



ZytoDot 2C SPEC 19q13/19p13 Probe

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

For the qualitative detection of the human chromosome 19q13.32-q13.33 deletions and 19p13.3 specific sequences by chromogenic *in situ* hybridization (CISH)

4250380P124QQ



In vitro diagnostic medical device
according to IVDR (EU) 2017/746

1. Intended purpose

The ZytoDot 2C SPEC 19q13/19p13 Probe (PD22) is intended to be used for the qualitative detection of deletions involving the human chromosomal region 19q13.32-q13.33 as well as chromosome 19p13.3 specific sequences in formalin-fixed, paraffin-embedded specimens, such as glioma by chromogenic *in situ* hybridization (CISH). The probe is intended to be used in combination with the ZytoDot 2C CISH Implementation Kit (Prod. No. C-3044-10/-40).

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

The probe is intended to be used as an aid to the differential diagnosis of glioma and therapeutic measures should not be initiated based on the test result alone.

2. Test principle

The chromogenic *in situ* hybridization (CISH) technique allows the detection and visualization of specific nucleic acid sequences in cell preparations. Hapten-labeled nucleotide fragments, so called CISH probes, and their complementary target sequences in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unbound and unbound probe fragments are removed by stringency washing steps. Duplex formation of the labeled probe can be visualized using primary (unmarked) antibodies, which are detected by secondary polymerized enzyme-conjugated antibodies. The enzymatic reaction with chromogenic substrates leads to the formation of colored precipitates. After counterstaining the nucleus with a nuclear dye, hybridized probe fragments are visualized by light microscopy.

3. Reagents provided

The ZytoDot 2C SPEC 19q13/19p13 Probe is composed of:

- Digoxigenin-labeled polynucleotides (~0.8 ng/ μ l), which target sequences mapping in 19p13.3* (chr19:815,938-962,244) (see Fig. 1).
- Dinitrophenyl-labeled polynucleotides (~0.8 ng/ μ l), which target sequences mapping in 19q13.32-q13.33* (chr19:47,857,776-48,339,398) (see Fig. 1).
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

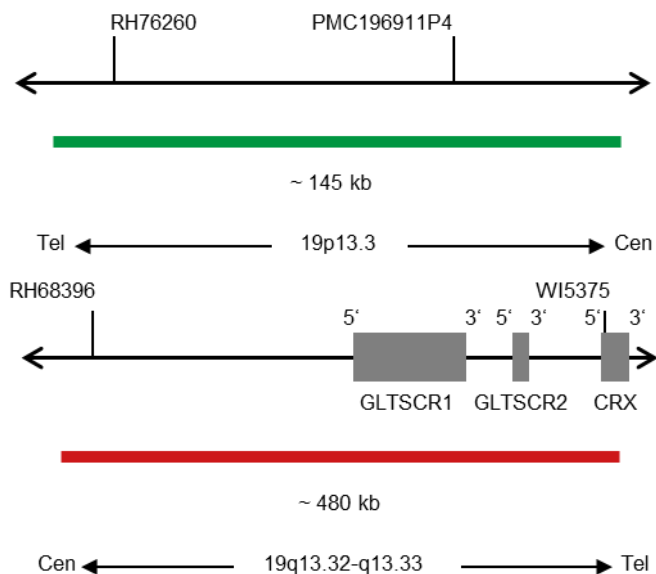


Fig. 1: Top: SPEC 19p13 Probe map; Bottom: SPEC 19q13 Probe map (not to scale)

The ZytoDot 2C SPEC 19q13/19p13 Probe is available in two sizes:

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

4. Materials required but not provided

- ZytoDot 2C CISH Implementation Kit (Prod. No. C-3044-10/-40)
- Positive and negative control specimens
- Microscope slides, positively charged
- Water bath (80 °C, 98 °C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable pipettes (10 μ l, 1000 μ l)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene
- Methanol 100%
- Hydrogen peroxide (H₂O₂) 30%
- Deionized or distilled water
- Coverslips (22 mm x 22 mm, 24 mm x 32 mm)
- Rubber cement, e.g., Fixogum Rubber Cement (Prod. No. E-4005-50/-125) or similar
- Adequately maintained light microscope (400-630x)

5. Storage and handling

Store at 2-8 °C in an upright position. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and precautions

- Read the instructions for use 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 and potentially infectious. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- The specimens must not be allowed to dry during the hybridization and washing steps.

