ZytoVision

Zyto*Fast*
DNA (+) Control Probe
(Digoxigenin-labeled)

REF T-1053-100 Σ 10 (0.1 ml)

For use as a positive control for the qualitative detection of human DNA sequences by chromogenic *in situ* hybridization (CISH)

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

1. Intended use

The Zyto*Fast* DNA (+) Control Probe (**PF23**) specific for human Alu repetitive sequences is intended to be used as a positive control for the qualitative detection of human DNA sequences 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* DNA (+) Control Probe (**PF23**) is composed of:

- Digoxigenin-labeled oligonucleotides (~ 0.14 ng/µl) targeting human Alu-repetitive sequences.
- Formamide based hybridization buffer

The Zyto*Fast* DNA (+) Control Probe is available in one size:

- T-1053-100: 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:

The hazard-determining component is formamide.

Danger

H351	Suspected of causing cancer.
H360FD	May damage fertility. May damage the unborn child.
H373	May cause damage to organs through prolonged or repeated exposure.
P201	Obtain special instructions before use.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.

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 (37°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 37°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* PLUS CISH Implementation Kits, hybridized Digoxigenin-labeled oligonucleotides appear as dark-blue pattern when detected by alkaline phosphatase (AP) and NBT/BCIP, as bright red pattern when detected by AP and Permanent Red, or as brown pattern when detected by horseradish peroxidase (HRP) and DAB.

A positive reactivity for human Alu repetitive sequences in target cells is indicated by a distinctly stained nucleus.

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.
- A negative or unspecific result 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* DNA (+) 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* DNA (+) Control Probe assessed by CISH was calculated to be 100.0 %.

Analytical specificity: The overall analytical specificity of the Zyto*Fast* DNA (+) 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.

Weak signals or no signals at all

Possible cause	Action

Cell or tissue sample has not been properly fixed	Optimize fixing time and fixative
Proteolysis, hybridization, denaturation, stringency wash or antibody-incubation temperature not correct	Check temperature of all technical devices used, using a calibrated thermometer. Use always the same number of slides in solutions with critical temperature
Proteolytic pretreatment not carried out properly	Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. Ascertain the optimum time for pepsin incubation in pre-tests
Hybridization time too short	Hybridize for at least 1 h; extend hybridization time if necessary
Too low concentrated Wash Buffer	Check concentration of Wash Buffer
Old dehydration solutions	Prepare fresh dehydration solutions
Probe evaporation	When using a hybridizer, the use of the wet stripes/water filled tanks is mandatory. When using a hybridization oven, the use of a humidity chamber is required. In addition, the coverslip should be sealed completely, e.g., with Fixogum, to prevent drying-out of the sample during hybridization
Insufficient preparation of chromogenic substrate	If kit Prod. No. T-1063-40 or Prod. No. T-1151-40 is used: Instead of preparing the color substrates by dropping, use a pipette
Incubation temperature for color substrates not correct	Check temperature of all technical devices used, using a calibrated thermometer
Counterstaining time too long	The counterstaining time depends on the nature of the specimen and should be optimized accordingly. Avoid dark counterstaining, because it may obscure positive staining signals
Bluing of counterstain not carried out properly	If kit Prod. No. T-1063-40 or Prod. No. T-1151-40 is used: Use cold running tap water for bluing; do not use warm or hot water or bluing reagents

Signals too strong

Possible cause	Action
Proteolytic pretreatment carried out too long	Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. Ascertain the optimum time for pepsin incubation in pre-tests
Substrate reaction is too intense	Shorten substrate incubation time; do not heat substrate solution above the temperature given in the instructions for use

Signals fade or merge

Possible cause	Action
An unsuitable mounting solution has been used	Use only the mounting solution provided with the kit or recommended by the instructions for use. Use solutions free of any impurities; do not use coverslip tape

Uneven or in some parts only very light staining

Possible cause	Action
Incomplete dewaxing	Use fresh solutions; check duration of dewaxing
Reagent volume too small	Ensure that the reagent volume is large enough to cover the tissue area
Air bubbles caught before hybridization or during mounting	Avoid air bubbles

Inconsistent results

Possible cause	Action
Insufficient drying before probe application	Extend air-drying
Too much water/wash buffer on tissue prior to application of pepsin, antibodies and/or color substrates	Ensure that excess liquid is removed from tissue section by blotting or shaking it off the slide. Small amounts of residual water/wash buffer do not interfere with the test
Variations in tissue fixation and embedding methods	Optimize fixation and embedding methods
Variations in tissue section thickness	Optimize sectioning

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 negative control probes, e.g., ZytoFast DNA (-) Control Probe (**PF24**), to ascertain tissue-specific background staining

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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