ZYTOVISION

ZytoDot
CISH Polymer Detection Kit

REF C-3005-40 ▽Σ 40

REF C-3005-10 ▽Σ 40

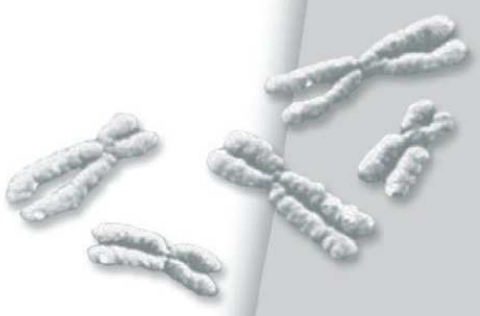
For the detection of DIG labeled probes by chromogenic *in situ* hybridization (CISH)

研究用です。診断用には使用できません

In vitro diagnostic medical device

according to EU directive 98/79/EC

人と科学のステキな未来へ
コスモ・バイオ株式会社



As of: January 1, 2010 (4.5)

Contents

1. Scope of Application

The ZytoDot CISH Polymer Detection Kit is designed to be used for the detection of digoxigenin (DIG)-labeled probes in either formalin-fixed, paraffin-embedded tissue or cell samples by chromogenic *in situ* hybridization (CISH).

DIG-labeled probes are detected using primary (unmarked) anti-DIG antibodies, secondary polymerized HRP-conjugated antibodies, and DAB (diaminobenzidine).

Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of 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 ZytoDot CISH Polymer Detection Kit

3.1 Components

The kit is made up of the following components:

Code	Component	Quantity		Container
		40 (Σ)	10	
BS1	Blocking Solution	4 ml	1 ml	Dropper bottle, orange cap
AB1	Mouse-Anti-DIG	4 ml	1 ml	Dropper bottle, pink cap
AB2	Anti-Mouse-HRP-Polymer	4 ml	1 ml	Dropper bottle, violet cap
SB1a	DAB Solution A	0.3 ml	0.1 ml	Dropper bottle, green cap
SB1b	DAB Solution B	10 ml	2 ml	Dropper bottle, grey cap
CS1	Mayer's Hematoxylin Solution	20 ml	4 ml	Screw-cap bottle, black
MT4	Mounting Solution (alcoholic)	4 ml	1 ml	Glass bottle, brown
	Instruction manual	1	1	

C-3005-40 (40 tests): All components are sufficient for 40 reactions.

C-3005-10 (10 tests): All components are sufficient for 10 reactions.

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 ZytoDot CISH Polymer Detection 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.

Prior to detection of hybridized digoxigenin-labeled probes we recommend the following procedures:

- ✓ *Tissue preparation:* Use 10% neutrally buffered formalin for 24 h at RT. Paraffin embedding should be carried out by standard processing. Prepare 2-5 µm microtome sections.
- ✓ *Pretreatment:* The pretreatment (dewax and proteolysis) of the tissue and cell section should be performed using established standard protocols. As a general rule, we recommend that the optimum time for proteolysis will be ascertained in pre-tests.
- ✓ *Hybridization:* Hybridization should be carried out in a humidity chamber overnight at 37°C. Wash slides before starting the detection.
- ✓ *Quenching:* Incubate slides for 10 min in 3% H_2O_2 in absolute methanol. Quenching can be performed after dewaxing the slides or after hybridization.

3.4 Additional Materials

The following reagents, materials, and equipment are not included in the kit:

Reagents and materials

- *Deionized or distilled water*
- *Ethanol 100%, denatured*
- *PBS/Tween*
- *Xylene*

Equipment

- *Coverslips (22 mm x 22 mm, 24 mm x 32 mm)*
- *Light microscope*
- *Staining jars, 50-80 ml*

4. The ZytoDot CISH Polymer Detection Kit Protocol

4.1 Preparatory Steps

- *Blocking Solution* **(BS1)**, *Mouse-Anti-DIG* **(AB1)**, *Anti-Mouse-HRP-Polymer* **(AB2)**, *Mayer's Hematoxylin Solution* **(CS1)**, *Mounting Solution (alcoholic)* **(MT4)**: Bring to room temperature before use.
- *Preparation of DAB Solution:* Prior to use, add dropwise DAB Solution B **(SB1b)** in a graduated cup up to 1 ml and add one drop DAB Solution A **(SB1a)**. The solution is stable for 2 h at room temperature (RT).

