For the qualitative detection of Digoxigenin/Dinitrophenyl-labeled ZytoDot 2C Probes by chromogenic in situ hybridization (CISH)

4. Reagents provided

The ZytoDot 2C Polymer Detection Kit is available in one size and is composed of:

<table>
<thead>
<tr>
<th>Code</th>
<th>Component</th>
<th>Quantity</th>
<th>Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB5</td>
<td>2x Wash Buffer TBS</td>
<td>2x 50 ml</td>
<td>Screw-cap bottle</td>
</tr>
<tr>
<td>AB14</td>
<td>Anti-DIG/DNP-Mix</td>
<td>4 ml</td>
<td>Dropper bottle, yellow cap</td>
</tr>
<tr>
<td>AB13</td>
<td>HRP/AP-Polymer-Mix</td>
<td>4 ml</td>
<td>Dropper bottle, blue cap</td>
</tr>
<tr>
<td>SB6a</td>
<td>AP-Red Solution A</td>
<td>0.4 ml</td>
<td>Dropper bottle, red cap (small)</td>
</tr>
<tr>
<td>SB6b</td>
<td>AP-Red Solution B</td>
<td>15 ml</td>
<td>Dropper bottle, red cap</td>
</tr>
<tr>
<td>SB7a</td>
<td>HRP-Green Solution A</td>
<td>0.8 ml</td>
<td>Dropper bottle, green cap (small)</td>
</tr>
<tr>
<td>SB7b</td>
<td>HRP-Green Solution B</td>
<td>15 ml</td>
<td>Dropper bottle, green cap</td>
</tr>
<tr>
<td>CS2</td>
<td>Nuclear Blue Solution</td>
<td>20 ml</td>
<td>Screw-cap bottle, black</td>
</tr>
<tr>
<td>MT4</td>
<td>Mounting Solution (alcoholic)</td>
<td>4 ml</td>
<td>Glass bottle, brown</td>
</tr>
<tr>
<td></td>
<td>AP-Red reaction vessel</td>
<td>2</td>
<td>Graduated cup, red lid</td>
</tr>
<tr>
<td></td>
<td>HRP-Green reaction vessel</td>
<td>2</td>
<td>Graduated cup, green lid</td>
</tr>
<tr>
<td></td>
<td>Instructions for use</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

C-3028-40 (40 tests): Components AB14, AB13, SB6a-b, SB7a-b, CS2, and MT4 are sufficient for 40 reactions. Component WB5 is sufficient for 28 staining jars of 70 ml each.

5. Materials required but not provided

- ZytoDot 2C CISH Probe
- 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 (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)

The ZytoDot 2C CISH Polymer Detection Kit is intended to be used in CISH procedures using ZytoVision Probes and kits. For information on materials required for CISH procedures, please refer to the instructions for use of the respective ZytoVision Probe and implementation kit.

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)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!

ZytoDot 2C CISH Polymer Detection Kit
If reagents come into contact with skin, rinse skin immediately with copious amounts of water!

A material safety data sheet is available on our homepage (www.zytovision.com).

Do not reuse reagents, unless reuse is explicitly permitted!

Avoid any cross-contamination and micro-bacterial contamination of the reagents!

The specimens must not be allowed to dry during the hybridization and washing steps!

Hazard and precautionary statements for AB13, AB14, SB7b, and WB5:
The hazard-determining component is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Warning

H317 May cause an allergic skin reaction.

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 IF ON SKIN: Wash with plenty of water.

P333+P313 IF skin irritation or rash occurs: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before reuse.

Hazard and precautionary statements for SB7a:
The hazard-determining components are methanol and hydrogen peroxide solution 30%.

Danger

H225 Highly flammable liquid and vapour.

H301 + H311 Toxic if swallowed, in contact with skin or if inhaled.

H370 Causes damage to organs.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P233 Keep container tightly closed.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P308+P311 IF exposed or concerned: Call a POISON CENTER/doctor.

P403+P235 Store in a well-ventilated place. Keep cool.

Hazard and precautionary statements for CS2:
The hazard-determining component is ethanediol, ethylene glycol.

Warning

H373 May cause damage to organs through prolonged or repeated exposure.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P314 Get medical advice/attention if you feel unwell.

