ZytoLight
SPEC ALK/EML4 TriCheck™ Probe

REF Z-2117-200	Σ 20 (0.2 ml)
REF Z-2117-50	Σ 5 (0.05 ml)

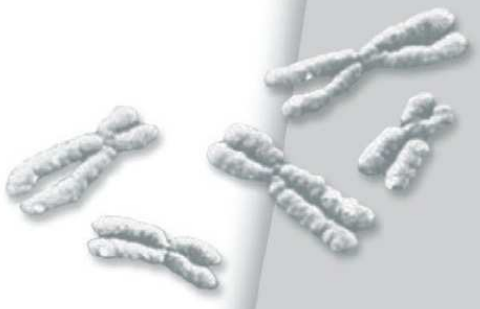
For the detection of ALK-EML4 rearrangements
by fluorescence *in situ* hybridization (FISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



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Fluorescence-labeled polynucleotide probe for the detection of ALK-EML4 rearrangements, ready to use

Product Description

Content: ZytoLight SPEC ALK/EML4 TriCheck™ Probe **(PL74)** in hybridization buffer. The probe contains green-labeled polynucleotides (ZyGreen: excitation at 503 nm and emission at 528 nm, similar to FITC), which target sequences mapping in 2p23 proximal to the ALK breakpoint region, orange-labeled poly-nucleotides (ZyOrange: excitation at 547 nm and emission at 572 nm, similar to rhodamine), which target sequences mapping in 2p23 distal to the ALK breakpoint region, and blue-labeled polynucleotides (ZyBlue: excitation at 418 nm and emission at 467 nm, similar to DEAC), which target the EML4 gene in 2p21.

Product: Z-2117-200: 0.2 ml (20 reactions of 10 µl each)
Z-2117-50: 0.05 ml (5 reactions of 10 µl each)

Specificity: The ZytoLight SPEC ALK/EML4 TriCheck™ Probe **(PL74)** is designed to be used for the detection of rearrangements involving the ALK gene at 2p23 and the EML4 gene at 2p21 in formalin-fixed, paraffin-embedded tissue or cells by fluorescence *in situ* hybridization (FISH).

Storage/Stability: The ZytoLight SPEC ALK/EML4 TriCheck™ Probe **(PL74)** 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 con-

text of the patient's clinical history with respect to further clinical and pathologic data of 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 the chromosomal region 2p21 and 2p23 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 ALK/EML4 TriCheck™ Probe **(PL74)** 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 ALK/EML4 TriCheck™ Probe **(PL74)**.

Results

With the use of appropriate sets, the hybridization signals of labeled ALK gene (2p23) appear green and orange, the hybridization signals of labeled EML4 gene (2p21) appear blue.

In interphases of normal cells or cells without ALK-EML4 rearrangements, two green/orange fusion signals appear when using an appropriate dual bandpass filter set, and two blue signals appear when using an appropriate single bandpass filter set (see fig. **1**).

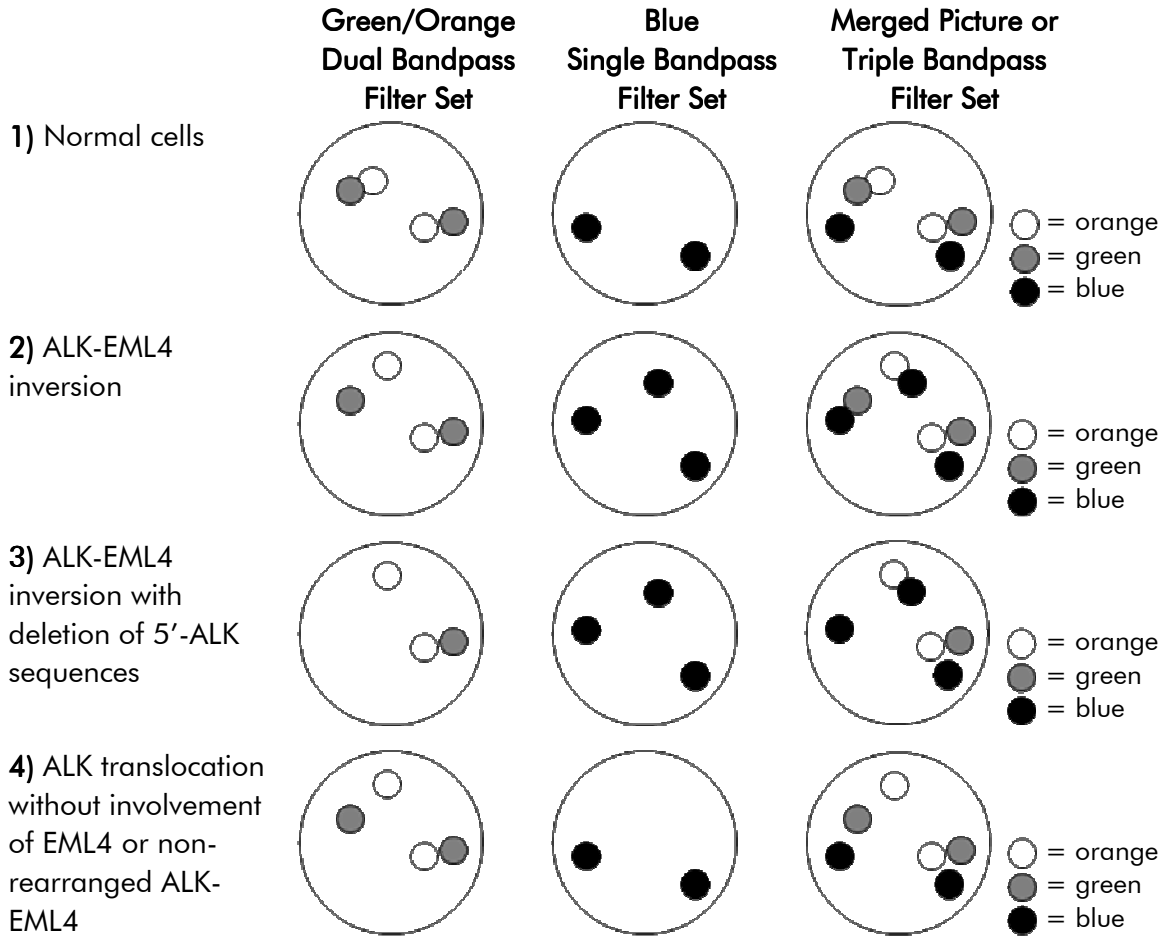
One 2p21-23 locus affected by an ALK-EML4 inversion is indicated by one separate green signal, one separate orange signal and an additional blue signal. The separate green and orange signals each co-localize with a blue signal (see fig. **2**).

One 2p21-23 locus affected by an ALK-EML4 inversion with deletion of 5'-ALK sequences is indicated by loss of one green signal and an additional blue signal. The orange signal co-localizes with a blue signal (see fig. **3**).

One separate green signal and one separate orange signal in combination with a normal number of blue signals (see fig. **4**) indicates either an ALK translocation without involvement of EML4 or, if the separate green and orange signals are located in close proximity, a non-rearranged 2p21-23 locus.

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 ALK-EML4 status 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

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As of: August 5, 2011 (4.6)

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