

ZytoDot
SPEC CCND1 Probe

REF C-3034-400

▽ 40 (0.4 ml)

For the detection of the human CCND1 gene by
chromogenic *in situ* hybridization (CISH)

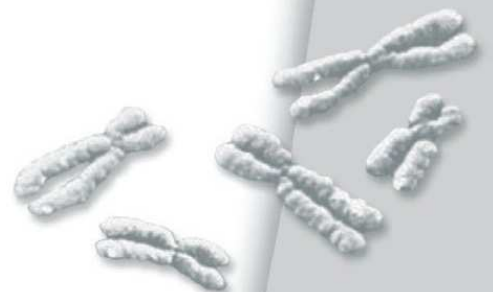
研究用です。診断用には
使用できません

In vitro diagnostic medical device

according to EU directive 98/79/EC



コスモ・バイオ株式会社
COSMO BIO Co., LTD.



Digoxigenin-labeled polynucleotide probe for the detection of
the human CCND1 gene by CISH,
ready to use

Product Description

- Content:** ZytoDot SPEC CCND1 Probe (PD19) in hybridization buffer. The probe contains labeled polynucleotides (digoxigenylated) which target sequences of the CCND1 gene.
- Product:** C-3034-400: 0.4 ml (40 reactions of 10 μ l each)
- Specificity:** The ZytoDot SPEC CCND1 Probe (PD19) is designed to be used for the detection of the human CCND1 gene in formalin-fixed, paraffin-embedded tissue or cells by chromogenic *in situ* hybridization (CISH).
- Storage/Stability:** The ZytoDot SPEC CCND1 Probe (PD19) must be stored at -16...-22°C (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 context 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 of the digoxigenin-labeled probe (with sequences of CCND1 in the test material) can be visualized using a primary (unmarked) anti-digoxigenin antibody, which is detected by a secondary polymerized enzyme-conjugated antibody. The enzymatic reaction of a chromogenic substrate leads to the formation of a color precipitate that is 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 SPEC CCND1 Probe (PD19) 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; 24 mm x 32 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 94-95°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 CISH system by ZytoVision. These systems were also used for the confirmation of appropriateness of the ZytoDot SPEC CCND1 Probe (PD19).

Results

In an interphase nucleus of normal cells or cells without aberrations of chromosome 11, two CCND1 specific punctual signals appear which can be clearly distinguished from the background. In cells with a gene amplification an increased number of gene specific signals or signal clusters 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.

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 11 and CCND1 gene copy number is known.

Our experts are available to answer your questions.

Literature

- Al-Kuraya K, et al. (2004) *Cancer Res* **64**: 8534-40.
- Courjal F, et al. (1997) *Cancer Res* **57**: 4360-7.
- Isola J, Tanner M (2004) *Methods Mol Med* **97**: 133-44.
- Motokura T, et al. (1991) *Nature* **350**: 512-5.
- Ormandy CJ, et al. (2003) *Breast Cancer Res Treat* **78**: 323-35.
- Speel EJ, et al. (1994) *J Histochem Cytochem* **42**: 1299-307.
- Schuuring E (1995) *Gene* **159**: 83-96.
- Tornillo L, et al. (2005) *Lab Invest* **85**: 921-31.
- Tsukamoto T, et al. (1991) *Int J Dev Biol* **35**: 25-32.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.
- Xiong Y, et al. (1991) *Cell* **65**: 691-9.

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