Fluorescence in situ hybridisation, FISH

DNA probes are used for fluorescence in-situ hybridization (FISH) to allow the analysis of chromosome structure or copy number variations. Colcemid prevents spindle formation during mitosis, and is used to arrest cells in metaphase. Many cell types that attach to plastic culture dishes round up in mitosis and can be dislodged by rinsing the surface.

Cell culture and preparation of slides

For adherent cells:

1-3 days before the experiment: Seed cells at an appropriate density in a petri dish to get exponentially growing cells (ca 80% confluency) at the day of harvest.

For suspension cells:

Peripheral blood was collected in the morning of day 0 and cultured in standard RPMI 1640 medium (see methodology sheet on chromosomal aberrations and micronuclei) for 72h.

10 µl/ml medium colcemid (Invitrogen 15212-012, 10 µg/ml) should be added for the last 3-4 h of culture.

The preparation of microscopic slides is the same as described in the protocol “Aberrations and Mn”. Slides are air dried overnight, then stored at -20°C (±5°C).

FISH protocol

A whole chromosome probe for chromosomes from Metasystems will be used. It paints chromosomes 1 in red, 2 in green and 4 in yellow. See attached product document.

Day 0

Slide preparation

1. Apply 10 µl of probe mixture.

2. Cover with coverslip 22 x 22 mm^2.

3. 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.

Day 1

Post-hybridization washes

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

1. Apply 10 µl of the DAPI (1 µg/ml) plus one drop antifade (Vectashield) and overlay with a 24 x 32 mm^2 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.

Analysis

Find the area of interest with a low magnification lens. Apply oil immersion, change to 100x lens. Take images in three channels (blue, red, green).

Expected results

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.

Solutions required for FISH

20xSSC buffer (1 liter)

1. Dissolve the following in 800 ml of distilled H_2O.
    - 175.3 g of NaCl (58.44 g mol-1)
    - 88.2 g of sodium citrate ($Na_3C_6H_5O_7$, 294.10 g mol-1)
2. Adjust the pH to 7.0 with a few drops of conc. HCl.
3. Adjust the volume to 1 L with additional distilled H_2O.
4. Sterilize by autoclaving.

0.4xSSC pH 7.0 (1 liter)

20 ml 20xSSC buffer

980 ml dH_2O

Adjust the pH to 7.0 with a few drops of conc. HCl

2xSSC, 0.05% Tween 20 pH 7.0 (1 liter)

100 ml 20xSSC buffer

900 ml dH_2O

0.5 ml Tween 20

Adjust the pH to 7.0 with a few drops of conc. HCl

Troubleshooting

Problem	Potential Cause(s)	Recommended Solution
No FISH signals are detected in the microscope.	• Reflected light shutter is closed / stop slider is in light path. • Fluorescent lamp is switched off. • Wrong fluorescence filter is in light path. • Objective is out of position. • Phototube is in camera position.	• Open shutter / move stop slider out of the light path. • Switch on fluorescent lamp. • Move correct filter into light path. • Swing objective into light path. • 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. • Focus plane cannot be adjusted properly. • Antifade layer is too thick for focusing.	• Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp. • Use enough immersion oil. Do not mix different immersion oils. Use immersion oil suitable for fluorescence. • 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. • Denaturation of chromosomes not adequate.	• Slides should not be older than two weeks. • Aging, baking or further fixation may inhibit the hybridization and is not recommended.
High diffuse background in green color channel.	• pH value of washing solutions is too low. • DAPI intensity is too high resulting in crosstalk to green single bandpass filter.	• Ensure that pH value is between 7.0 and 7.5 of solutions. Some green fluorophores are sensitive to pH below 7. • Reduce DAPI concentration in the DAPI/antifade solution.
If the recommended measures do not solve the problem, or your problem is not listed, please contact MetaSystems.		

Customer Support

Please contact MetaSystems GmbH (contact details, see below) or our authorized distributor in your country.
MetaSystems disclaims any proprietary interest in the marks and names of others.

M

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Revision: Rev S 160129

Symbols Used

Symbol	Description	Symbol	Description
V	This symbol marks a product as an “In Vitro Diagnostic Medical Device”.	Y	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION.
M	Manufacturer	h	Reference number
X	No of tests	g	Lot number
H	Expiry date	I	Temperature limitation for storage. Lower and upper limits are indicated.

Manufactured by MetaSystems GmbH,
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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.

CV

I -20°C (±5°C)

H YYYY-MM

g XXXXX

h D-0328-200-MC

200µl (X20)

Whole Chromosome Paint

XCP Mix #1-#2-#4, 3-colors

MetaSystems

XCyting Chromosome Paints

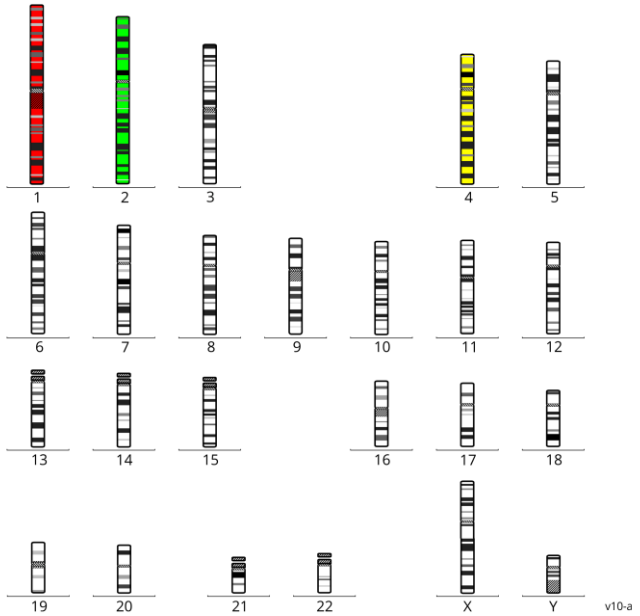
For Professional Use Only

Further information available at www.metasystems.de

Product	Label	Order No.	Pack Size
XCP Mix #1-#2-#4, 3-colors	green/orange	D-0328-200-MC	200µl

Probe mix containing whole chromosome paints (XCP) specific for chromosome 1 labeled with an orange emitting fluorophore, chromosome 2 with a green emitting fluorophore and chromosome 4 with a combination of the 2 fluorophores.

Probe Diagram:



V 160323

Materials Provided

200µl of XCP Mix #1-#2-#4, 3-colors, premixed 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 probe kits produced by MetaSystems 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 probe kits 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 at -20°C (±5°C).

Shipping

MetaSystems' DNA probes are shipped at room temperature.

Equipment Necessary but not Supplied

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

Fluorescence Microscope Recommendation

For optimal visualization of the probe we recommend:

- Fluorescence Illumination: Metal halide fluorescence illumination systems or conventional 100 watt mercury lamp illuminators may be used.
- Objectives: x10/x20 and x63/x100 suitable for epi-fluorescent illumination.

Fluorescence Filters Recommendations for MetaSystems' DNA probes:

- For viewing/counting use a MetaSystems triple or quad bandpass filter set.
- For capturing images, suitable single bandpass filters for the respective fluorochromes should be used. For details please inquire.

Sample Preparation

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 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-9002-500-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:

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.