ZytoVision

ZytoDot 2C
Glioma 1p/19q Probe Set

REF	C-3076-10	Σ	10
REF	C-3076-40	Σ	40

For the qualitative detection of deletions involving the human chromosomal region 1p36.31 as well as deletions involving the human chromosomal region 19q13.32-q13.33 by chromogenic *in situ* hybridization (CISH)

4250380N9183

CE 0483

IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The ZytoDot 2C Glioma 1p/19q Probe Set is intended to be used for the qualitative detection of deletions involving the human chromosomal region 1p36.31 as well as deletions involving the human chromosomal region 19q13.32-q13.33 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, unspecific 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 Glioma 1p/19q Probe Set is a set comprising two separate probes:

- ZytoDot 2C SPEC 1p36/1q25 Probe (Prod. No. C-3036-100/-400)
- ZytoDot 2C SPEC 19q13/19p13 Probe (Prod. No. C-3037-100/-400)

The ZytoDot 2C SPEC 1p36/1q25 Probe (**PD21**) is composed of:

- Digoxigenin-labeled polynucleotides (~1.7 ng/µl), which target sequences mapping in 1q25.3* (chr1:184,562,510-184,752,938) (see Fig. 1).
- Dinitrophenyl-labeled polynucleotides (~1.7 ng/µl), which target sequences mapping in 1p36.31* (chr1:5,808,946-6,176,336) (see Fig. 1).
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

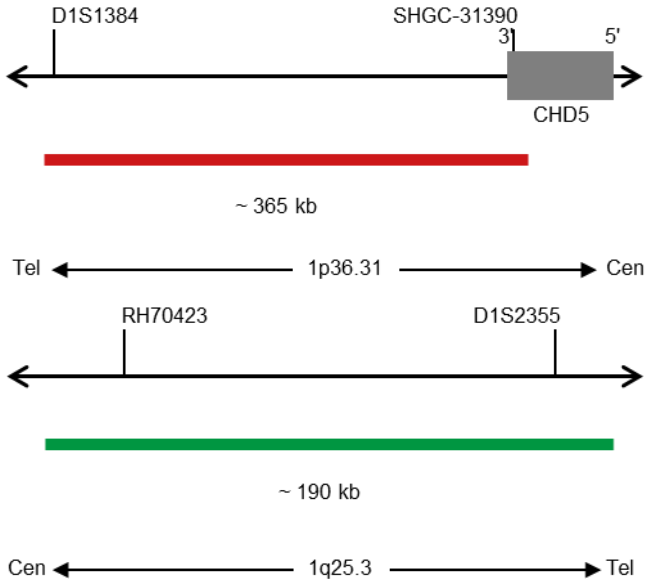

Fig. 1: Top: SPEC 1p36 Probe map; Bottom: SPEC 1q25 Probe map (not to scale)

The ZytoDot 2C SPEC 19q13/19p13 Probe (**PD22**) is composed of:

- Digoxigenin-labeled polynucleotides (~0.8 ng/µl), which target sequences mapping in 19p13.3* (chr19:815,938-962,244) (see Fig. 2).
- Dinitrophenyl-labeled polynucleotides (~0.8 ng/µl), which target sequences mapping in 19q13.32-q13.33* (chr19:47,857,776-48,339,398) (see Fig. 2).
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

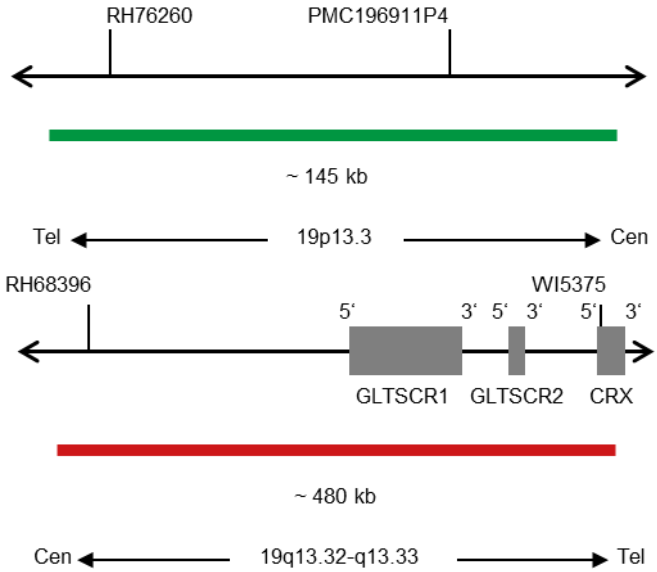

Fig. 2: Top: SPEC 19q13 Probe map; Bottom: SPEC 19p13 Probe map (not to scale)

The ZytoDot 2C Glioma 1p/19q Probe Set is available in two sizes:

- C-3076-10: The individual probes are sufficient for 10 reactions of 10 µl each.
- C-3076-40: The individual probes are sufficient for 40 reactions of 10 µl 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 µl, 1000 µl)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene
- Methanol 100%
- Hydrogen peroxide (H_2O_2) 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, counter-staining, mounting, microscopy) according to the instructions for use of the ZytoDot 2C CISH Implementation Kit.

