ZYTOVISION

ZytoLight SPEC 1p12 Probe

REF Z-2101-200 Σ 20 (0.2 ml)

For the qualitative detection of human chromosome 1p12 specific sequences by fluorescence *in situ* hybridization (FISH)

For research use only.
Not for use in diagnostic procedures

1. Intended purpose

The ZytoLight SPEC 1p12 Probe (**PL59**) is intended to be used for the qualitative detection of human chromosome 1p12 specific sequences in formalin-fixed, paraffin-embedded specimens by fluorescence *in situ* hybridization (FISH). The probe is intended to be used in combination with the ZytoLight FISH-Tissue Implementation Kit (Prod. No. Z-2028-5-R/-20-R).

2. Test principle

The fluorescence *in situ* hybridization (FISH) technique allows for the detection and visualization of specific nucleic acid sequences in cell preparations. Fluorescently-labeled DNA fragments, so called FISH probes, and their complementary target DNA strands 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. After counterstaining the DNA with DAPI, hybridized probe fragments are visualized using a fluorescence microscope equipped with excitation and emission filters specific for the fluorochromes with which the FISH probe fragments have been directly labeled.

3. Reagents provided

The ZytoLight SPEC 1p12 Probe is composed of:

- ZyOrange (excitation 547 nm/emission 572 nm) labeled polynucleotides (~4.5 ng/µl), which target sequences mapping in 1p12* (chr1:119,537,102-119,823,147) (see Fig. 1).
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

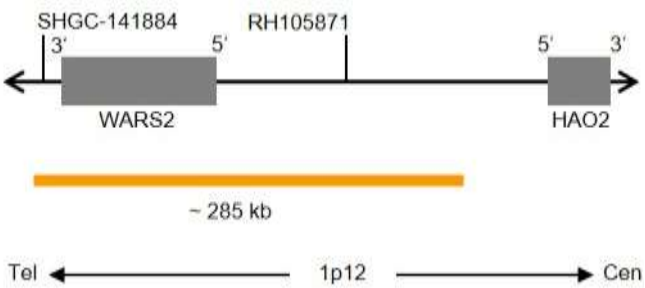

Fig. 1: SPEC 1p12 Probe map (not to scale)

The ZytoLight SPEC 1p12 Probe is available in one size:

- Z-2101-200: 0.2 ml (20 reactions of 10 µl each)

4. Materials required but not provided

- ZytoLight FISH-Tissue Implementation Kit (Prod. No. Z-2028-5-R/-20-R)
- Positive and negative control specimens
- Microscope slides, positively charged
- Water bath (37 °C, 98 °C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable pipettes (10 µl, 25 µl)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene
- Deionized or distilled water
- Coverslips (22 mm x 22 mm, 24 mm x 60 mm)
- Rubber cement, e.g., Fixogum Rubber Cement (Prod. No.E-4005-50/-125) or similar
- Adequately maintained fluorescence microscope (400-1000x)
- Immersion oil approved for fluorescence microscopy
- Appropriate filter sets

5. Storage and handling

Store at 2-8 °C in an upright position protected from light. Use protected from light. 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 probe should not be exposed to light, especially strong light, for a longer period of time, i.e., all steps should be accomplished, where possible, in the dark and/or using lightproof containers.

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.
P202	Do not handle until all safety precautions have been read and understood.
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.

7. Limitations

- For research use only.
- For professional use only.
- For non-automated 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 loci described in chapter 3. "Reagents provided".

8. Interfering substances

Red blood cells present in the specimen might exhibit autofluorescence which hinders signal recognition.

The following fixatives are incompatible with FISH:

- 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 ZytoLight FISH-Tissue Implementation Kit.

10. Preparatory treatment of the device

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25 °C) before use, protect from light. Prior to opening the vial, mix by vortexing and spin down briefly.

11. Assay procedure

Specimen pretreatment
Perform specimen pretreatment (dewaxing, proteolysis) according to the instructions for use of the ZytoLight FISH-Tissue 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 10 min at 75 °C.
4. Transfer slides to a humidity chamber and hybridize overnight at 37 °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, counter-staining, fluorescence microscopy) according to the instructions for use of the ZytoLight FISH-Tissue Implementation Kit.

12. 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: Non-neoplastic cells within the specimen that exhibit normal signal pattern, e.g., fibroblasts.

External control: Validated positive and negative control specimens.

13. Disposal

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

14. 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
Cell or tissue sample not fixed properly	Optimize fixing time and fixative or apply a post-fixation step as described in "assay procedure" of the manual of the ZytoLight FISH-Tissue Implementation Kit
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
Inappropriate filter sets used	Use filter sets appropriate for the fluochromes of the probe. *Triple-bandpass filter sets provide less light compared to single or dual-bandpass filter sets. Consequently, the signals may appear fainter using these triple-bandpass filter sets*

Cross hybridization signals; noisy background

Possible cause	Action
Incomplete dewaxing	Use fresh solutions; check duration of dewaxing
Proteolytic pretreatment too strong	Reduce pepsin incubation time
Slides cooled to room temperature before hybridization	Transfer the slides quickly to 37 °C

Morphology degraded

Possible cause	Action
Cell or tissue sample has not been fixed properly	Optimize fixing time and fixative or apply a post-fixation step as described in "assay procedure" of the manual of the ZytoLight FISH-Tissue Implementation Kit
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, decrease if necessary
Insufficient drying before probe application	Extend air-drying

Overlapping nuclei

Possible cause	Action
Inappropriate thickness of tissue sections	Prepare 2-4 µm microtome sections

Specimen floats off the slide

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time

Weak counterstain

Possible cause	Action
Low concentrated DAPI solution	Use DAPI/DuraTect-Solution (ultra) instead
DAPI incubation time too short	Adjust DAPI incubation time

15. Literature

- Christiansen H, et al (1992) *Genes Chromosomes Cancer* 5: 141-9.
- Ichikawa D, et al. (1996) *Cancer* 77: 2064-9.
- Kievits T, et al. (1990) *Cytogenet Cell Genet* 53: 134-6.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

16. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com

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