ZytoVision

Zyto*Fast*
human Ig-kappa Probe
(Digoxigenin-labeled)

REF T-1115-400 Σ 40 (0.4 ml)

For the qualitative detection of human Ig-kappa (κ) light chain mRNA by chromogenic *in situ* hybridization (CISH)

4250380P102QE

CE
0124
IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The Zyto*Fast* human Ig-kappa Probe (**PF30**) is intended to be used for the qualitative detection of human Ig-kappa (κ) light chain mRNA in formalin-fixed, paraffin-embedded specimens, such as multiple myeloma, by chromogenic *in situ* hybridization (CISH). The probe is intended to be used in combination with the Zyto*Fast* PLUS CISH Implementation Kit HRP-DAB (Prod. No. T-1063-40).

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

The probe is intended to be used as an aid to the differential diagnosis of multiple myeloma and therapeutic measures should not be initiated based on the test result alone.

2. 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.

3. Reagents provided

The Zyto*Fast* human Ig-kappa Probe is composed of:

- Digoxigenin-labeled oligonucleotides (~0.2 ng/µl), which target mRNA sequences encoding Ig-kappa light chain constant regions.

The Zyto*Fast* human Ig-kappa Probe is available in one size:

- T-1115-400: 0.4 ml (40 reactions of 10 µl each)

4. Materials required but not provided

- Zyto*Fast* PLUS CISH Implementation Kit HRP-DAB (Prod. No. T-1063-40)
- 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)

5. 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.

6. 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)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- 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.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated 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/human geneticist 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/human geneticist 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 probe should be used only for detecting sequence described in chapter 3. "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. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

8. 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

9. Preparation of specimens

Prepare specimens as described in the instructions for use of the ZytoFast PLUS CISH Implementation Kit HRP-DAB.

10. 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.

11. Assay procedure

Specimen pretreatment
Perform specimen pretreatment (e.g., dewaxing, proteolysis) according to the instructions for use of the respective ZytoFast CISH Implementation Kit.

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
Perform post-hybridization processing (washing, detection, counter-staining, mounting, microscopy) according to the instructions for use of the respective ZytoFast CISH Implementation Kit.

12. Interpretation of results

Using the ZytoFast PLUS CISH Implementation Kit HRP-DAB, hybridized Digoxigenin-labeled oligonucleotides appear as brown pattern when detected by horseradish peroxidase (HRP) and DAB.

A positive reactivity in plasma B-cells is indicated by cytoplasmic staining.

In lymphoid tissue, the normal kappa-to-lambda ratio is roughly 2:1, an indication for monoclonality is given if the kappa-to-lambda ratio is >3:1 or <0.3:1.

Please note:

- Visualization of signals should be performed at least at 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 16 "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.

13. 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.

Internal control: A plasma B-cell within the specimen should show either Ig-kappa or Ig-lambda staining pattern. Cells within the specimen not belonging to the B-cell type, e.g. fibroblasts, should show no staining pattern.

External control: Validated positive and negative control specimens.

14. Performance characteristics

14.1 Analytical performance

Analytical sensitivity:	100% (95% CI 97.0 – 100.0)
Analytical specificity:	100% (95% CI 97.0 – 100.0)

14.2 Clinical performance

Diagnostic sensitivity:	100% (95% CI 86.3 -100.0) vs. IHC 100% vs. RAPID RISH Biocare/ Serum-free light chain restriction assay 88.24% vs. IHC
Diagnostic specificity:	100% (95% CI 86.3 -100.0) vs. IHC

15. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to www.zytovision.com for more information.

Weak signals or no signals at all

Possible cause	Action
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
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	Instead of preparing the color substrates by dropping, use a pipette
Counterstaining time too long	Avoid dark counterstaining, because it may obscure positive staining signals
Bluing of counterstain not carried out properly	Use cold running tap water for bluing; do not use warm or hot water or bluing reagents

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 times
Reagent volume too small	Ensure that the reagent volume is large enough to cover the tissue area

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

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
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 not carried out properly	Optimize pepsin incubation time
Slides cooled to room temperature before hybridization	Transfer the slides quickly to hybridization temperature

Overlapping signals

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

Specimen floats off the slide

Possible cause	Action
Proteolytic pretreatment too strong	Shorten pepsin incubation time

17. Literature

- Lang G. (2010) *Journal of Histotechnology*.
- Sen A, et al. (2020) *Med J Armed Forces India*.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, Oxford University Press (1992) ISBN 0 19 963327 4.

18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com
For the summary of safety and performance, please refer to www.zytovision.com.

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