ZytoDot 2C
SPEC 19q13/19p13 Probe

Optimized for use with Clear-it™ Stringency Buffer by ZytoVision

REF C-3037-400  ▼  40 (0.4 ml)
REF C-3037-100  ▼  10 (0.1 ml)

For the detection of human 19q13 and 19p13 specific sequences by chromogenic in situ hybridization (CISH)

CE

In vitro diagnostic medical device
according to EU directive 98/79/EC
Digoxigenin and DNP labeled polynucleotide probe for the detection of human chromosome 19q13 and 19p13 specific sequences by CISH, ready to use

Product Description

Content: ZytoDot 2C SPEC 19q13/19p13 Probe (PD22) in hybridization buffer. The probe contains DNP-labeled polynucleotides, which target chromosome 19q13 specific sequences and digoxigenin-labeled polynucleotides, which target chromosome 19p13 specific sequences.

Product: C-3037-400: 0.4 ml (40 reactions of 10 µl each)  
C-3037-100: 0.1 ml (10 reactions of 10 µl each)

Specificity: The ZytoDot 2C SPEC 19q13/19p13 Probe (PD22) is designed to be used for the detection of chromosome 19q13 and 19p13 specific sequences in formalin-fixed, paraffin-embedded tissue or cells by chromogenic in situ hybridization (CISH).

Storage/Stability: The ZytoDot 2C SPEC 19q13/19p13 Probe (PD22) 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 the 19q13 and 19p13 regions 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 19q13/19p13 Probe (PD22)* and pipette 10 µl 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. For improved post-hybridization stringency wash the probe was optimized for use with the *Clear-it™ Stringency Buffer (WB9)* by ZytoVision (separately available, prod. no. WB-0009-500) replacing the Wash Buffer SSC (WB1) in the *ZytoDot 2C CISH system*. These systems were also used for the confirmation of appropriateness of the *ZytoDot 2C SPEC 19q13/19p13 Probe (PD22).*
Results

In an interphase nucleus of normal cells or cells without aberrations of chromosome 19, two red 19q13 signals and two green 19p13 signals, which can be clearly distinguished from the background, appear when using a ZytoDot 2C CISH detection system by ZytoVision. Overlapping signals occasionally can be seen as dark dots of undefined color. In cells with a deletion of the 19q13 locus, a different signal pattern is visible in interphases. Color and appearance of the signals may vary when a different detection system is used.

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 19q13/19p13 Probe (PD22) which recognize the 19p13 chromosomal region 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 19 copy number is known.

Our experts are available to answer your questions.
Literature


As of: May 24, 2013 (5.7)

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