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-RevD220412-230524

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Danger

H360FD, H351, H373
P201, P260, P280, P308+P313, P501

UDI

(01)04251315814417
(10)XXXX
(17)YYMMDD
(240)D-0320-050-FI
(91)RUO
(92)10

RUO

-25°C -15°C

LOT XXXX

YYYY-MM-DD

REF D-0320-050-FI 10

50µl (Σ 5)

XCP 20 green

Whole Chromosome Paint

MetaSystems Probes

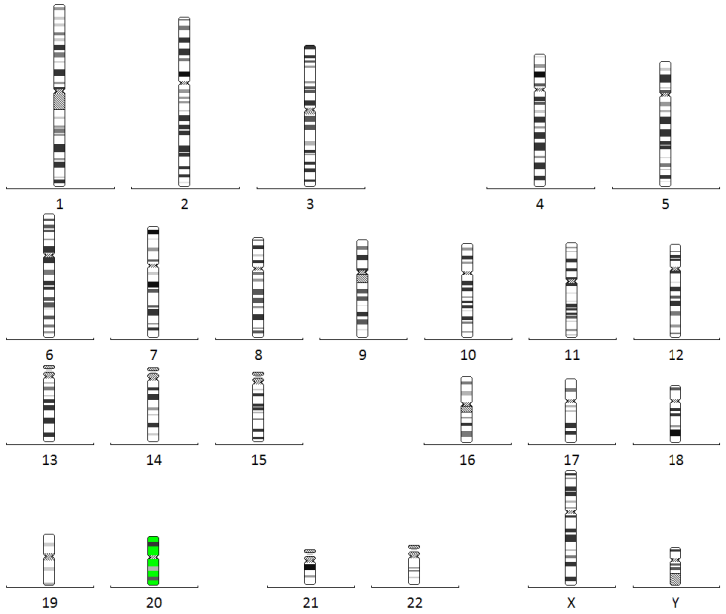
For Professional Use Only

Further information available at www.metasystems-probes.com

Product	Label	Order No.	Pack Size
XCP 20 green	green	D-0320-050-FI	50µl

XCyting Chromosome Paint specific for chromosome 20 labeled with a green emitting fluorochrome. Up to four XCPs can be mixed together which will provide reliable but slightly reduced signal strength compared to single applications.

Probe Diagram:



Materials Provided

50µl of XCP 20 green, the probe is dissolved in hybridization solution (formamide, dextran sulfate, saline-sodium citrate) and ready to use.

Intended Use

XCP 20 green 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 viewing/counting use an appropriate 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
Gold	525 nm	551 nm
Orange	552 nm	576 nm
Red	595 nm	615 nm
Near Infrared	644 nm	669 nm

Absorption and Emission depending on the label used in the product.

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 a lack of signal.
- **Before opening:** Spin briefly to collect probe mix at the bottom of the tube.

FISH Protocol for DNA Probes

Slide Preparation

1. Spot cell sample onto cleaned microscope slide. Allow to air dry. If you are not using the slides the same day, store at -20°C (±5°C).
2. Apply 10 µl of probe mixture. For combination of two XCPs use 5µl of each probe per hybridization and mix well. For a three color mix of three different chromosomes use 2.5µl of a first XCP in green, 2.5µl of a second XCP in orange, 2.5µl each of a third XCP in green and orange per hybridization and mix well. This results in a green, an orange, and a yellow chromosome class when viewing in a triple filter or after capturing.
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

- 0.4X SSC (pH 7.0 – 7.5) at 72ºC (±1°C)
- 2X 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 0.4X SSC (pH 7.0) at 72ºC (±1°C) **for 2 min**.
3. Drain slide and wash in 2X 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

Procedure:

1. Apply 10 µl of the DAPI/antifade and overlay with a 24 x 32 mm² coverslip.
2. Allow penetration of DAPI/antifade **for 10min**.
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:

When XCP 20 green is hybridized to a normal human metaphase spread, the two respective homologue chromosomes show hybridization signals along the entire length. At the centromeric and heterochromatic regions signals are reduced or suppressed. Translocations with involvement of the respective chromosome result in split signals and chromosomes with partially unlabeled regions.