## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Potential Cause(s)</th>
<th>Recommended Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No RFI signals are detected in the microscope.</td>
<td>- Reflected light shutter is not closed/stop slider is in light path. - Fluorescent lamp is switched off. - Wrong fluorescence filters in light path. - Objectives out of position. -Photobleaching in camera position.</td>
<td>- Open shutter/move stop slider out of the light path. - Switch on fluorescent lamp. - Move correct filter into light path. - Swing objective into light path. - Direct light path to eyepieces.</td>
</tr>
<tr>
<td>Hybridization signals become weak after a while.</td>
<td>- Immersion oil soaked in between slide and cover slip.</td>
<td>- Replace coverslip and DAPI/antifade. Use 24 x 32 mm2 coverslip even if only a small region is hybridized.</td>
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<tr>
<td>Diffuse signals.</td>
<td>- Preparation is not adequately illuminated. - Focus plane cannot be adjusted properly. - Antifade layer is too thick for focusing.</td>
<td>- Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp. Use immersion oil. Do not mix immersion oil. Use immersion oil suitable for fluorescence. Do not use too much DAPI/antifade. 10 μl per slide (24 x 32 mm2 coverslip) are sufficient.</td>
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<tr>
<td>Weak signals.</td>
<td>- Chromosome slide preparations too old. - Denaturation of chromosomes not adequate.</td>
<td>- Slides should not be older than two weeks. - Aging, baking or further fixation may inhibit the hybridization and is not recommended.</td>
</tr>
<tr>
<td>High diffuse background in green color channel.</td>
<td>- pH value of washing solutions is too low. - DAPI intensity is too high resulting in cross-talk to green single bandpass filter.</td>
<td>- Ensure that pH value is between 7.0 and 7.5 of solutions. Some green fluorophores are sensitive to pH below 7. - Reduce DAPI concentration in the DAPI/antifade solution.</td>
</tr>
</tbody>
</table>

If the recommended measures do not solve the problem, or your problem is not listed, please contact MetaSystems Probes.

## Customer Support

Please contact MetaSystems Probes GmbH (contact details see below) or our authorized distributor in your country. MetaSystems Probes disclaims any proprietary interest in the marks and names of others.

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## Revision: Rev A 160617

### Symbols Used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>This symbol marks a product as an “In Vitro Diagnostic Medical Device”.</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Manufacturer</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Reference number</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>No of tests</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Lot number</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Expiry date</td>
</tr>
<tr>
<td><img src="image" alt="Symbol" /></td>
<td>Temperature limitation for storage. Lower and upper limits are indicated.</td>
</tr>
</tbody>
</table>

### Further information available at www.metasystems-probes.com

<table>
<thead>
<tr>
<th>Product</th>
<th>Label</th>
<th>Order No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL BCR/ABL1 plus</td>
<td>green/orange</td>
<td>D-5052-100-OG</td>
<td>100μl</td>
</tr>
</tbody>
</table>

The XL BCR/ABL1 plus probe is specific for the 19;22. The orange labeled probe hybridizes to an extended region spanning the ABL1 locus on 9q34, and a green labeled probe hybridizes specifically to extended region spanning the BCR gene on 22q11. The probe is designed as a dual-color, dual-fusion assay.

### Probe Diagram:

![Probe Diagram](image)

**Chromosome 9**  **Chromosome 22**
Materials Provided
100μL of XL-BCR/ABL1 plus, the probe mix is dissolved in hybridization solution and ready to use.

Intended Use
DNA FISH probes are intended for fluorescence in-situ hybridization (FISH) for the analysis of chromosomal aberrations on fixed cells from human tissue suitable for cytogenetic investigation. Hybridized to metaphase and/or interphase nuclei FISH probes allow the analysis of chromosome structure or copy number variations to detect acquired genetic alterations according to the Global Medical Device Nomenclature (G MDN) CT039. FISH analysis is used as an adjunct test to other diagnostic investigations and not to be used as sole base for diagnosis or therapy decisions.

Safety Instructions
All probes produced by MetaSystems Probes are for professional use only and should be used by qualified and trained personnel only. In order to ensure safe operation and reproducible results please observe the safety notices and caution signs below.

CAUTION: Formamide is toxic and a potential teratogen!
MetaSystems probes contain formamide. Formamide is toxic and a teratogen. May cause harm to the unborn child. Do not breathe vapours; avoid skin contact!

CAUTION: Hot water bath and hot plates!
For denaturation and hybridization hot water baths and hot plates are used with temperatures of >37°C. Be careful not to get in direct contact with hot surfaces or liquids.

