ZYTOVISION

# Zyto*Dot*
# Pretreatment Kit

REF C-3004-40 Σ 40

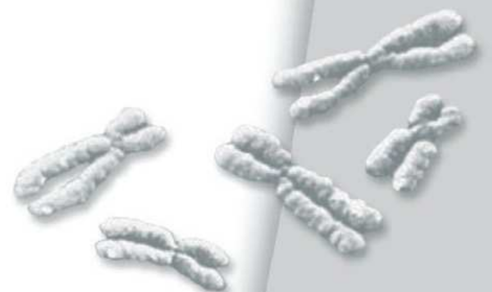
For pretreatment prior to
chromogenic *in situ* hybridization (CISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



# 1. Scope of Application

The Zyto*Dot* 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).

Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

# 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. 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 <u>Zyto*Dot* Pretreatment Kit</u>

## 3.1 Components

The kit is made up of the following components:

| Code | Component | Quantity | Container |
|---|---|---|---|
| | | Σ 40 | |
| PT2 | Heat Pretreatment Solution EDTA | 500 ml | Screw-cap bottle (large) |
| ES1 | Pepsin Solution | 4 ml | Dropper bottle, white cap |
| | Instruction manual | 1 | |

Component **(ES1)** is sufficient for 40 reactions. Component **(PT2)** is sufficient for 7 staining jars of 70 ml each.

## 3.2 Storage and Shelf Life

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

## 3.3 Test Material

The Zyto*Dot* Pretreatment Kit has been optimized for the use with formalin-fixed, paraffin-embedded tissue and cell samples. When test material is used that has been fixed or embedded in a different manner (e.g. methanol/glacial-acetic-acid-fixed cells or blood smears) the test protocol may need to be adapted by the user. Our specialists are available to support you whenever adjustments are necessary.

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 3-5 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.*

# 4. Pretreatment

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

**2.** Incubate slides for 2x 5 min in xylene

**3.** Incubate in 3x 3 min in 100% ethanol

**4.** Wash 3x 2 min in deionized or distilled water

**5.** Heat Heat Pretreatment Solution EDTA **(PT2)** in a covered staining jar standing in a boiling water bath to at least 95°C

**6.** Place slides in the Heat Pretreatment Solution EDTA **(PT2)** and incubate for 15 min

**7.** Transfer slides immediately to deionized or distilled water, wash 3x 2 min and drain off or blot off the water

**8.** Bring Pepsin Solution **(ES1)** to room temperature before use

**9.** Apply (dropwise) Pepsin Solution **(ES1)** to the tissue/cell section and incubate for 5 min 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 3-10 min for tissue and cell samples. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.*

**10.** Wash 3x 2 min in deionized or distilled water

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

Air dry sections.

Further processing, such as denaturation, hybridization, washing, detection, and counter-staining, can be completed according to the user's needs. For a particularly user-friendly performance, we recommend the use of a Zyto*Dot* CISH system by ZytoVision.

# 5. Literature

Isola J, Tanner M (2004) *Methods Mol Med* **97**: 133-44.

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Speel EJ, et al. (1994) *J Histochem Cytochem* **42**: 1299-307.

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

Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

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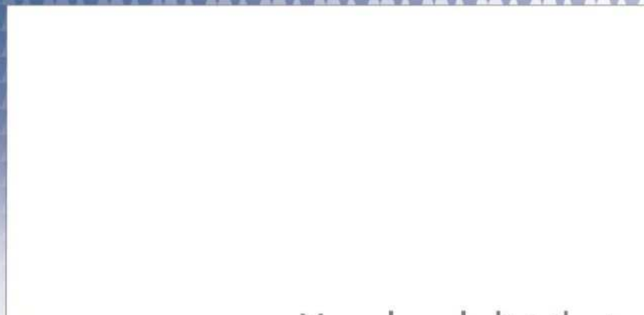
ZytoVision GmbH · Fischkai 1
D - 27572 Bremerhaven · Germany
Phone: +49 (0) 471/4832 - 300
Fax: +49 (0) 471/4832 - 509
www.zytovision.com
info@zytovision.com



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