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.

4. Reagents provided
The **ZytoDot 2C SPEC FGFR2/CEN 10 Probe** is composed of:

- Digoxigenin-labeled polynucleotides (~1.1 ng/µl), which target sequences mapping in 10q26.12-10q26.13* (chr10:123,080,085-123,492,398) harboring the FGFR2 gene region (see Fig. 1).
- Dinitrophenyl-labeled polynucleotides (~1.1 ng/µl), which target sequences mapping in 10p11.1-q11.1 specific for the alpha satellite centromeric region D10Z1 of chromosome 10.
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

5. Materials required but not provided
- **ZytoDot 2C CISH Implementation Kit** (Prod. No. E-3044-10/-40)
- 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 (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)

6. 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.

7. 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)!
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.

8. 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 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 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 4. “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.

9. 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
- Holland’s fixative
- Non-buffered formalin

10. Preparation of specimens
Recommendations:
- Avoid cross-contamination of samples in any step of preparation as this may lead to erroneous results.
- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Sample size ≤ 0.5 cm².
- Use premium quality paraffin.
- Embedding should be carried out at temperatures lower than 65°C.
- Prepare 3-5 µm microtome sections.
- Use positively charged microscope slides.
- Fix tissue sections for 2-16 h at 50-60°C.

11. 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) and mix briefly before use.

12. Assay procedure
Specimen pretreatment
Perform specimen pretreatment (e.g., dewaxing, proteolysis) according to the instructions for use of the ZytocDot 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 ZytocDot 2C CISH Implementation Kit.

13. Interpretation of results
Using the ZytocDot 2C CISH Implementation Kit, hybridization signals of Digoxigenin-labeled polynucleotides appear as dark green colored distinct dots (FGFR2 gene region), and Dinitrophenyl-labeled polynucleotides appear as bright red colored distinct dots (CEN 10).

Normal situation: In interphases of normal cells or cells without an amplification involving the FGFR2 gene region, two distinct dot-shaped green and two distinct dot-shaped red signals appear (see Fig. 2).

Aberant situation: In cells with an amplification of the FGFR2 gene region, an increased number of green signals or green signal clusters will be observed (see Fig. 2).
14. **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.

15. **Performance characteristics**

The performance of the probe was determined by comparison against the corresponding IVD approved FISH probe. The concordance was 100%.

**Accuracy**: The accuracy was calculated as 100%.

**Analytical sensitivity**: The analytical sensitivity was calculated as 100%.

**Analytical specificity**: The analytical specificity was calculated as 100%.

16. **Disposal**

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

17. **Troubleshooting**

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

<table>
<thead>
<tr>
<th><strong>Weak signals or no signals at all</strong></th>
<th><strong>Possible cause</strong></th>
<th><strong>Action</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell or tissue sample has not been properly fixed</td>
<td>Optimize fixing time and fixative</td>
<td></td>
</tr>
<tr>
<td>Heat pretreatment, proteolysis, hybridization, denaturation, stringency wash or antibody-incubation temperature not correct</td>
<td>Check temperature of all technical devices used, using a calibrated thermometer. Use always the same number of slides in solutions with critical temperature</td>
<td></td>
</tr>
<tr>
<td>Proteolytic pretreatment not carried out properly</td>
<td>Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. Ascertain the optimum time for pepsin incubation in pre-tests</td>
<td></td>
</tr>
<tr>
<td>Hybridization time too short</td>
<td>Hybrdize for at least 12 h; extend hybridization time if necessary</td>
<td></td>
</tr>
<tr>
<td>Old dehydration solutions</td>
<td>Prepare fresh dehydration solutions</td>
<td></td>
</tr>
<tr>
<td>Probe evaporation</td>
<td>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 Fixaqum, to prevent drying-out of the sample during hybridization</td>
<td></td>
</tr>
<tr>
<td><strong>Signals too strong</strong></td>
<td><strong>Possible cause</strong></td>
<td><strong>Action</strong></td>
</tr>
<tr>
<td>Proteolytic pretreatment carried out too long</td>
<td>Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. Ascertain the optimum time for pepsin incubation in pre-tests</td>
<td></td>
</tr>
<tr>
<td>AP-Red Solution incubation time not correct</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>HRP-Green solution incubation time not correct</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>

**Red signals too weak**

<table>
<thead>
<tr>
<th><strong>Possible cause</strong></th>
<th><strong>Action</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-Red Solution was exposed to strong direct light</td>
<td>Prepare and use AP-Red Solution protected from strong direct light</td>
</tr>
<tr>
<td>AP-Red Solution was prepared too early</td>
<td>Prepare prior to immediate use</td>
</tr>
<tr>
<td>AP-Red Solution incubation time not correct</td>
<td>If required, the incubation time can be extended up to 15 min</td>
</tr>
</tbody>
</table>
Inconsistent results

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient preparation of chromogenic substrate</td>
<td>Do not increase volume of Solution A</td>
</tr>
<tr>
<td>Green signals too weak</td>
<td></td>
</tr>
<tr>
<td>Possible cause</td>
<td>Action</td>
</tr>
<tr>
<td>Incubation time of any washing steps after staining with HRP-Green too long</td>
<td>Do not exceed given incubation times</td>
</tr>
<tr>
<td>HRP-Green solution incubation time not correct</td>
<td>If required, the incubation time can be extended up to 15 min</td>
</tr>
<tr>
<td>Insufficient preparation of chromogenic substrate</td>
<td>Do not increase volume of Solution A</td>
</tr>
<tr>
<td>Signals fade or merge</td>
<td></td>
</tr>
<tr>
<td>Possible cause</td>
<td>Action</td>
</tr>
<tr>
<td>An unsuitable mounting solution has been used</td>
<td>Use only the mounting solution provided with the kit or xylene-based mounting solutions free of any impurities; do not use coverslip tape</td>
</tr>
<tr>
<td>Sections were not dehydrated properly</td>
<td>Use fresh ethanol and xylene solutions; use only xylene of “pure” quality</td>
</tr>
<tr>
<td>Uneven or in some parts only very light staining</td>
<td></td>
</tr>
<tr>
<td>Possible cause</td>
<td>Action</td>
</tr>
<tr>
<td>Incomplete dewaxing</td>
<td>Use fresh solutions; check duration of dewaxing times</td>
</tr>
<tr>
<td>Reagent volume too small</td>
<td>Ensure that the reagent volume is large enough to cover the tissue area</td>
</tr>
<tr>
<td>Air bubbles caught before hybridization or during mounting</td>
<td>Avoid air bubbles</td>
</tr>
<tr>
<td>Inconsistency of hybridization signals; noisy background</td>
<td></td>
</tr>
<tr>
<td>Possible cause</td>
<td>Action</td>
</tr>
<tr>
<td>Stringency wash temperature not correct</td>
<td>Check temperature of the technical devices used, using a calibrated thermometer. Use always the same number of slides in the jar. We recommend not to use more than eight slides per jar for heat incubation steps</td>
</tr>
<tr>
<td>Slides not thoroughly rinsed</td>
<td>Use fresh and sufficient wash buffer and deionized or distilled water where indicated</td>
</tr>
</tbody>
</table>

Specimen floats off the slide

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsuitable slide coating</td>
<td>Use appropriate (positively charged) slides</td>
</tr>
<tr>
<td>Proteolytic pretreatment too strong</td>
<td>Shorten pepsin incubation time</td>
</tr>
</tbody>
</table>

Overlapping signals

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inappropriate thickness of tissue sections</td>
<td>Prepare 3-5 µm microtome sections</td>
</tr>
</tbody>
</table>

18. Literature


19. Revision

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

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