Zyto*Dot* 2C
SPEC DDIT3 Break Apart Probe

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

REF C-3047-100 Σ 10 (0.1 ml)

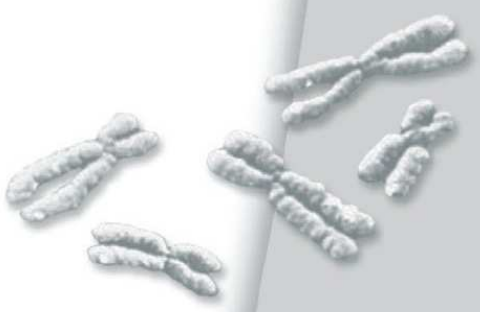
For the detection of translocations involving the DDIT3 gene at 12q13.3 by chromogenic *in situ* hybridization (CISH)

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



Digoxigenin and DNP labeled polynucleotide probe for the detection of translocations involving the DDIT3 gene at 12q13.3 by CISH, ready to use

Product Description

Content: ZytoDot 2C SPEC DDIT3 Break Apart Probe **(PD27)** in hybridization buffer. The probe contains digoxigenin-labeled polynucleotides, which target sequences mapping in 12q13.3-q14.1 distal to the DDIT3 gene and DNP-labeled polynucleotides, which target sequences mapping in 12q13.3 proximal to the DDIT3 gene.

Product: C-3047-100: 0.1 ml (10 reactions of 10 µl each)

Specificity: The ZytoDot 2C SPEC DDIT3 Break Apart Probe **(PD27)** is designed to be used for the detection of translocations involving the DDIT3 gene at 12q13.3 in formalin-fixed, paraffin-embedded tissue or cells by chromogenic *in situ* hybridization (CISH).

Storage/Stability: The ZytoDot 2C SPEC DDIT3 Break Apart Probe **(PD27)** 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 chromosomal region 12q13.3-q14.1 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 DDIT3 Break Apart Probe **(PD27)** 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 best results 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 DDIT3 Break Apart Probe **(PD27)**.

Results

In an interphase nucleus of normal cells or cells without a translocation involving the 12q13.3 band, two green/red fusion signals, which can be clearly distinguished from the background, appear when using a Zyto*Dot* 2C CISH detection system by ZytoVision. Overlapping signals occasionally can be seen as dark dots of undefined color. One 12q13.3 locus affected by a translocation is indicated by one separate green and one separate red signal. 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.

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 12q13.3 status is known.

Our experts are available to answer your questions.

Literature

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