FISH protocol for formalin-fixed, paraffin-embedded (FFPE) tissue

Protocol

Note: Ready-to-use product. Do not reconstitute or dilute with hybridization buffer.

The DNA-FISH Probe is optimized for use on 4-5 μm thick sections from formalin-fixed, paraffin-embedded tissue. Alternative fixatives require testing and calibration/alteration of protocol. Excessive or insufficient fixation time may result in suboptimal FISH results.

Slide Preparation

IMPORTANT: Sections should be mounted on coated slides and baked overnight (12-18 hours) at 55°C before hybridization.

Slide Pretreatment

1. Deparaffinize the slide in 3 changes of CitriSolv™ at RT, 10 min each.
   Procedure Note: Changes of CitriSolv™ may be reused no more than twice. However, the last change should be previously unused.
2. Dehydrate the slide in 2 changes of 100% EtOH at RT, 5 min each. Air dry.
   Procedure Note: The slide may be held dry at RT for several hours.
3. Incubate the slide in 0.2 N HCl at RT for 20 min.
4. Rinse the slide in once change of dH₂O and 2 changes of 2X SSC for 5 min each at RT.
5. Incubate the slide in prewarmed 1N NaSCN solution for 10 min at 80°C.
6. Rinse the slide in once change of dH₂O and 2 changes of 2X SSC for 5 min each at RT.
7. Place the slide in a 37°C humid chamber, layer 1 mL of 0.4% Pepsin on the target area on the slide, cover with parafilm and incubate for 10 min.
   Procedure Note: Depending on fixation and age of section, this time may need to be adjusted.
8. Rinse the slide in dH₂O for 1 min at RT.
9. Rinse the slide in 2 changes of 2X SSC for 5 min each at RT.
10. Incubate the slide for 10 min in 0.4% Pepsin.
   Procedure Note: Depending on fixation and age of section, this time may need to be adjusted.
11. Rinse the slide in 2 changes of 2X SSC for 5 min each at RT. Dip briefly in dH₂O and air dry.
12. Incubate the slide in 10% buffered formalin for 15 mins at RT.
13. Rinse the slide in 2 changes of 2X SSC for 5 min each at RT. Dip briefly in dH₂O and air dry.

Probe Denaturation / Hybridization

1. Vortex the DNA-FISH Probe briefly and spin the tube in a microcentrifuge.
2. Apply 10 μL of Probe to the target area on slide and cover with a cover-slip (22x22 mm).
   Procedure Note: Care should be taken to avoid air bubbles. Smaller or larger coverslips may be used with proportional change in DNA-FISH Probe volume.
3. Seal edges of cover-slip thoroughly with rubber cement.
4. Co-denature the slide with the probe for 5 min at 90°C on a temperature controlled hot plate.
5. Incubate for 12-18 hours in a humidified environment at 37°C in dark.
Post Hybridization Washing

Procedure Note: Do not allow slides to dry before washes are complete.

1. Remove the rubber cement with forceps.
2. Remove cover-slip by soaking in 2X SSC at RT.
3. Wash the slide in two changes for 5 min in 2X SSC/0.1% TWEEN 20 at 45°C.
4. Briefly rinse the slide in dH₂O and air dry slide out of direct light.
5. Apply 20 μL of DAPI/antifade solution to the hybridized area and cover with a cover-slip (25x25 mm).
   Procedure Note: Depending on fixation, age of section, and the pre treatment conditions, Green background can be seen. If the Green background is excessive or interferes with the scoring, slides can be rewash at higher stringency. The stringency can be increased by increasing the wash time in step 4 and/or temperature (up to 65°C).

Filter Requirement

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation max</th>
<th>Emission max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>496 nm</td>
<td>520 nm</td>
</tr>
<tr>
<td>Red</td>
<td>580 nm</td>
<td>603 nm</td>
</tr>
<tr>
<td>DAPI</td>
<td>360 nm</td>
<td>460 nm</td>
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</table>

Storage of Slides: Store hybridized slides at -20°C protected from direct light.

Storage of DNA-FISH Probe: Store at -20°C protected from light until the expiry date as indicated on the label.

DNA-FISH Probes manufactured by Cancer Genetics Italia S.r.l. and packaged in Milan, Italy.

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