Troubleshooting

Problem	Potential Cause(s)	Recommended Solution
No FISH signals are detected in the microscope.	• Reflected light shutter closed / stop slider in light path.	• Open shutter / move stop slider out of the light path.
	• Fluorescent lamp is switched off.	• Switch on fluorescent lamp.
	• Wrong fluorescence filter is in light path.	• Move correct filter into light path.
	• Objective is out of position.	• Swing objective into light path.
	• Phototube is in camera position.	• Direct light path to eyepieces.
Hybridization signals become weak after a while.	• Immersion oil soaked in-between slide and coverslip.	• Replace coverslip and DAPI/antifade. Use 24 x 32 mm² coverslip even if only a small region is hybridized.
Diffuse signals.	• Preparation is not adequately illuminated.	• Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp.
	• Focus plane cannot be adjusted properly.	• Use enough immersion oil. Do not mix different immersion oils. Use immersion oil suitable for fluorescence.
	• Antifade layer is too thick for focusing.	• Do not use too much DAPI/antifade. 10 µl per slide (24 x 32 mm² coverslip) are sufficient.
Weak signals.	• Chromosome slide preparation is too old.	• Slides should not be older than two weeks.
	• Denaturation of chromosomes is not adequate.	• Aging, baking or further fixation may inhibit the hybridization and is not recommended. • Increase denaturation temperature up to 80°C
	• A multi bandpass filter is used for viewing.	• Use a dedicated single bandpass filter.
Weak aqua or green signals or high diffuse background in green color channel.	• DAPI intensity is too high resulting in crosstalk to AQUA filter or GREEN filter.	• Use DAPI/antifade of low concentration.
	• pH value of washing solutions is too low.	• Ensure that pH value is between 7.0 and 7.5 of solutions. Some green fluorophores are very sensible to pH below 7.
High unspecific background	• Remaining cytoplasmic proteins of the cells may impair the hybridization.	• Pretreat slides with Pepsin.
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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Symbols Used

Symbol	Description	Symbol	Description
IVD	This symbol marks a product as an "In Vitro Diagnostic Medical Device".	⚠	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION.
	Manufacturer	REF	Reference number
Σ	No of tests	LOT	Lot number
	Expiry date		Temperature limitation for storage. Lower and upper limits are indicated.

Revision: General-CE-IVD-RevC200506-240116v10.1

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FORMAMIDE
Danger. May damage the unborn child. Suspected of causing cancer. May cause damage to organs through prolonged/repeated exposure. Obtain special instructions before use. Do not breathe vapours. Wear protective gloves/protective clothing. IF exposed or concerned: Get medical advice.

Gefahr. Kann das Kind im Mutterleib schädigen. Kann vermutlich Krebs erzeugen. Kann die Organe schädigen bei längerer/wiederholter Exposition. Vor Gebrauch besondere Anweisungen einholen. Dampf nicht einatmen. Schutzhandschuhe/Schutzkleidung tragen. BEI Exposition oder Verdacht: Ärztlichen Rat einholen.

Danger. Peut nuire au foetus. Susceptible de provoquer le cancer. Risque présumeé d'effets graves pour les organes à la suite d'expositions répétées ou d'une exposition prolongée. Se procurer les instructions avant utilisation. Ne pas respirer les vapeurs. Porter des gants de protection/ des vêtements de protection. EN CAS d'exposition prouvée ou suspectée: consulter un médicin.

IVD
-25°C -15°C
LOT XXXXX
YYYY-MM-DD
REF D-5146-100-OG 10
XL IGH/MAFB DF
Dual Fusion Probe
100µl (Σ 10)
MetaSystems Probes

