Zyto*Light*
SPEC 13/CEN 18/SPEC 21 Triple Color Probe

REF Z-2095-200	Σ 20 (0.2 ml)
REF Z-2095-50	Σ 20 (0.05 ml)

For the detection of human chromosome 13 specific sequences, alpha-satellites of chromosome 18, and chromosome 21 specific sequences by fluorescence *in situ* hybridization (FISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC

Fluorescence-labeled polynucleotide probe for the detection of human chromosome 13 and 21 specific sequences, and alpha-satellites of chromosome 18 centromeres, ready to use

Product Description

Content: ZytoLight SPEC 13/CEN 18/SPEC 21 Triple Color Probe **(PL143)** in hybridization buffer. The probe contains green-labeled polynucleotides (ZyGreen: excitation at 503 nm and emission at 528 nm, similar to FITC), which target chromosome 13 specific sequences, blue-labeled polynucleotides (ZyBlue: excitation at 418 nm and emission at 467 nm, similar to DEAC) which target alpha-satellite-sequences of the centromere of chromosome 18, and orange-labeled poly-nucleotides (ZyOrange: excitation at 547 nm and emission at 572 nm, similar to rhodamine), which target chromosome 21 specific sequences.

Product: Z-2095-200: 0.2 ml (20 reactions of 10 µl each)
Z-2095-50: 0.05 ml (5 reactions of 10 µl each)

Specificity: The ZytoLight SPEC 13/CEN 18/SPEC 21 Triple Color Probe **(PL143)** is designed to be used for the detection of human chromosome 13q12 specific sequences as well as chromosome 18 alpha-satellites and chromosome 21q22 specific sequences in formalin-fixed, paraffin-embedded tissue or cells by fluorescence *in situ* hybridization (FISH).

Storage/Stability: The ZytoLight SPEC 13/CEN 18/SPEC 21 Triple Color Probe **(PL143)** must be stored at -16...-22°C in the dark (short-time storage at 2...8°C is possible) 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 the 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 (with sequences of chromosomes 13 and 21, and chromosome 18 alpha-satellites in the test material) is directly detected by using the tags of fluorescence-labeled polynucleotides.

Instructions

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

Denaturation and hybridization of probe:

1. Pipette 10 µl ZytoLight SPEC 13/CEN 18/SPEC 21 Triple Color Probe **(PL143)** each onto individual samples

A gentle warming of the probe, as well as using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure of the probe to light.

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 (±2°C) for 10 min, e.g. on a hot plate

Depending upon the age of the sample and variations in the fixation stage, it may be necessary to optimize the denaturing temperature (73°C-77°C).

4. Transfer the slide to a humidity chamber and hybridize overnight at 37°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 and counter-staining, can be completed according to the user's needs. For a particularly user-friendly performance, we recommend the use of a ZytoLight FISH system by ZytoVision. These systems were also used for the confirmation of appropriateness of the ZytoLight SPEC 13/CEN 18/SPEC 21 Triple Color Probe **(PL143)**.

Results

With the use of appropriate filter sets, the hybridization signals of labeled chromosome 13 specific sequences appear green; the hybridization signals of labeled alpha-satellite-sequences of the centromere of chromosome 18 appear blue, and the hybridization signals of labeled chromosome 21 specific sequences appear orange. In interphases of normal cells or cells without aberrations of chromosomes 13, 18, and 21, two chromosome 13, two chromosome 18, and two chromosome 21 signals appear. In cells with an aneuploidy of one of the chromosomes mentioned above, a different signal pattern is visible in interphases.

In order to judge the specificity of the signals, every hybridization should be accompanied by controls. We recommend using at least one control sample in which the chromosome 13, 18, and 21 copy number is known.

Care should be taken not to evaluate overlapping cells, in order to avoid false results, e.g. an amplification of genes. Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance equal to or less than the diameter of one signal, should be counted as one signal.

Our experts are available to answer your questions.

Literature

Kievits T, et al. (1990) *Cytogenet Cell Genet* **53**: 134-6.

Waye JS, Willard HF (1987) *Nucleic Acids Res* **15**: 7549-69.

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

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