ATTENTION: Good Laboratory Practice!
Use in accordance with the principles of good laboratory practice.

ATTENTION: Waste Disposal!
All hazardous materials should be disposed of according to local/national regulation for hazardous waste disposal.

Storage and Handling
Probes should be stored in the dark at 20°C (±5°C). Performance of the probe has been shown to be unaffected for up to 20 freezethaw cycles.

Shipping
MetaSystems’ DNA probes are shipped at room temperature.

Equipment Necessary but not Supplied
- Water bath with accurate temperature control
- Variable micro-pipettes with volumes ranging from 1 μl to 1 ml, calibrated
- Thermometer
- pH meter, calibrated
- Timer
- Coplin jars (glass or plastic)
- Storage box
- Fluorescence microscope with suitable filters (see below)

Fluorescence Microscope Recommendation
- Fluorescence Illumination: Metal halide fluorescence illumination systems or conventional 100 Watt mercury lamp illuminators
- Objectives suitable for epifluorescent illumination
- Fluorescence Filters: For viewing/counting use a MetaSystems triple or quad bandpass filter set. For capturing images, suitable single bandpass filters for the respective fluorochromes should be used. Please inquire.

Fluorochrome Specification

<table>
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<tr>
<th>Label</th>
<th>Absorption max.</th>
<th>Emission max.</th>
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<tbody>
<tr>
<td>Blue</td>
<td>426 nm</td>
<td>480 nm</td>
</tr>
<tr>
<td>Green</td>
<td>505 nm</td>
<td>530 nm</td>
</tr>
<tr>
<td>Orange</td>
<td>552 nm</td>
<td>576 nm</td>
</tr>
</tbody>
</table>

Sample Preparation
General Comments
- MetaSystems probes are designed for use on cytogenetic samples which are fixed in 3:1 methanol/acetic acid and should be prepared according to the laboratory or institution guidelines.
- Prepare specimen according to standard cytogenetic procedures.

Stability of Hybridized Slides
- Hybridized FISH slides can be analysed for at least six months if stored in the dark at -20°C (±5°C).

Additional Procedural Recommendations
- The use of a calibrated thermometer is strongly recommended for measuring temperatures of solutions, water baths, and incubators, as these temperatures are critical for optimum product performance.
- Carefully check the temperature of preheated solutions.
- Carefully check the pH value of all solutions. It must be in the range of 7.0 – 7.5 at room temperature.
- The wash concentrations (stringency) pH and temperature are important, as low stringency can result in non-specific binding of the probe and too high stringency can result in lack of signals.
- Before opening: Spin briefly to collect probe mix at the bottom of the tube.

FISH Protocol for MetaSystems’ DNA Probes

Slide Preparation
1. Spot cell sample onto cleaned microscope slide. Allow to air dry. If you are not using these slides the same day, store at -20°C (±5°C).
2. Apply 1 μl of probe mixture.
3. Cover with coverslip 22 x 22 mm².
4. Seal with rubber cement.

Denaturation
1. Denature sample and probe simultaneously by heating slide on a hotplate at 75°C (±1°C) for 2 min.

Hybridization
1. Incubate in a humidified chamber at 37°C (±1°C) overnight.

Post-Hybridization Washes

Solutions Required
- 0.4 x SSC, pH 7.0 – 7.5 at 72°C (±1°C)
- 2 x SSC, 0.06% Tween20, pH 7.0 at room temperature

Protocol
1. Remove coverslip and all traces of glue carefully.
2. Wash slide in 0.4 x SSC, pH 7.0 at 72°C (±1°C) for 2 min.
3. Drain slide and wash in 2 x SSC, 0.05% Tween20, pH 7.0 at room temperature for 30 seconds.
4. Rinse briefly in distilled water to avoid crystal formation and let air dry.

Counterstain

- DAPI/antifade (e.g. MetaSystems DAPI/antifade, D-0002-500-DAA)
- Hoechst 33258/antifade (e.g. MetaSystems Hoechst 33258/antifade, D-0002-500-DAA)

Protocol
1. Apply 10 μl of the DAPI/antifade and overlay with a 24 x 32 mm² coverslip.
2. Allow the penetration of DAPI/antifade for 10 min.
3. Proceed with microscopy and analysis.
4. Store slides at -20°C (±5°C). Hybridization signals are fine for at least six months.

Expected Results

Normal Cell: Two green (23) and two orange (23) signals.

Aberrant Cell (typical result): One green (1G), one orange (1C), and two green-orange fusion (2GO) (adjacent green and orange) signals.

Only the most frequent signal constellations are shown, other relevant signal patterns may be observed.