried out in accordance with local regulations!
- ✓ A material safety data sheet is available on request for the professional user!

3. The Pepsin Solution

The following components are included:

Code	Component	Quantity	Container
-	Pepsin Solution	8 ml	Dropper bottle, white cap
	Instruction manual	1	

The Pepsin Solution is sufficient for approx. 50 reactions.

4. Storage and Shelf Life

The Pepsin Solution must be stored at 2-8°C. If these storage conditions are followed, the Pepsin Solution will function, without loss of performance, at least until the expiry date printed on the label.

5. Test Material

The Pepsin Solution has been optimized for use with formalin-fixed, paraffin-embedded tissue and cell samples. We recommend the following tissue preparation:

- ✓ Fixation in 10% neutrally buffered formalin for 24 h at RT

  In order to achieve optimum and uniform fixation and paraffin embedding, the sample size should not exceed 0.5 cm^3.

- ✓ Standard processing and paraffin embedding

  Use premium quality paraffin. Infiltration and embedding should be carried out at temperatures lower than 65°C.

- ✓ Prepare 2-5 µm microtome sections

  Draw up the sections onto silane-coated or adhesion slides (e.g. HistoBond®) and fix for 2-16 h at 50-60°C.

- ✓ Pretreatment (first steps)

  The first steps of pretreatment (dewax and heat pre-treatment in a buffer) of the tissue and cell section should be performed using established standard protocols.

6. Instruction

1. After heat pretreatment transfer slides immediately to deionized or distilled water and wash 3x 2 mins

2. Bring Pepsin Solution to room temperature before use

3. Apply (dropwise) Pepsin Solution to the air-dried tissue/cell section and incubate for 10 mins at RT in a humidity chamber

Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections and nature of tissue/cells, different incubation times may be required. As an incubation guideline we recommend an incubation time of 10-30 minutes for

tissue samples and 5-10 minutes for cell samples. As a general rule, we recommend that the optimum time for proteolysis will be ascertained in pre-tests.

4. Wash 3x 2 mins in deionized or distilled water

5. Dehydration: in 70%, 85%, 95% and 2x 100% ethanol each for 2 mins

Air dry sections.

6. Proceed with *in situ* hybridization (ISH), i.e., application of the probe, hybridization and detection

The final experimental results of an ISH experiment are strongly influenced by upstream and downstream experimental steps, i.e., tissue fixation, heat pre-treatment in a buffer, denaturation of DNA probe, hybridization, washing, and detection. For a particularly user-friendly performance we recommend the use of ZytoVision's Zyto*Fast* ISH systems. These systems were also used for the confirmation of appropriateness of the Pepsin Solution.

Our experts are available to answer your questions.

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