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. Intended use

The Zyto*Dot* Pretreatment Kit is intended to be used for heat pretreatment and enzyme digestion of formalin-fixed, paraffin-embedded specimens prior to chromogenic *in situ* hybridization (CISH). The kit is intended to be used in combination with a Zyto*Dot* Implementation kit (Prod. No. C-3044-40 or Prod. No. C-3018-40) and a respective Zyto*Dot* CISH Probe.

Interpretation of the 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. Clinical relevance

Genetic aberrations, e.g., deletions and/or amplifications, are associated with various human neoplasms. Chromosomal aneuploidies are observed in many congenital disorders.

3. Test principle

The chromogenic *in situ* hybridization (CISH) technique allows the detection and visualization of specific nucleic acid sequences in cell preparations. Hapten-labeled nucleotide fragments, so called CISH probes, and their complementary target sequences in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. Duplex formation of the labeled probe can be visualized using primary (unmarked) antibodies, which are detected by secondary polymerized enzyme-conjugated antibodies. The enzymatic reaction with chromogenic substrates leads to the formation of colored precipitates. After counterstaining the nucleus with a nuclear dye, hybridized probe fragments are visualized by light microscopy.

4. Reagents provided

The Zyto*Dot* Pretreatment Kit is available in one size and is composed of:

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

C-3004-40 (40 tests): Component **ES1** is sufficient for 40 reactions. Component **PT2** is sufficient for 7 staining jars of 70 ml each.

5. Materials required but not provided

- Zyto*Dot* CISH Probe
- Zyto*Dot* CISH Implementation Kit (Prod. No.-C-3018-40) or Zyto*Dot 2C* CISH Implementation Kit (Prod. No. C-3044-10/-40)
- Positive and negative control tissue
- Microscope slides, positively charged
- Water bath (80°C, 98°C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable pipettes (10 µl, 1000 µl)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene
- Methanol 100%
- Hydrogen peroxide (H_2O_2) 30%
- Deionized or distilled water
- Coverslips (22 mm x 22 mm, 24 mm x 32 mm)
- Rubber cement, e.g., Fixogum Rubber Cement (Prod. No. E-4005-50/-125) or similar
- Adequately maintained light microscope (400-630x)

The Zyto*Dot* Pretreatment Kit is intended to be used in CISH procedures using ZytoVision Probes and kits. For information on materials required for CISH procedures, please refer to the instructions for use of the respective ZytoVision Probe and implementation kit.

6. Storage and handling

Store at 2-8°C in an upright position. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

7. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- This product contains substances (in low concentrations and volumes) that are harmful to health and potentially infectious. 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 amounts of water!
- A material safety data sheet is available on our homepage (www.zytovision.com).
- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid any cross-contamination and micro-bacterial contamination of the reagents!
- The specimens must not be allowed to dry during the hybridization and washing steps!

Special labeling of ES1

EUH208	Contains Pepsin A. May produce an allergic reaction.
EUH210	Safety data sheet available on request.
	< 20 % of the mixture consists of ingredient(s) of unknown acute toxicity (inhalation).

Hazard and precautionary statements for PT2

The hazard determining component is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

For further information concerning this point, please refer to the instructions for use of the respective ZytoVision Probe and implementation kit.

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the CISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user.

9. Interfering substances

Refer to the instructions for use of the respective implementation kit.

10. Preparation of specimens

Refer to the instructions for use of the respective implementation kit.

11. Preparatory treatment of the device

The product is ready-to-use. No reconstitution, mixing, or dilution is required.

12. Assay procedure

For detailed information on how to perform CISH with Zyto*Dot* products, including heat pretreatment and proteolysis with the Zyto*Dot* Pretreatment Kit, please refer to the instructions for use of the respective implementation kit.

Preparatory steps

(1) *Heat Pretreatment Solution EDTA* (**PT2**): Heat to 98°C in a covered staining jar.

(2) *Preparation of 3% H_2O_2:* Dilute 1 part 30% H_2O_2 in 9 parts 100% methanol.

Pretreatment (dewax/proteolysis)

(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 slides for 3x 3 min in 100% ethanol.
(4) Incubate slides for 5 min in 3% H_2O_2.
(5) Wash slides 2x 1 min in deionized or distilled water.
(6) Incubate for 15 min in pre-warmed Heat Pretreatment Solution EDTA (**PT2**) at 98°C.

Use eight slides per staining jar (add dummy slides if needed).

(7) Transfer slides immediately to deionized or distilled water and wash for 2x 2 min.
(8) Apply (dropwise) Pepsin Solution (**ES1**) to the specimen and incubate for 5-15 min at 37°C in a humidity chamber.

As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.

(9) Immerse slides in deionized or distilled water.
(10) Dehydration in: 70%, 90%, and 100% ethanol, each for 1 min.
(11) Air dry sections.

Perform application of the Zyto*Dot* CISH Probe and post-hybridization processing (washing, detection, counter-staining, mounting, microscopy) according to the instructions for use of the respective implementation kit.

13. Interpretation of results

Please refer to the instructions for use of the respective Zyto*Dot* CISH Probe.

14. Recommended quality control procedures

Please refer to the instructions for use of the respective Zyto*Dot* CISH Probe.

15. Performance characteristics

Please refer to the instructions for use of the respective Zyto*Dot* CISH Probe.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to the instructions for use of the respective Zyto*Dot* CISH Probe and implementation kit for further information.

18. Literature

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

Our experts are available to answer your questions.
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