4.2 Detection

1. Wash 3x 2 min in a PBS/Tween buffer (not provided)

2. Apply Blocking Solution **(BS1)** dropwise (3-4 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)** dropwise (3-4 drops per slide) to the slides and incubate for 30 min at RT

5. Wash 3x 2 min in a PBS/Tween buffer (not provided)

6. Apply Anti-Mouse-HRP-Polymer **(AB2)** dropwise (3-4 drops per slide) to the slides and incubate for 30 min at RT

7. Wash 3x 2 min in a PBS/Tween buffer (not provided)

8. During the wash steps, prepare DAB Solution by adding DAB Solution B **(SB1b)** dropwise in a graduated cup up to 1 ml and add one drop DAB Solution A **(SB1a)**

9. Apply DAB Solution dropwise (3-4 drops per slide) to the slides and incubate for 30 min at RT

10. Transfer slides into a staining jar and wash 2 min in running tap water

11. Counterstain the tissue or cell samples for 8-10 s with Mayer's Hematoxylin Solution **(CS1)**

12. Transfer slides into a staining jar and wash 2 min in running tap water

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

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

Air dry sections for approximately 15 min

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)** and air dry the slides for approx. 30 min

16. Evaluation of the sample material is carried out by light microscopy

5. Interpretation of Results

Probes labeled with digoxigenin (DIG) will result in permanent DAB brown-colored distinct dot-shaped signals, which can be clearly distinguished from the background counterstained with hematoxylin.

In normal diploid nuclei without chromosome aberrations, 2 dot-shaped signals with smooth, rounded edges will be visible per nucleus, except for probes targeting sex chromosomes resulting in 0 to 2 dot-shaped signals per probe, depending on the gender. Due to mitosis, additional signals may be visible even in a small percentage of non-neoplastic cells. Occasionally, nuclei with missing signals may be observed in paraffin-embedded tissue sections.

Visualization of signals should be performed using at least a 40x objective resulting in easily visible signals.

The counterstaining time depends on the nature of the tissue or cells used and should therefore be optimized. Avoid dark counterstaining, as this may obscure positive staining signals. Absent or weak signals may be the result of a too short incubation with Mayer's Hematoxylin Solution.

The final experimental results are also strongly influenced by the preceding experimental steps, i.e., tissue fixation, pretreatment, denaturation of DNA probe, hybridization, and washing. For a particularly user-friendly performance we recommend the use of a Zyto*Dot* CISH system by ZytoVision.

For troubleshooting, please refer to chapter 7.

6. Literature

Hopman AHN, et al. (1997) *Histochem Cell Biol* **108**: 291-8.

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

Shipley J (2006) *J Pathol* **210**: 1-2.

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

7. Problems and Possible Causes

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Problem	Possible cause	Action
Streaks on the slide after stopping the pepsin treatment	Precipitation	Wash section immediately in deionized or distilled water
Weak signal or no signal at all	No target sequences available	Use controls
	Cell or tissue sample has not been properly fixed	Optimization of fixing time
	Proteolytic pretreatment not carried out properly	Optimization of incubation time
	Denaturing temperature not correct	Check temperature; increase or decrease if necessary
	Hybridization temperature not correct	Check temperature
	Hybridization time too short	Extension of hybridization time
	Incubation with chromogenic substrate too short	Extension of incubation time
	Too dark counterstaining	Optimize counterstaining time
Uneven and in some parts only very light staining	Incomplete dewaxing	Use fresh solutions; check length of dewaxing times
Cross hybridization signals; strong background staining	Probe volume per area too high	Reduce probe volume per section/area, distribute probe dropwise to avoid local concentration
	Proteolytic pretreatment too strong	Optimization of incubation time
	Dehydration of sections between the individual incubation steps	Prevent dehydration
	Washing temperature following hybridization too low	Check temperature
Section floats off the slide	Proteolytic pretreatment too strong	Shortening of incubation time
	Unsuitable slide coating	Use appropriate slides

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

Your local distributor