Hazard and precautionary statements:

The hazard-determining component is formamide.



Danger

| | |
|-----------|--|
| H351 | Suspected of causing cancer. |
| H360FD | May damage fertility. May damage the unborn child. |
| H373 | May cause damage to organs through prolonged or repeated exposure. |
| P201 | Obtain special instructions before use. |
| P202 | Do not handle until all safety precautions have been read and understood. |
| P260 | Do not breathe dust/fume/gas/mist/vapours/spray. |
| P280 | Wear protective gloves/protective clothing/eye protection/face protection. |
| P308+P313 | IF exposed or concerned: Get medical advice/attention. |
| P405 | Store locked up. |

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated use only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist/human geneticist to be familiar with the CISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist/human geneticist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.
- The probe should be used only for detecting loci described in chapter 3. "Reagents provided".
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

8. Interfering substances

The following fixatives are incompatible with ISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin

9. Preparation of specimens

Prepare specimens as described in the instructions for use of the [ZytoDot 2C CISH Implementation Kit](#).

10. Preparatory treatment of the device

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25 °C) before use, protect from light. Prior to opening the vial, mix by vortexing and spin down briefly.

11. Assay procedure

Specimen pretreatment

Perform specimen pretreatment (e.g., dewaxing, proteolysis) according to the instructions for use of the [ZytoDot 2C CISH Implementation Kit](#).

Denaturation and hybridization

1. Pipette 10 µl of the probe onto each pretreated specimen.
2. Cover specimens with a 22 mm x 22 mm coverslip (avoid trapped bubbles) and seal the coverslip.

We recommend using rubber cement (e.g., Fixogum) for sealing.

3. Place slides on a hot plate or hybridizer and denature specimens for 5 min at 79 °C.
4. Transfer slides to a humidity chamber and hybridize overnight at 37 °C (e.g., in a hybridization oven).

It is essential that specimens do not dry out during the hybridization step.

Post-hybridization

Perform post-hybridization processing (washing, detection, counterstaining, mounting, microscopy) according to the instructions for use of the [ZytoDot 2C CISH Implementation Kit](#).

12. Interpretation of results

Using the [ZytoDot 2C CISH Implementation Kit](#), hybridization signals of Digoxigenin-labeled polynucleotides appear as dark green colored distinct dots (19p13 locus), and Dinitrophenyl-labeled polynucleotides appear as bright red colored distinct dots (19q13 locus).

Normal situation: In interphases of normal cells or cells without deletion involving the 19q13 locus, two distinct dot-shaped red and two distinct dot-shaped green signals appear (see Fig. 2).

Aberrant situation: In a cell with deletion affecting the 19q13 locus, a reduced number of red signals will be observed. Deletions affecting only parts of the 19q13 locus might result in a normal signal pattern with red signals of reduced size (see Fig. 2).

Overlapping signals may appear as brown signals.

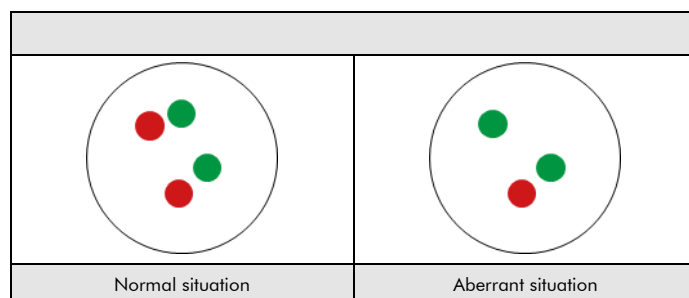


Fig. 2: Expected results in normal and aberrant nuclei

Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

Please note:

- Due to decondensed chromatin, single CISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance ≤ 1 signal diameter, should be counted as one signal.
- Prior to signal enumeration, the specimen should be scanned for any possible intratumoral heterogeneity at 100- to 200-fold magnification.
- Visualization of signals should be performed at least at 400-fold magnification resulting in easily visible signals. A 630-fold magnification is recommended for probes detecting chromosomal breaks. Do not use contrast enhancing filter lenses as this might distort the signal color. To obtain signals in bright colors, open the aperture diaphragm. Be sure to focus up and down when evaluating a nucleus, as red and green signals might be located on top of each other.
- Do not evaluate areas of necrosis, overlapping nuclei, over-digested nuclei and nuclei with weak signal intensity.
- 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 specimens due to cutting artefacts.
- A negative or unspecific result can be caused by multiple factors (see chapter 16 "Troubleshooting").
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

13. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by internal and external controls. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

Internal control: Non-neoplastic cells within the specimen that exhibit normal signal pattern, e.g., fibroblasts.

