Zyto*Light*
SPEC ERG/TMPRSS2 TriCheck™
Probe

REF Z-2135-200 Σ 20 (0.2 ml)

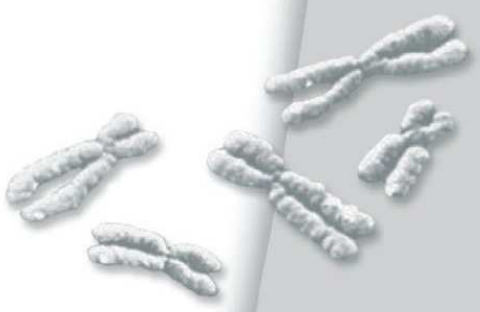
For the detection of ERG-TMPRSS2 rearrangements
by fluorescence *in situ* hybridization (FISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



Fluorescence-labeled polynucleotide probe for the detection of ERG-TMPRSS2 rearrangements, ready to use

Product Description

Content: ZytoLight SPEC ERG/TMPRSS2 TriCheck™ Probe **(PL92)** 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 21q22.2 distal to the ERG breakpoint region, orange-labeled polynucleotides (ZyOrange: excitation at 547 nm and emission at 572 nm, similar to rhodamine), which target sequences mapping in 21q22.13-q22.2 proximal to the ERG breakpoint region, and blue-labeled poly-nucleotides (ZyBlue: excitation at 418 nm and emission at 467 nm, similar to DEAC), which target sequences mapping in 21q22.3 distal to the TMPRSS2 break-point region.

Product: Z-2135-200: 0.2 ml (20 reactions of 10 µl each)

Specificity: The ZytoLight SPEC ERG/TMPRSS2 TriCheck™ Probe **(PL92)** is designed to be used for the detection of rearrangements involving the ERG gene at 21q22.2 and the TMPRSS2 gene at 21q22.3 in formalin-fixed, paraffin-embedded tissue or cells by fluorescence *in situ* hybridization (FISH).

Storage/Stability: The ZytoLight SPEC ERG/TMPRSS2 TriCheck™ Probe **(PL92)** must be stored at 2...8°C protected from light 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). Inter-

pretation 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 the chromosomal regions 21q22.13-q22.3 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 Zyto*Light* SPEC ERG/TMPRSS2 TriCheck™ Probe **(PL92)** 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 Zyto*Light* FISH system by ZytoVision. These systems were also used for the confirmation of appropriateness of the Zyto*Light* SPEC ERG/TMPRSS2 TriCheck™ Probe **(PL92)**.

Results

With the use of appropriate filter sets, the hybridization signals of the labeled ERG gene (21q22.2) appear green and orange, the hybridization signals of the labeled TMPRSS2 gene (21q22.3) appear blue.

In interphases of normal cells or cells without ERG-TMPRSS2 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).

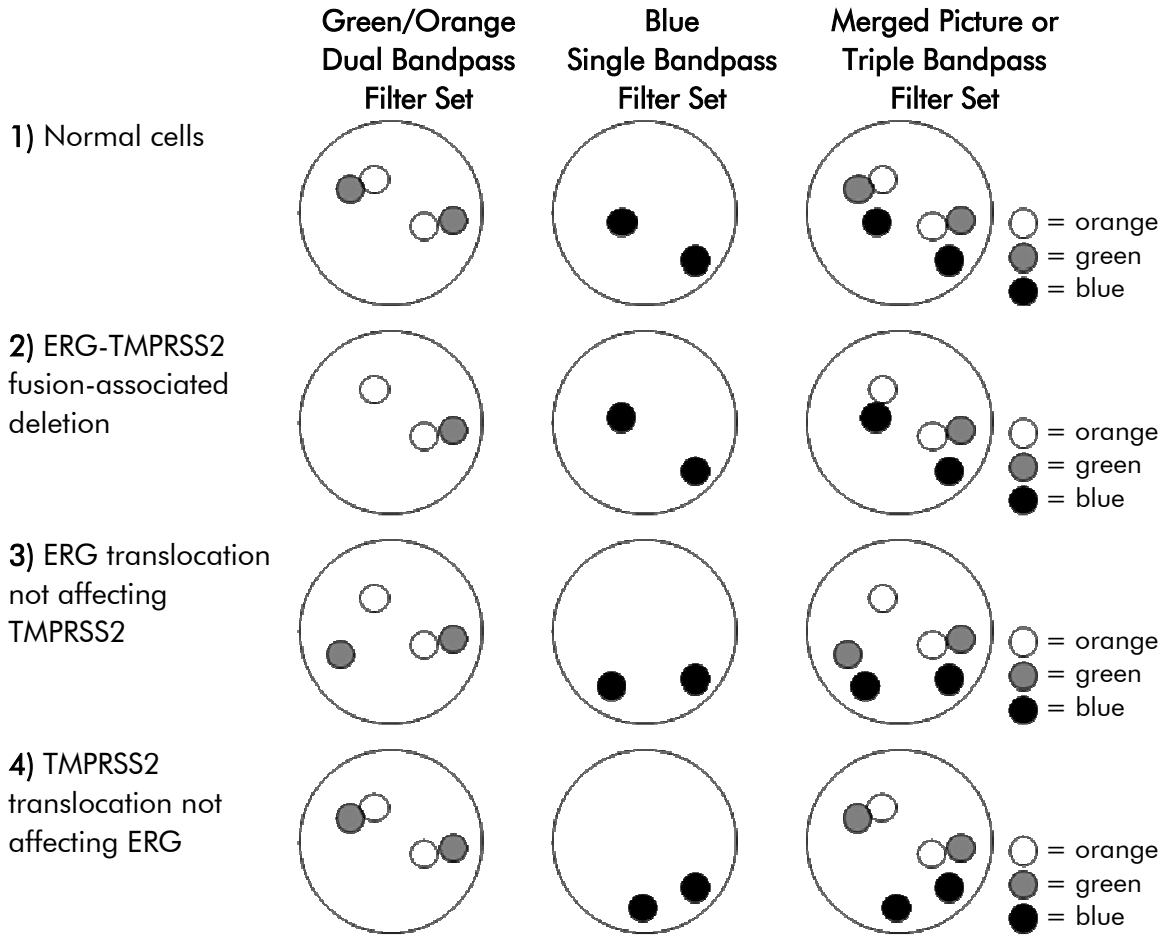
One 21q22.13-q22.3 locus affected by a 21q22.2 deletion resulting in the TMPRSS2-ERG fusion is indicated by one separate orange signal co-localizing with one blue signal and the loss of one green signal (see fig. 2).

An ERG translocation without involvement of TMPRSS2 is indicated by the split of one green/orange fusion signal on the derivative chromosome resulting in one separate green signal, in close proximity to one blue signal, and one separate orange signal (see fig. 3).

A TMPRSS2 translocation without involvement of ERG is indicated by one separate blue signal not co-localizing with the green/orange fusion signal (see fig. 4).

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 ERG-TMPRSS2 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: November 14, 2014 (5.2)

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