ZYTOVISION

Zyto*Dot*
CISH Polymer Detection Kit

REF C-3005-40 Σ 40

For the qualitative detection of
Digoxigenin-labeled Zyto*Dot* probes
by 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* CISH Polymer Detection Kit is intended to be used for the qualitative detection of Digoxigenin-labeled Zyto*Dot* CISH Probes in formalin-fixed, paraffin-embedded specimens by chromogenic *in situ* hybridization (CISH). The kit is intended to be used in combination with the Zyto*Dot* CISH Implementation Kit (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* CISH Polymer Detection Kit is available in one size and is composed of:

Code	Component	Quantity Σ 40	Container
BS1	Blocking Solution	4 ml	Dropper bottle, orange cap
AB1	Mouse-Anti-DIG	4 ml	Dropper bottle, pink cap
AB2	Anti-Mouse-HRP-Polymer	4 ml	Dropper bottle, violet cap
SB1a	DAB Solution A	0.3 ml	Dropper bottle, green cap
SB1b	DAB Solution B	10 ml	Dropper bottle, grey cap
CS1	Mayer's Hematoxylin Solution	20 ml	Screw-cap bottle, black
MT4	Mounting Solution (alcoholic)	4 ml	Glass bottle, brown
	Instructions for use	1	

C-3005-40 (40 tests): Components **BS1, AB1, AB2, SB1a, SB1b, CS1, and MT4** are sufficient for 40 reactions.

5. Materials required but not provided

- Zyto*Dot* CISH Probe
- Zyto*Dot* CISH Implementation Kit (Prod. No.-C-3018-40)
- 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* CISH Polymer Detection 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!

Hazards and precautionary statements for BS1, AB1, and AB2:

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 soap and water.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Hazards and precautionary statements for SB1a:

The hazard-determining component is biphenyl-3,3',4,4'tetrayltetraamine; diaminobenzidine.

Danger

H350	May cause cancer.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.

Hazards and precautionary statements for SB1b:

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).

Danger

H317	May cause an allergic skin reaction.
H360D	May damage the unborn child.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P362+P364	Take off contaminated clothing and wash it before reuse.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.

Hazards and precaution statements for MT4:

The hazard-determining component is Xylene.

Danger

H226	Flammable liquid and vapour.
H304	May be fatal if swallowed and enters airways.
H302	Harmful if swallowed.
H332	Harmful if inhaled.
H335	May cause respiratory irritation.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H373	May cause damage to organs through prolonged or repeated exposure.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
EUH208	Contains methyl 2-methylprop-2-enoate; methyl 2-methylpropenoate; methyl methacrylate. May produce an allergic reaction.

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 ZytoDot CISH Implementation Kit.

10. Preparation of specimens

Refer to the instructions for use of the ZytoDot CISH 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 the detection of Digoxigenin-labeled probes with the Zyto*Dot* CISH Polymer Detection Kit, please refer to the instructions for use of the respective implementation kit.

Detection

(1) Immerse slides in Wash Buffer PBS/Tween.

(2) Apply Blocking Solution (**BS1**) (1-2 drops per slide) to the slides and incubate for 10 min at RT.

(3) Blot off Blocking Solution (**BS1**), **but do not rinse!**

(4) Apply Mouse-Anti-DIG (**AB1**) (1-2 drops per slide) to the slides and incubate for 30 min at RT.

(5) Wash slides 3x 1 min in PBS/Tween.

(6) Apply Anti-Mouse-HRP-Polymer (**AB2**) (1-2 drops per slide) to the slides and incubate for 30 min at RT.

(7) Wash slides 3x 1 min in PBS/Tween.

(8) Prepare DAB Solution (working solution): fill 1 ml DAB Solution B (**SB1b**) in a graduated cup and add one drop (30 µl) DAB Solution A (**SB1a**). Mix well.

(9) Apply DAB Solution (1-2 drops per slide) to the slides and incubate for 30 min at RT.

(10) Transfer slides into a staining jar and wash 2 min under cold running tap water.

(11) Counterstain specimens for 5-10 sec with Mayer's Hematoxylin Solution (**CS1**).

(12) Transfer slides into a staining jar and wash 2 min under cold running tap water.

(13) Dehydration in: 70%, 90%, and 100% ethanol, each for 1 min.

(14) Incubate slides for 2x 2 min in xylene (use very pure xylene).

(15) Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm; 24 mm x 32 mm) by using Mounting Solution (alcoholic) (**MT4**). Allow 20-30 min for the coverslip to become immobilized.

(16) Evaluate stained specimens by using light microscopy.

13. Interpretation of results

The hybridization signals of Digoxigenin-labeled polynucleotides appear as brown- to dark brown colored distinct dots. For further information please refer to the instructions for use of the respective Zyto*Dot* CISH Probe.

14. Recommended quality control procedures

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

15. Performance characteristics

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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