



ZytoDot AP-Red Solution Set

REF C-3038-100 100

For the use in chromogenic *in situ* hybridization (CISH)
procedures



In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The ZytoDot AP-Red Solution Set is intended to be used as a substrate for an AP-conjugated antibody in chromogenic *in situ* hybridization (CISH) applications. The kit is intended to be used in combination with the ZytoDot 2C CISH Implementation Kit (Prod. No. C-3044-40).

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., translocations, 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 ZytoDot AP-Red Solution Set is available in one size and is composed of:

Code	Component	Quantity	Container
		100	
SB6a	AP-Red Solution A	0.4 ml	Dropper bottle, red cap (small)
SB6b	AP-Red Solution B	15 ml	Dropper bottle, red cap
	Instructions for use	1	

C-3038-100 (100 tests): Components **SB6a-b** are sufficient for 100 reactions.

5. Materials required but not provided

- ZytoDot 2C CISH Probe
- ZytoDot 2C CISH Implementation Kit (Prod. No.-C-3044-40)
- 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 μ l, 1000 μ l)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene
- Methanol 100%
- Hydrogen peroxide (H₂O₂) 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 ZytoDot AP-Red Solution Set 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 SB6b:

EUH210 Safety data sheet available on request.

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 2C CISH Implementation Kit](#).

10. Preparation of specimens

Refer to the instructions for use of the [ZytoDot 2C CISH Implementation Kit](#).

11. Preparatory treatment of the device

Prior to immediate use, fill 1 ml AP-Red Solution B (**SB6b**) in a graduated cup and add one drop (30 µl) of AP-Red Solution A (**SB6a**). Mix well.

12. Assay procedure

For detailed information on how to perform CISH with ZytoDot products, including detection steps with the [ZytoDot AP-Red Solution Set](#), please refer to the instructions for use of the [ZytoDot 2C CISH Implementation Kit](#).

13. Interpretation of results

Refer to the instructions for use of the respective *ZytoDot 2C CISH Probe*.

14. Recommended quality control procedures

Refer to the instructions for use of the respective *ZytoDot 2C CISH Probe*.

15. Performance characteristics

Refer to the instructions for use of the respective *ZytoDot 2C 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 *ZytoDot 2C CISH Probe* and implementation kit for further information.

18. Literature

- Lojda, Z., et al. (1964) *Z Zellforsch Microsk Anat Histochem*. 48:428–454
- Speel EJ, et al. (1994) *J Histochem Cytochem* 42: 1299-307.
- 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.
Please contact helptech@zytovision.com



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

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