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.	• The immersion oil has penetrated between the glass slide and cover glass.	• 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.
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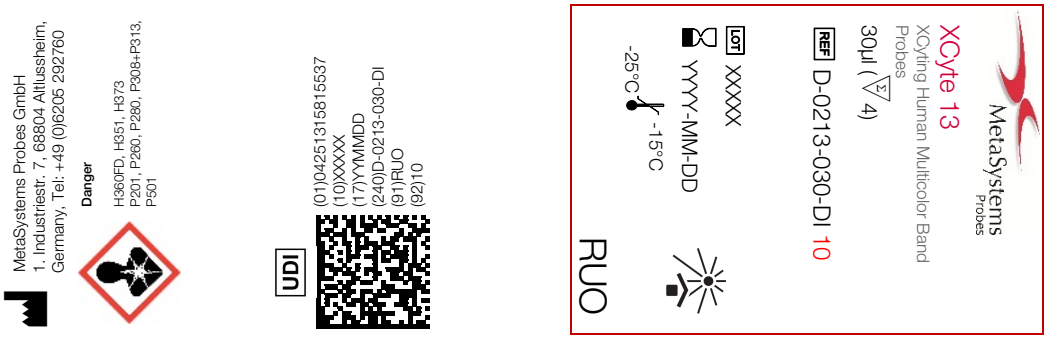
Symbols Used

Symbol	Description	Symbol	Description
RUO	This symbol marks a product as for “Research Use Only”.	⚠	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
	Expiration date		Temperature limitation for storage. Lower and upper limits are indicated.
UDI	Unique Device Identification		Keep away from sunlight. Indicates a device that needs protection from light sources.
	Danger		

Revision: RUO-Multicolor-RevD220412-220407



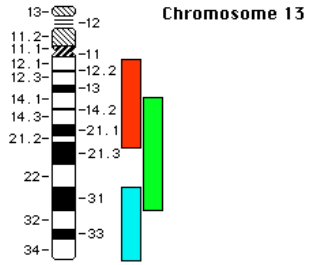
For Professional Use Only

Further information available at www.metasystems-probes.com

Product	Label	Order No.	Pack Size
XCyte 13	multicolor	D-0213-030-DI	30µl

Chromosome 13 specific mix of partially overlapping region-specific paints, labeled with different fluorochromes. The excitation/emission spectra are comparable to the common aqua, green and orange fluorochromes.

Probe Diagram:



Materials Provided

30µl of XCyte 13, the probe is dissolved in hybridization solution (formamide, dextran sulfate, saline-sodium citrate) and ready to use.

Intended Use

XCyte 13 is intended for fluorescence in-situ hybridization (FISH) for the analysis of chromosomal aberrations on fixed cytogenetic specimen. This probe is intended for research use only. This product is not intended for diagnostic use

Safety Instructions

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

DANGER

Contains:	Formamide
Hazard statements:	H360FD May damage fertility. May damage the unborn child. H351 Suspected of causing cancer. H373 May cause damage to organs through prolonged or repeated exposure.
Precaution statements:	P201 Obtain special instructions before use. P260 Do not breathe dust/fume/gas/mist/vapors/spray. P280 Wear protective gloves/protective clothing. P308+ P313 IF exposed or concerned: Get medical advice/attention. P501 Dispose of contents/container to in accordance with local/regional/national/international regulations.
	Special labeling: Restricted to professional users.

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.

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

Products produced by MetaSystems Probes are shipped at room temperature.

