ZYTOVISION

Zyto*Fast*
RNA (-) Control Probe
(Digoxigenin-labeled)

REF T-1119-400 Σ 40 (0.4 ml)

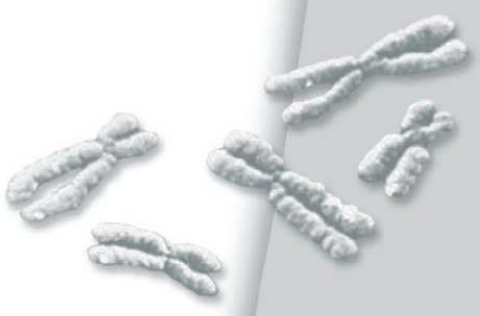
For assessing
the unspecific background staining within specimens
by chromogenic *in situ* hybridization (CISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



Digoxigenin-labeled oligonucleotide probe for assessing the unspecific background staining within specimens by CISH, ready to use

Product Description

Content: Zyto*Fast* RNA (-) Control Probe **(PF33)** in hybridization buffer. The probe contains digoxigenin-labeled oligonucleotides with GC contents of 40-70% without known consensus to any naturally occurring sequences.

Product: T-1119-400: 0.4 ml (40 reactions of 10 µl each)

Specificity: The Zyto*Fast* RNA (-) Control Probe **(PF33)** is designed to be used for assessing the unspecific background staining in formalin-fixed, paraffin-embedded tissue or cells by chromogenic *in situ* hybridization (CISH).

Storage/Stability: The Zyto*Fast* RNA (-) Control Probe **(PF33)** must be stored at 2...8°C and is stable through the expiry date printed on the label.

Use: This product is designed for *in vitro* diagnostic use (according to EU directive 98/79/EC). 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!

Safety Precautions: Read the operating instructions 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. 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!

A material safety data sheet is available on request for the professional user!

Principle of the Method

The presence of certain nucleic acid sequences in cells or tissue can be detected by *in situ* hybridization using labeled DNA probes. The hybridization results in duplex formation of sequences present in the test object with the labeled DNA probe.

Duplex formation of the digoxigenin-labeled probe with RNA sequences in the test material is indirectly detected by using enzyme-conjugated antibodies directed against digoxigenin or unconjugated antibodies detected by a secondary polymerized enzyme-conjugated antibody. The enzymatic reaction of a chromogenic substrate leads to the formation of a color precipitate that is visualized by light microscopy.

Instructions

Pretreatment (dewaxing, proteolysis, post-fixation) should be carried out according to the needs of the user.

Bring probe to hybridization temperature before use.

Denaturation and hybridization of probe:

1. Vortex the Zyto*Fast* RNA (-) Control Probe **(PF33)** and pipette 10 µl each onto individual samples

Distribute dropwise on the whole target area to avoid local concentration of probe. Alternatively, add probe to the center of a coverslip and place it upside down on target area.

2. Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm). Seal the coverslip, e.g. with a layer of hot glue from an adhesive pistol or with rubber cement

3. Denature the slides at 75°C for 5 min, e.g. on a hot plate

4. Transfer the slides to a humidity chamber and hybridize for 60 min at 55°C (e.g. in a hybridization oven)

It is essential that the tissue/cell samples do not dry out during the hybridization step.

Further processing, such as washing, detection, and counterstaining, can be completed according to the user's needs. For a particularly user-friendly performance, we recommend the use of a Zyto*Fast* CISH system by ZytoVision. These systems were also used for the confirmation of appropriateness of the Zyto*Fast* RNA (-) Control Probe **(PF33)**.

Results

The ZytoFast RNA (-) Control Probe **(PF33)** consists of a set of random sequence oligonucleotides with GC contents of 40-70% without known consensus to any naturally occurring sequences. This probe should not result in positive staining signals and is to be used to assess the unspecific background staining within specimens.

Visualization of signals should be performed by light microscopy using a 10x or 20x objective. For signal evaluation, necrotic, degenerated or over-digested cells should be avoided as these cells often stain nonspecifically.

In order to judge the specificity of the hybridization signals and to confirm the correct performance of the method, any hybridization should be accompanied by controls. We recommend using at least one control sample containing both true positive and negative staining cells.

Our experts are available to answer your questions.

Literature

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

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