Hazard and precautionary statements for MT4:
The hazard-determining component is xylene.

Warning

H226 Flammable liquid and vapour.

H312 + H332 Harmful in contact with skin or if inhaled.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

H373 May cause damage to organs through prolonged or repeated exposure.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P403+P235 Store in a well-ventilated place. Keep cool.

EUH208 Contains methyl 2-methylprop-2-enoate; methyl 2-methylpropenoate; methyl methacrylate. May produce an allergic reaction.

For further information concerning this point, please refer to the instructions for use of the respective ZytoVision Probe and implementation kit.

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 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

Refer to the instructions for use of the ZytoDot 2C CISH Implementation Kit.

10. Preparation of specimens

Refer to the instructions for use of the ZytoDot 2C CISH Implementation Kit.
11. Preparatory treatment of the device

1. Preparation of 1x Wash Buffer TBS: Dilute 1 part of 20x Wash Buffer TBS (WB5) in 19 parts deionized or distilled water.

2. Anti-DIG/DNP-Mix [AB14], HRP/AP-Polymer-Mix [AB13], AP-Red Solution A (SB6a), AP-Red Solution B (SB6b), HRP-Green Solution A (SB7a), HRP-Green Solution B (SB7b), Nuclear Blue Solution (CS2), Mounting Solution (alcoholic) (MT4): Bring to room temperature before use.

Components SB7a and SB7b may form precipitates, which do not affect the staining quality.

12. Assay procedure

Specimen pretreatment

For detailed information on how to perform CISH with ZytoDot products, including the detection of Digoxigenin/Dinitrophenyl-labeled probes with the ZytoDot 2C CISH Polymer Detection Kit, please refer to the instructions for use of the ZytoDot 2C CISH Implementation Kit.

Detection

1. Wash slides 2x 1 min in deionized or distilled water.

2. Immune slides in 1x Wash Buffer TBS.

3. Apply Anti-DIG/DNP-Mix (AB14) (1-2 drops per slide) to the slides and incubate for 15 min at 37°C in a humidity chamber.

4. Wash slides 3x 1 min in 1x Wash Buffer TBS.

5. Apply HRP/AP-Polymer-Mix (AB13) (1-2 drops per slide) to the slides and incubate for 15 min at 37°C in a humidity chamber.

6. Wash slides 3x 1 min in 1x Wash Buffer TBS.


8. Apply AP-Red Solution (1-2 drops per slide) to the slides and incubate for 10 min at RT.

9. During the incubation, prepare HRP-Green Solution (working solution): fill 1 ml HRP-Green Solution B (SB7b) in a graduated cup and add two drops (2x 20 µl) HRP-Green Solution A (SB7a). Mix well.

10. Wash slides for 2 min in deionized or distilled water.

11. Apply HRP-Green Solution dropwise (1-2 drops per slide) to the slides and incubate for 15 min at room temperature.

12. Wash slides for 2 min in deionized or distilled water.

13. Counterstain specimens for 2 min with Nuclear Blue Solution (CS2).

14. Transfer slides into a staining jar and wash 2 min under cold running tap water.

15. Dehydrate 3x 30 s in 100% ethanol (use very pure ethanol).

16. Incubate slides for 2x 30 s in xylene (use very pure xylene).

Do not prolong or shorten the incubation time as this might result in loss of signals!

17. Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm; 24 mm x 32 mm) by using Mounting Solution (alcoholic) (MT4). Allow 20-30 min for the coverslip to become immobilized.

18. Evaluate stained specimens by using light microscopy.

13. Interpretation of results

Using the ZytoDot 2C CISH Implementation Kit, the hybridization signals of Digoxigenin-labeled polynucleotides appear as dark green colored distinct dots, and Dinitrophenyl-labeled polynucleotides appear as bright red colored distinct dot. For further information, please refer to the instructions of use of the respective ZytoDot Probe.

14. Recommended quality control procedures

Refer to the instructions for use of the respective ZytoDot 2C CISH Probe.

15. Performance characteristics

Refer to the instructions for use of the respective ZytoDot 2C CISH Probe.

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. Please refer to the instructions for use of the respective ZytoDot 2C CISH Probe and implementation kit for further information.

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.