12. Interpretation of results

ZytoDot 2C SPEC 1p36/1q25 Probe:

Using the ZytoDot 2C CISH Implementation Kit, hybridization signals of Digoxigenin-labeled polynucleotides appear as dark green colored distinct dots (1q25 locus), and Dinitrophenyl-labeled polynucleotides appear as bright red colored distinct dots (1p36 locus).

Normal situation: In interphases of normal cells or cells without deletion involving the 1p36 locus, two distinct dot-shaped red and two distinct dot-shaped green signals appear (see Fig. 3).

Aberrant situation: In a cell with deletion affecting the 1p36 locus, a reduced number of red signals will be observed. Deletions affecting only parts of the 1p36 locus might result in a normal signal pattern with red signals of reduced size (see Fig. 3).

Overlapping signals may appear as brown signals.

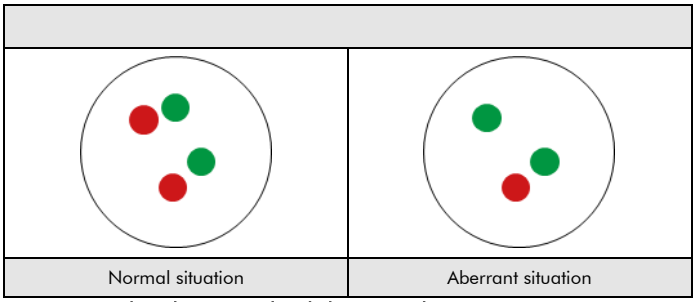

Fig. 3: Expected results in normal and aberrant nuclei

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

ZytoDot 2C SPEC 19q13/19p13 Probe:

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. 4).

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. 4).

Overlapping signals may appear as brown signals.

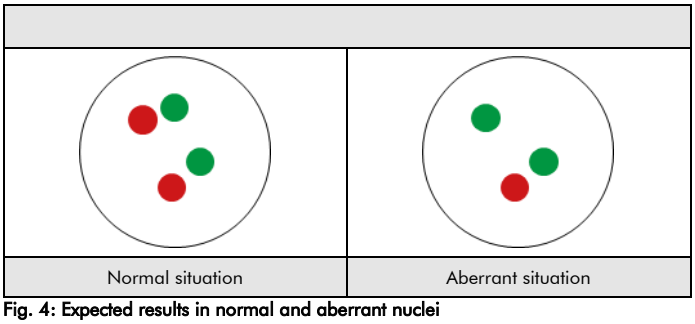

Fig. 4: Expected results in normal and aberrant nuclei

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

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

Day-to-Day-Reproducability 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:	**ZytoDot 2C SPEC 1p36/1q25 Probe**: 100% (95% CI 91.6 – 100.0) vs FISH **ZytoDot 2C SPEC 19q13/19p13 Probe:** 100% (95% CI 91.4 – 100.0) vs. FISH
Diagnostic specificity:	**ZytoDot 2C SPEC 1p36/1q25 Probe:** 100% (95% CI 91.6 – 100.0) vs FISH **ZytoDot 2C SPEC 19q13/19p13 Probe:** 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.

Weak signals or no signals at all

Possible cause	Action
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
Probe evaporation	When using a hybridizer, the use of the wet stripes/water filled tanks is mandatory. When using a hybridization oven, the use of a humidity chamber is required. In addition, the coverslip should be sealed completely, e.g., with Fixogum, to prevent drying-out of the sample during hybridization
Counterstaining time too long	Avoid dark counterstaining, because it may obscure positive staining signals
Bluing of counterstain not carried out properly	Use cold running tap water for bluing; do not use warm or hot water, or bluing reagents

Signals too strong

Possible cause	Action
Proteolytic pretreatment carried out too long	Optimize pepsin incubation time, increase or decrease if necessary
AP-Red Solution incubation time not correct	If required, the incubation time can be shortened down to 5 min. Do not heat substrate solution over 25 °C; incubate at room temperature only
HRP-Green solution incubation time not correct	If required, the incubation time can be shortened down to 7 min. Do not heat substrate solution over 25 °C; incubate at room temperature only

Red signals too weak

Possible cause	Action
AP-Red Solution was exposed to strong direct light	Prepare and use AP-Red Solution protected from strong direct light
AP-Red Solution was prepared too early	Prepare prior to immediate use
AP-Red Solution incubation time not correct	If required, the incubation time can be extended up to 15 min
Insufficient preparation of chromogenic substrate	Do not increase volume of Solution A

Green signals too weak

Possible cause	Action
Incubation time of any washing steps after staining with HRP-Green too long	Do not exceed given incubation times
HRP-Green solution incubation time not correct	If required, the incubation time can be extended up to15 min
Insufficient preparation of chromogenic substrate	Do not increase volume of Solution A

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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