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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 fœtus. Susceptible de provoquer le cancer. Risque présumé 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 médecin.

## XCyting AneuScore Probes

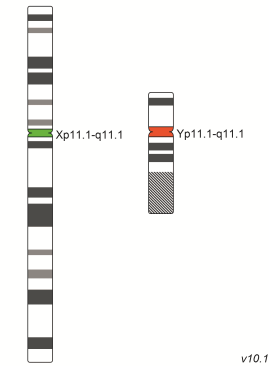
**For Professional Use Only**

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

Product	Label	Order No.	Pack Size
XA X/Y	green / orange	D-5608-100-OG	100µl

The XA X/Y mix of specific probes allows detecting copy number variations for chromosomes X and Y. The probe mix is composed of repetitive sequences which hybridize to the centromeric region of chromosomes X in green and Y in orange.

Probe Diagram:



Chromosomes X, and Y

## Materials Provided





100µl (∇ 10), XA X/Y is dissolved in hybridization solution and ready to use.

## Intended Use

All DNA FISH probes provided by MetaSystems Probes are intended for fluorescence in-situ hybridization (FISH) experiments for cytogenetic studies. XA X/Y allows the diagnosis and monitoring of chromosomal abnormalities of a constitutional nature i.e. present in all cells of the body (according to the Global Medical Device Nomenclature (GMDN) CT 826). The provided FISH assay is to be used as adjunct with other tests, such as fetal cell karyotyping and not as stand-alone. XA X/Y is not intended for the detection of structural rearrangement (e.g. translocations) or numerical aberrations of other chromosomes.

## 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.

	<b>CAUTION: Formamide is toxic and a potential teratogen</b> 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.
	<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.
	<b>ATTENTION: Waste Disposal!</b> 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 mm<sup>2</sup> and 24 x 32 mm<sup>2</sup>
- 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

## 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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URL: www.metasystems-probes.com

Revision: XA-CE-RevC200506-220407v10.1

## 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>Immersion oil soaked in-between slide and coverslip.</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> </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>Use DAPI/antifade of low concentration.</li> <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.		

## Symbols Used

Symbol	Description
	This symbol marks a product as an "In Vitro Diagnostic Medical Device".
	Manufacturer
	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION.
	Reference number
	Lot number
	Temperature limitation for storage. Lower and upper limits are indicated.
	This symbol indicates the number of tests.
	Expiry date

## Sample Preparation

### Solutions required (not supplied):

- Water, double distilled,
- Trypsin/EDTA (0.05% Trypsin, 0.02% EDTA\*4Na in Hanks' Balanced Salt Solution without Ca<sup>2+</sup> and Mg<sup>2+</sup>, 37°C),
- 0.075 M KCl, 37°C,
- Carnoy's fixative (3:1 Methanol/Acetic Acid, fresh prepared, -20°C),
- Microscopic slides,
- 2X SSC at 37°C,
- Ethanol series: 100%, 95%, 70%, room temperature.

### Procedure:

- Centrifuge 2-5 ml of amniotic fluid (not bloody) for 8 minutes at 1000 rpm.
- Remove supernatant and resuspend pellet by carefully tapping the tube.
- Add 5 ml Trypsin/EDTA and incubate for 30 minutes at 37°C (±1°C).
- Centrifuge for 8 minutes at 1000 rpm, remove supernatant and resuspend pellet by carefully tapping the tube.
- Add 5 ml of 0.075 M KCl and incubate for 20 minutes at 37°C (±1°C).
- Add slowly 2 ml of fresh prepared fixative (3:1 Methanol/Acetic Acid, -20°C) and mix.
- Centrifuge for 8 minutes at 1000 rpm, remove supernatant and resuspend pellet by carefully tapping the tube.
- Add 5 ml of fixative and incubate for 30 minutes in a refrigerator (2°C-6°C).
- Centrifuge for 8 minutes at 1000 rpm, remove supernatant and dissolve pellet by carefully tapping the tube.
- Add 50-100 µl of fixative and resuspend pellet carefully.
- Drop 10 µl of cell suspension per area on a clean dust-free glass slide (mark areas with a diamond pen). Optimal results are obtained at 20-25°C and 45-55% humidity. Air dry at room temperature and check cell density under a phase contrast microscope.
- If cell density is too low, add another 10 µl of cell suspension and allow to air dry.
- Incubate slide for 15-30 minutes in 2X SSC at 37°C (±1°C).
- Dehydrate slides in an ethanol series (70%, 85%, and 100%) 2 minutes each. Air dry.
- Proceed with the prenatal probe kit FISH protocol as provided with the individual probe or probe set.

### General Comments

- MetaSystems probes are designed for use on cytogenetic samples fixed in Carnoy's fixative 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 MetaSystems' DNA Probes

## Slide Preparation

1. Use slides prepared according to sample preparation.
2. Apply 10 µl of probe
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% 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

### Solutions required:

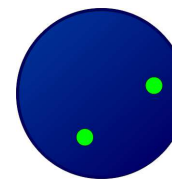
- DAPI/antifade (e.g. MetaSystems DAPI/antifade, D-0902-500-DA)

### Procedure:

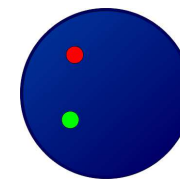
1. Apply 10 µl of DAPI/antifade and cover with a 24 x 32 mm<sup>2</sup> coverslip.
2. Allow penetration of DAPI/antifade for 10min.
3. Proceed with microscopy and analysis.
4. Store slides at -20°C (±5°C). Hybridization signals are fine for at least six months.

## Expected Results

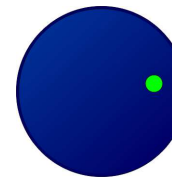
Normal Cell:  
46,XX  
Two green (2G) signals.



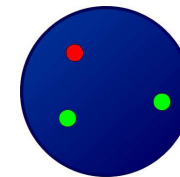
Normal Cell:  
46,XY  
One green (1G) and one orange (1O) signal.



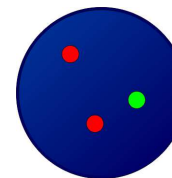
Aberrant Cell:  
45,X0  
One green (1G) signal.  
Mosaics with 46, XX occur frequently.



Aberrant Cell:  
47,XXY  
Two green (2G) and one orange (1O) signal.  
May exist as mosaic with 46, XY or 46, XX.



Aberrant Cell:  
47,YYY  
One green (1G) and two orange (2O) signals.  
Additional X-Chromosomes are possible (e.g. 48, XYYY; 52, XXXXXYY).



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