

ZytoDot 2C
SPEC PTEN/CEN 10 Probe

REF C-3053-400

Σ 40 (0.4 ml)

For the detection of the human PTEN gene and alpha-satellites of chromosome 10 by chromogenic *in situ* hybridization (CISH)



IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



Digoxigenin and DNP labeled polynucleotide probe for the detection of the human PTEN gene and alpha-satellites of chromosome 10 centromeres by CISH, ready to use

Product Description

- Content:** ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33) in hybridization buffer. The probe contains digoxigenin-labeled polynucleotides, which target sequences of the PTEN gene and DNP-labeled polynucleotides, which target alpha-satellites of the centromere of chromosome 10.
- Product:** C-3053-400: 0.4 ml (40 reactions of 10 µl each)
- Specificity:** The ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33) is designed to be used for the detection of the human PTEN gene as well as chromosome 10 alpha-satellites in formalin-fixed, paraffin-embedded tissue or cells by chromogenic *in situ* hybridization (CISH).
- Storage/Stability:** The ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33) must be stored at 2...8°C 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 of the labeled probe (with sequences of PTEN and chromosome 10 in the test material) can be visualized using primary (unmarked) antibodies, which are detected by secondary polymerized enzyme-conjugated antibodies. The enzymatic reactions of chromogenic substrates lead to the formation of colored signals that can be visualized by light microscopy.

Instructions

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

Denaturation and hybridization of probe:

- 1.** Vortex the ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33) and pipette 10 μ l each onto individual samples

Distribute dropwise on the whole target area to avoid local concentration of probe. Alternatively, add probe to the center of a coverslip and place it upside down on target area. 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.

- 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 78-80°C for 5 min, e.g. on a hot plate

- 4.** Transfer the slides 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, detection, and counter-staining, can be completed according to the user's needs. For a particularly user-friendly performance, we recommend the use of a ZytoDot 2C CISH system by ZytoVision. These systems were also used for the confirmation of appropriateness of the ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33).

Results

In an interphase nucleus of normal cells or cells without aberrations of chromosome 10, two PTEN and two centromere 10 specific punctual signals appear which can be clearly distinguished from the background (color and appearance of the signals depend on the detection system that is used). In cells with a gene deletion a reduced number of gene specific signals are visible.

Due to mitosis, additional signals may be visible even in a small percentage of non-neoplastic cells. Occasionally, nuclei with missing signals may be observed in paraffin-embedded tissue sections.

The polynucleotides contained in the ZytoDot 2C SPEC PTEN/CEN 10 Probe (PD33) which recognize the alpha-satellite-sequences of the centromere of chromosome 10 function in themselves as an internal control that a successful hybridization has occurred, as well as proving the integrity of the cellular DNA.

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 10 and PTEN gene copy number is known.

Our experts are available to answer your questions.

Literature

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