



Zyto*Dot* Pretreatment Kit

50 reactions

For pretreatment prior to
chromogenic *in situ* hybridization (CISH)

FOR RESEARCH USE ONLY

Product No.: [C-3004](#)

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1. Scope of Application

The [ZytoDot Pretreatment Kit](#) is designed to be used for heat pretreatment and enzyme digestion of formalin-fixed, paraffin-embedded tissue or cell samples prior to chromogenic *in situ* hybridization (CISH).

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 (EDTA, 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!
- ✓ The following risk and safety phrases apply to component 1 (Pretreatment Solution) containing EDTA: R22 Harmful if swallowed!
- ✓ A material safety data sheet is available on request for the professional user!

3. The [ZytoDot Pretreatment Kit](#)

The kit is made up of the following components:

Code	Component	Quantity	Container
1	Pretreatment Solution	1000 ml	Screw-cap bottle (large)
2	Pepsin Solution	8 ml	Dropper bottle, white cap
	Instruction manual	1	

Component 1 is sufficient for 12 staining jars of 80 ml each. Component 2 is sufficient for approx. 50 reactions.

4. Storage and Shelf Life

The components of the [ZytoDot Pretreatment Kit](#) must be stored at 2-8°C. If these storage conditions are followed, the components of the [ZytoDot Pretreatment Kit](#) will function, without loss of performance, at least until the expiry date printed on the label.

5. Test Material

The [ZytoDot Pretreatment Kit](#) 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 for 10 mins at 70°C (e.g. on a hot plate)
- 2.** Incubate slides 2x 5 mins in xylene
- 3.** Incubate 3x 3 mins in 100% ethanol
- 4.** Wash 3x 2 mins in deionized or distilled water
- 5.** Heat Pretreatment Solution (**1**) in a staining jar standing in a boiling water bath to at least 95°C
- 6.** Place slides in the Pretreatment Solution (**1**) and incubate for 15 mins
- 7.** Transfer slides immediately to deionized or distilled water and wash 3x 2 mins
- 8.** Bring Pepsin Solution (**2**) to room temperature before use
- 9.** Apply (dropwise) Pepsin Solution (**2**) 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.

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

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

Air dry sections.

12. Proceed with chromogenic *in situ* hybridization (CISH), 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 dot-shaped HER2 signals in a nucleus of a normal cell.

Absent or weak signals in nuclei, which are not counterstained, may be the result of an over-digestion during pepsin incubation (due to loss of nuclei and chromosome structure). Absent or weak signals in nuclei, which are counterstained, may be due to an under-digestion during pepsin incubation.

The final experimental results are also strongly influenced by the downstream experimental steps, i.e., denaturation of DNA probe, hybridization, washing, and detection. For a particularly user-friendly performance we recommend the use of ZytoVision's *ZytoDot* hybridization systems.

Our experts are available to answer your questions.

Literature:

Ezaki T (1996) Kaibogaku Zasshi 71: 615-28.

Tsukamoto T, et al. (1991) Int J Dev Biol 35: 25-32.

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

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