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

REF T-1119-100 Σ 10 (0.1 ml)

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

For research use only.
Not for use in diagnostic procedures.

1. Intended use

The Zyto*Fast* RNA (-) Control Probe (**PF33**) is intended to be used for the qualitative detection of unspecific background staining in formalin-fixed, paraffin-embedded specimens by chromogenic *in situ* hybridization (CISH). The probe is intended to be used in combination with one of the Zyto*Fast* PLUS CISH Implementation Kits, either the Zyto*Fast* PLUS CISH Implementation Kit AP-NBT/BCIP (Prod. No. T-1061-40), the Zyto*Fast* PLUS CISH Implementation Kit HRP-DAB (Prod. No. T-1063-40), or the Zyto*Fast* PLUS CISH Implementation Kit AP-Permanent Red (Prod. No. T-1151-40).

2. Clinical relevance

This product is for research use only and not for diagnostic procedures.

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 subsequently 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*Fast* RNA (-) Control Probe (**PF33**) is composed of:

- Digoxigenin-labeled oligonucleotides (~ 0.14 ng/µl) with GC contents of 40-70 % without known consensus to any naturally occurring sequences.

The Zyto*Fast* RNA (-) Control Probe is available in one size:

- T-1119-100: 0.1 ml (10 reactions of 10 µl each)

5. Materials required but not provided

- Zyto*Fast* PLUS CISH Implementation Kit AP-NBT/BCIP (Prod. No. T-1061-40) or Zyto*Fast* PLUS CISH Implementation Kit HRP-DAB (Prod. No. T-1063-40) or Zyto*Fast* PLUS CISH Implementation Kit AP-Permanent Red (Prod. No. T-1151-40)
- Positive and negative control specimens
- Microscope slides, positively charged
- Water bath (55°C, 98°C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable calibrated pipettes (10 µl, 100 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 (100-200x)

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!
- 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!

Hazard and precautionary statements:

This probe is not classified as hazardous according to Regulation (EC) No. 1272/2008.

8. Limitations

- For research use only.
- For professional use only.
- 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 probe should be used only for detecting target sequences described in 4. "Reagents provided".
- 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

The following fixatives are incompatible with ISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin

10. Preparation of specimens

Recommendations:

- Avoid cross-contamination of samples in any step of preparation as this may lead to erroneous results.
- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Sample size ≤ 0.5 cm^3.
- Use premium quality paraffin.
- Embedding should be carried out at temperatures lower than 65°C.
- Prepare 3-5 µm microtome sections.
- Use positively charged microscope slides.
- Fix tissue sections for 2-16 h at 50-60°C.

11. Preparatory treatment of the device

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Bring probe to hybridization temperature (55°C) and mix thoroughly before use.

12. Assay procedure

Specimen pretreatment
Perform specimen pretreatment (e.g., dewaxing, proteolysis) according to the instructions for use of the Zyto*Fast* PLUS CISH Implementation Kits.

Denaturation and hybridization

1. Pipette 10 µl of the probe onto each pretreated specimen.
2. Cover specimens with a 22 mm x 22 mm coverslip (avoid trapped bubbles) and seal the coverslip.

We recommend using rubber cement (e.g., Fixogum) for sealing.

3. Place slides on a hot plate or hybridizer and denature specimens for 5 min at 75°C.
4. Transfer slides to a humidity chamber and hybridize for 1 h at 55°C (e.g., in a hybridization oven).

It is essential that specimens do not dry out during the hybridization step.

Post-hybridization and detection
Perform post-hybridization processing (washing, detection, counter-staining, mounting, microscopy) according to the instructions for use of the respective Zyto*Fast* PLUS CISH Implementation Kit.

13. Interpretation of results

Using the Zyto*Fast* RNA (-) Control Probe, no positive staining signals should appear. The probe is to be used to assess the unspecific background staining within specimens.

Please note:

- Visualization of signals should be performed using at least a 100-fold magnification resulting in easily visible signals.
- Do not evaluate areas of necrosis, overlapping nuclei, over-digested nuclei and nuclei with weak signal intensity.
- An unexpected staining can be caused by multiple factors (see chapter 17 "Troubleshooting").
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

14. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by internal and external controls. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

15. Performance characteristics

In order to assess the analytical performance of the Zyto*Fast* RNA (-) Control Probe, chromogenic *in situ* hybridizations were performed on formalin-fixed, paraffin-embedded (FFPE) specimens in combination with all Zyto*Fast* PLUS CISH Implementation Kits.

Analytical sensitivity: The overall analytical sensitivity of Zyto*Fast* RNA (-) Control Probe assessed by CISH was calculated to be 100.0 %.

Analytical specificity: The overall analytical specificity of the Zyto*Fast* RNA (-) Control Probe assessed by CISH was calculated to be 100.0 %.

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.

Tissue morphology degraded

Possible cause	Action
Cell or tissue sample has not been properly fixed	Optimize fixing time and fixative
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time

Noisy background

Possible cause	Action
Stringency wash temperature not correct	Check temperature of the technical devices used, using a calibrated thermometer. Use always the same number of slides in the jar. We recommend not to use more than eight slides per jar for heat incubation steps
Slides not thoroughly rinsed	Use fresh and sufficient wash buffer and deionized or distilled water where indicated
Sections dried out any time during or after hybridization	Avoid sections being dried out; use humidity chamber; seal coverslip properly
Prolonged substrate incubation time	Shorten substrate incubation time
Incomplete dewaxing	Use fresh solutions; check duration of dewaxing

Proteolytic pretreatment too strong	Optimize pepsin incubation time
Slides cooled to room temperature before hybridization	Transfer the slides quickly to hybridization temperature
Tissue-antibody interaction	Use an alternative detection system circumventing the observed interaction

Overlapping nuclei

Possible cause	Action
Inappropriate thickness of tissue sections	Prepare 3-5 µm microtome sections

Specimen floats off the slide

Possible cause	Action
Unsuitable slide coating	Use appropriate (positively charged) slides
Proteolytic pretreatment too strong	Shorten pepsin incubation time

18. Literature

- 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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