Equipment Necessary but not Supplied

- Water bath with accurate temperature control
- Humidified chamber 37°C (±1°C)
- Variable micro-pipettes with volumes ranging from 1 µl to 1 ml, calibrated
- Hotplate 75°C (±1°C), with a solid plate and accurate temperature control up to 80°C
- Thermometer
- pH meter, calibrated
- Timer
- Freezer -20°C (±5°C)
- Coplin jars (glass or plastic)
- Microcentrifuge
- Gloves
- Forceps
- DAPI/antifade
- Fluorescence microscope with suitable filters (see below)
- Imaging System
- Immersion oil, recommended by the microscope manufacturer (fluorescence grade)
- DAPI/antifade
  Rubber Cement
- Coverslips (glass):
  22 x 22 mm² and 24 x 32 mm²

Fluorescence Microscope Recommendation

- Fluorescence Illumination: Metal halide fluorescence illumination systems or conventional 100 watt mercury lamp illuminators.
- Objectives: 10x/20x and 63x/100x suitable for epi-fluorescent illumination.
- Fluorescence Filters: For capturing images use suitable single bandpass filters for the respective fluorochromes. For all multi-color probes bandpass filters with narrow band characteristic should be employed to minimize (avoid) spectral cross-talking between fluorochromes. Please inquire.
- Imaging System: For mFISH/mBAND probes an appropriate imaging system with a color karyotyping software should be used.

References

Denaturation procedure adapted from (modified):
Fritz et al, Hum Genet (1998)103:441-449; Rieder et al, Leukemia (1998)9:1473-1481)

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 two weeks if stored in the dark at temperatures below -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.
- 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 a lack of signal.
- **Before opening:** Spin briefly to collect probe mix at the bottom of the tube.

Slide Denaturation

Solutions required:

- 0.1x SSC, pH 7.0 - 7.5 (you will need solution at room temperature and at 4°C)
- 2x SSC, pH 7,0 - 7.5 (you will need solution at 70°C and at 4°C)
- NaOH 0.07mol/l, room temperature
- Ethanol series: 100%, 95%, 70%, room temperature

Procedure:

1. Put a coplin jar with 0.1 x SSC and 2 x SSC into the refrigerator.
2. Prewarm a coplin jar with 2x SSC to 70°C (±1°C) in a water bath.
3. Put slides into 2x SSC at 70°C (±1°C) and incubate slide for **30min**.
4. Remove coplin jar from water bath, let cool down to room temperature for approx. **20min.**
5. Transfer slide to 0.1x SSC at room temperature for **1min.**
6. Denature slide in 0.07N NaOH at room temperature for **1min**.
7. Put slide into 0.1x SSC 4°C for **1min.**
8. Put slide into 2x SSC, 4°C for **1min.**
9. Transfer to a coplin jar with 70% ethanol for **1min.**
10. Subsequently, transfer to a coplin jar with 95% and 100% ethanol, incubate for **1min** each.
11. Let air dry.

Probe Denaturation and Hybridization

Procedure:

1. Prepare probe cocktail according to the intended hybridization area: 7µl for a 18 x 18 mm² coverslip, 10µl for a 22 x 22 mm² coverslip, or 12µl for a 24 x 24 mm² cover slip.
2. Denature probe by incubating at 75°C (±1°C) for 5min.
3. Put on ice briefly.
4. Incubate at 37°C (±1°C) **for 30min**.
5. Spin briefly to collect probe cocktail.
6. Pipette denatured and prehybridized probe cocktail onto the denatured chromosome preparation.
7. Overlay with cover slip and seal with rubber cement.
8. Incubate **1 - 2 days** in a humidified chamber at 37°C (±1°C).

Posthybridization Washing

Solutions required:

- 0.4x SSC, pH 7.0 - 7.5, 72°C (±1ºC)
- 2x SSCT (2 x SSC, pH 7.0 - 7.5 containing 0.05% Tween20), room temperature

Procedure:

1. Carefully remove rubber cement and cover slips.
2. Place slides in prewarmed (72°C, ±1ºC) 0.4x SSC **for 2min**.
3. Incubate slides in 2x SSCT **for 1/2min**.

Counterstain

Solutions required:

- DAPI/antifade (250ng/ml)

Procedure:

1. Wash briefly in double distilled water to avoid crystal formation and let air dry.
2. Apply 20µl of the DAPI/antifade and overlay with a 24 x 60 mm² cover slip.
3. Allow penetration of DAPI/antifade **for 10min**. Proceed with microscopy and analysis or store slides at –20°C (±5°C).