XCyting Locus-Specific Probes

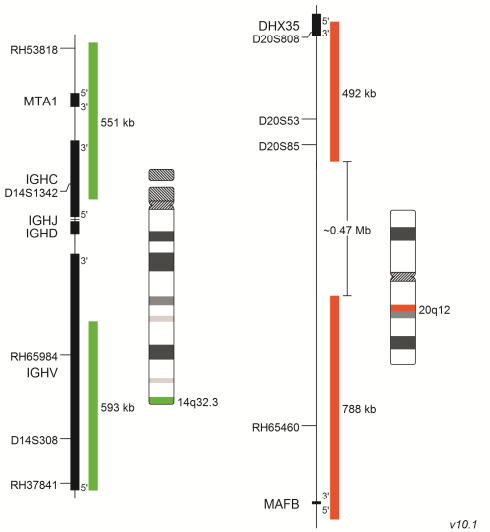
For Professional Use Only

Further information available at www.metasystems-probes.com

Product	Label	Order No.	Pack Size
XL IGH/MAFB DF	green/orange	D-5146-100-OG	100µl

XL IGH/MAFB DF probe is designed as a dual fusion probe. The orange labeled probe covers the MAFB gene and flanks the breakpoint at 20q12, the green labeled probe flanks the IGH breakpoint region at 14q32.

Probe Diagram:



Chromosome 14 **Chromosome 20**

Materials Provided

100µl of XL IGH/MAFB DF, 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 (GMDN) CT929. 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! Wear gloves and a lab coat. In case of contact with skin or eyes, wash immediately with water.
⚠	**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. Wear gloves and a lab coat. In case of contact with skin, cool immediately with cold water.
⚠	**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). Probe performance has been shown to be unaffected for up to 20 freeze-thaw 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)
- Hotplate 75°C (±1°C), with a solid plate and accurate temperature control up to 80°C
- Freezer -20°C (±5°C)
- Humidified chamber 37°C (±1°C)
- Forceps
- Gloves
- Microcentrifuge
- Fluorescence microscope with suitable filters (see below)
- Immersion oil, recommended by the microscope manufacturer (fluorescence grade)
- Imaging System, e. g. Isis (MetaSystems)
- Coverslips (glass): 22 x 22 mm² and 24 x 32 mm²
- Rubber Cement
- DAPI/antifade

Fluorescence Microscope Recommendation

- Fluorescence Illumination: Metal halide fluorescence illumination systems or conventional 100 watt mercury lamp illuminators
- Objectives suitable for epi-fluorescent illumination.
- Fluorescence Filters: For viewing/counting use a MetaSystems triple or quad bandpass filter set or appropriate single bandpass filter. For capturing images use suitable single bandpass filters for the respective fluorochromes. Please inquire.

Fluorochrome Specification

Label	Absorption max.	Emission max.
Blue (aqua)	426 nm	480 nm
Green	505 nm	530 nm
Orange	552 nm	576 nm

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 analyzed 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 10 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 75ºC (±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 72ºC (±1°C)
- 2 x SSC, 0.05% Tween-20 (pH 7.0) at room temperature

Procedure

1. Remove coverslip and all traces of glue carefully.
2. Wash slide in 0.4 x SSC (pH 7.0) at 72ºC (±1°C) **for 2 min**.
3. Drain slide and wash in 2 x SSC, 0.05% Tween-20 (pH 7.0) at room temperature **for 30 seconds**.
4. Rinse briefly in distilled water to avoid crystal formation and let air dry.

Counterstain

Solutions required:

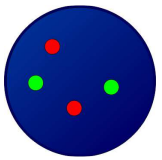
- DAPI/antifade (e.g. MetaSystems DAPI/antifade, D-0902-500-DA)

Procedure:

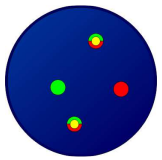
1. Apply 10 µl of the DAPI/antifade and overlay with a 24 x 32 mm² coverslip.
2. Allow the penetration of DAPI/antifade **for 10min**.
3. Proceed with microscoping and analysis.
4. Store slides at –20°C (±5°C). Hybridization signals are fine for at least six months.

Expected Results

Normal Cell:
Two green (2G) and two orange (2O) signals.



Aberrant Cell (typical results):
One green (1G), one orange (1O), and two green-orange colocalization/fusion signals (2GO) resulting from a reciprocal translocation between the relevant loci.



Faint green cross-hybridizations may be observed at 15q11.2 and 16p11.2 due to IGH pseudogenes.
Only the most frequent signal constellations are shown, other relevant signal patterns may be observed.