Zyto*Light*

FISH Pretreatment Solution Citric I

1000 ml

For pretreatment prior to fluorescence *in situ* hybridization (FISH)

FOR RESEARCH USE ONLY

Product No.: Z-2058

Manufacturer: **ZytoVision GmbH**, Fischkai 1, D-27572 Bremerhaven
Phone: +49 (0) 471-4832 300
Fax: +49 (0) 471-4832 509
info@zytovision.com, http://www.zytovision.com

1. Scope of Application

The FISH Pretreatment Solution Citric I is designed to be used for heat pretreatment prior to enzyme digestion of formalin-fixed, paraffin-embedded tissue or cell samples prior to fluorescence *in situ* hybridization (FISH).

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 (Citric acid). 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 FISH Pretreatment Solution Citric I

The following components are included:

Code	Component	Quantity	Container
-	FISH Pretreatment Solution Citric I	1000 ml	Screw-cap bottle (large)
	Instruction manual	1	

Component 1 is sufficient for 12 staining jars of 80 ml each.

4. Storage and Shelf Life

The FISH Pretreatment Solution Citric I must be stored RT. If these storage conditions are followed, the FISH Pretreatment Solution Citric I will function, without loss of performance, at least until the expiry date printed on the label.

5. Test Material

The FISH Pretreatment Solution Citric I 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 cm³.

✓ 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.

6. Pretreatment

1. Incubate slides for 10 mins at 70°C (e.g. on a hot plate)

2. Incubate slides 2x 10 mins in xylene

3. Incubate each for 5 mins in 100%, 100%, 90% and 70% Ethanol

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

5. Incubate for 15 mins in pre-warmed FISH Pretreatment Solution Citric I at 98°C

We recommend not to use more than six slides per staining jar.

6. Transfer slides immediately to deionized or distilled water and wash for 2x 2 mins

7. Bring a Pepsin Solution (not included) to room temperature before use

8. Apply (dropwise) Pepsin Solution to the air-dried tissue/cell section and incubate for 10 mins at 37°C 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.

9. Wash for 5 mins in 2x SSC and 1 min in distilled or deionized water

10. Dehydration: in 70%, 90% and 100% Ethanol each for 1 min

Air dry sections.

11. Proceed with fluorescence *in situ* hybridization (FISH), i.e., application of the probe, hybridization and detection

7. Results

Using appropriate probes a positive staining can be obtained on appropriate cells as for example two HER2 signals in a nucleus of a normal cell.

Absent or weak signals in nuclei may be the result of an over-digestion (due to loss of nuclei and chromosome structure) or under-digestion (no penetration of probe) during pepsin incubation.

The final experimental results are also strongly influenced by the downstream experimental steps, i.e., denaturation of DNA probe, hybridization, and washing. For a particularly user-friendly performance we recommend the use of ZytoVision's Zyto*Light* FISH systems. These systems were also used for the confirmation of appropriateness of FISH Pretreatment Solution Citric I.

Our experts are available to answer your questions.

Literature:

Wilkinson DG (1992) Oxford University Press; ISBN 0 19 963327 4

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