

## Troubleshooting

Problem	Potential Cause(s)	Recommended Solution
No FISH signals are detected in the microscope.	<ul style="list-style-type: none"> <li>Reflected light shutter closed / stop slider in light path.</li> <li>Fluorescent lamp is switched off.</li> <li>Wrong fluorescence filter is in light path.</li> <li>Objective is out of position.</li> <li>Phototube is in camera position.</li> </ul>	<ul style="list-style-type: none"> <li>Open shutter / move stop slider out of the light path.</li> <li>Switch on fluorescent lamp.</li> <li>Move correct filter into light path.</li> <li>Swing objective into light path.</li> <li>Direct light path to eyepieces.</li> </ul>
Hybridization signals become weak after a while.	<ul style="list-style-type: none"> <li>The immersion oil has penetrated between the glass slide and cover glass.</li> </ul>	<ul style="list-style-type: none"> <li>Replace coverslip and DAPI/antifade. Use 24 x 32 mm<sup>2</sup> coverslip even if only a small region is hybridized.</li> </ul>
Diffuse signals.	<ul style="list-style-type: none"> <li>Preparation is not adequately illuminated.</li> <li>Focus plane cannot be adjusted properly.</li> <li>Antifade layer is too thick for focusing.</li> </ul>	<ul style="list-style-type: none"> <li>Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp.</li> <li>Use enough immersion oil. Do not mix different immersion oils. Use immersion oil suitable for fluorescence.</li> <li>Do not use too much DAPI/antifade. 10 µl per slide (24 x 32 mm<sup>2</sup> coverslip) are sufficient.</li> </ul>
Weak signals.	<ul style="list-style-type: none"> <li>Chromosome slide preparation is too old.</li> <li>Denaturation of chromosomes is not adequate.</li> <li>A multi bandpass filter is used for viewing.</li> </ul>	<ul style="list-style-type: none"> <li>Slides should not be older than two weeks.</li> <li>Aging, baking or further fixation may inhibit the hybridization and is not recommended.</li> <li>Increase denaturation temperature up to 80°C.</li> <li>Use a dedicated single bandpass filter.</li> <li>Use DAPI/antifade of low concentration.</li> </ul>
Weak aqua or green signals or high diffuse background in green color channel.	<ul style="list-style-type: none"> <li>DAPI intensity is too high resulting in crosstalk to AQUA filter or GREEN filter.</li> <li>pH value of washing solutions is too low.</li> </ul>	<ul style="list-style-type: none"> <li>Ensure that pH value is between 7.0 and 7.5 of solutions. Some green fluorophores are very sensible to pH below 7.</li> </ul>
High unspecific background	<ul style="list-style-type: none"> <li>Remaining cytoplasmic proteins of the cells may impair the hybridization.</li> </ul>	<ul style="list-style-type: none"> <li>Pretreat slides with Pepsin.</li> </ul>

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. All trademarks are property of their respective owners.



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## Symbols Used

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		Reference number
	No of tests		Lot number
	Expiration date		Temperature limitation for storage. Lower and upper limits are indicated.
	Unique Device Identification		Keep away from sunlight. Indicates a device that needs protection from light sources.
	Danger		

Revision: RUO-RevD220412-220407v10.1

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Danger



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

UDI



(01)042513115159803  
(10)XXXXXX  
(17)YYMMDD  
(240)D-5441-050-OG  
(91)RUO  
(92)10

**XL 21q22/XCP 21**

50µl ( )

**REF** D-5441-050-OG 10

**LOT** XXXXX  
YYYY-MM-DD

-25°C -15°C

**RUO**

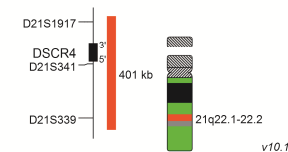
## For Professional Use Only

Further information available at [www.metasystems-probes.com](http://www.metasystems-probes.com)

Product	Label	Order No.	Pack Size
XL 21q22/XCP 21	orange/green	D-5441-050-OG	50µl

The XL 21q22 / XCP 21 is a combination of a whole chromosome paint 21 in green with an orange labeled locus-specific probe for 21q22. This probe allows analysis of copy numbers and structural aberrations of chromosome 21 on metaphase chromosomes. This probe is not intended for use to analyse Trisomie 21 in prenatal diagnosis.

### Probe Diagram:



## Chromosome 21

## Materials Provided




50µl of XL 21q22/XCP 21, the probe is dissolved in hybridization solution (formamide, dextran sulfate, saline-sodium citrate) and ready to use.

## Intended Use

XL 21q22/XCP 21 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.

	<b>DANGER</b>
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.
	<b>CAUTION: Hot water bath and hot plates!</b> 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.
	<b>ATTENTION: Good Laboratory Practice!</b> 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 mm<sup>2</sup> and 24 x 32 mm<sup>2</sup>

## 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.
3. Cover with coverslip 22 x 22 mm<sup>2</sup>.
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

- 0.4X SSC (pH 7.0 – 7.5) at 72°C (±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 72°C (±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 mm<sup>2</sup> 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

XL 21q22/XCP 21 hybridized to normal human metaphase spreads will result in green signals along the entire length of chromosome 21 combined with orange signals at 21q22 (Down Syndrome Critical Region 4). A translocation with involvement of chromosome 21 will result in a split of a green signal. In this case the green signal will be partially visible on the der(21) and the respective partner chromosome of the translocation. Depending on the localization of the breakpoint on the der(21), the signal of the locus-specific probe remains on the der(21) or will be observed on the translocation partner.

Cross-hybridizations for the XCP 21, in green only, may occur in the centromeric and short-arm regions of acrocentric chromosomes and to a minor extent on the centromere of chromosome 20 and the p-terminal region of chromosome 18.