External control: Validated positive and negative control specimens.

14. Performance characteristics

14.1 Analytical performance

The performance of the probe was determined by comparison against the corresponding IVD approved FISH probe.

| | |
|--------------------------------|----------------------------|
| Analytical sensitivity: | 100% (95% CI 98.5 – 100.0) |
| Analytical specificity: | 100% (95% CI 97.0 – 100.0) |

Day-to-Day-Reproducibility was tested comparing the results obtained from one examiner on 10 different days and evaluating the agreement.

| | Percent Agreement |
|-------------------|--------------------------|
| negative specimen | 100% |
| positive specimen | 100% |

Repeatability was tested comparing the replicated results obtained from one examiner on 10 different days and evaluating the agreement.

| | Percent Agreement |
|-------------------|--------------------------|
| negative specimen | 100% |
| positive specimen | 100% |

14.2 Clinical performance

| | |
|--------------------------------|-------------------------------------|
| Diagnostic sensitivity: | 100% (95% CI 91.4 – 100.0) vs. FISH |
| Diagnostic specificity: | 100% (95% CI 91.4 – 100.0) vs. FISH |

15. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to www.zytovision.com for more information.

Signals fade or merge

| Possible cause | Action |
|---|---|
| An unsuitable mounting solution has been used | Use only the mounting solution provided with the kit or xylene-based mounting solutions free of any impurities; do not use coverslip tape |
| Sections were not dehydrated properly | Use fresh ethanol and xylene solutions; use only xylene of "pure" quality |

Uneven or in some parts only very light staining

| Possible cause | Action |
|--------------------------|---|
| Incomplete dewaxing | Use fresh solutions; check duration of dewaxing times |
| Reagent volume too small | Ensure that the reagent volume is large enough to cover the tissue area |

Inconsistent results

| Possible cause | Action |
|---|--|
| Insufficient drying before probe application | Extend air-drying |
| Too much water/wash buffer on tissue prior to application of pepsin, antibodies and/or color substrates | Ensure that excess liquid is removed from tissue section by blotting or shaking it off the slide. Small amounts of residual water/wash buffer do not interfere with the test |
| Variations in tissue fixation and embedding methods | Optimize fixation and embedding methods |
| Variations in tissue section thickness | Optimize sectioning |

Morphology degraded

| Possible cause | Action |
|---|-----------------------------------|
| Cell or tissue sample has not been properly fixed | Optimize fixing time and fixative |
| Proteolytic pretreatment not carried out too long | Decrease pepsin incubation time |

Cross hybridization signals; noisy background

| Possible cause | Action |
|---|---|
| Sections dried out any time during or after hybridization | Avoid sections being dried out; use humidity chamber; seal coverslip properly |
| Prolonged substrate incubation time | Shorten substrate incubation time |
| Incomplete dewaxing | Use fresh solutions; check duration of dewaxing |
| Proteolytic pretreatment too strong | Optimize pepsin incubation time |
| Slides cooled to room temperature before hybridization | Transfer the slides quickly to hybridization temperature |

Overlapping signals

| Possible cause | Action |
|--|--|
| Inappropriate thickness of tissue sections | Prepare 3-5 μm microtome sections |

Specimen floats off the slide

| Possible cause | Action |
|-------------------------------------|--------------------------------|
| Proteolytic pretreatment too strong | Shorten pepsin incubation time |

17. Literature

- Lass U, et al. (2013) *Brain Pathol* 23: 311-8.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

18. Revision

| Revision | Description of the change |
|----------|--|
| 5.1.1 | Change of notified body 14. Performance characteristics add accuracy |



www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions. Please contact helptech@zytovision.com. For the summary of safety and performance, please refer to www.zytovision.com.



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