MetaSystems‘ Multi-color probe kits

Product	Ref.-No.	Pack Size
24XCyte		
 CE	D-0125-060-DI	60µl probe cocktail
	D-0125-120-DI	120µl probe cocktail
	D-0125-600-DI	600µl probe cocktail
21XMouse		
 CE	D-0425-060-DI	60µl probe cocktail
	D-0425-120-DI	120µl probe cocktail
	D-0425-600-DI	600µl probe cocktail
22XRat		
 CE	D-1525-060-DI	60µl probe cocktail
	D-1525-120-DI	120µl probe cocktail
	D-1525-600-DI	600µl probe cocktail

The kits contain different chromosome painting probes specific for the individual chromosomes. Each paint is labeled with 1 of 5 different fluorochromes or a unique combination of them. The excitation/emission spectra of the fluorochromes are equivalent to FITC, Orange, TexasRed®, Aqua and Cy™5. The details of the labeling scheme are given below.

IVD Inside the EU: For in-vitro diagnostic purpose only!
For professional use only!!

Outside the EU: For research purpose only!

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REF J-9020-125-EN

General Information

Fluorescence *In Situ* Hybridization (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei of fixed cultured or uncultured cytogenetic samples. The technique uses DNA probes that hybridize to entire chromosomes or single unique sequences, and serves as a powerful adjunct to classic cytogenetics. Target DNA, after fixation and denaturation is available for annealing to a similarly denatured, fluorescent labeled DNA probe which has a complementary sequence. Following hybridizations, unbound and non-specifically bound DNA probe is removed by formamide-free stringent washes and the DNA is counter-stained for visualization. Fluorescence microscopy then allows the visualization of the hybridized probe on the target material.

Important Informations – Precautions and Warnings

- In the EU, MetaSystems' probes are classified as for *in vitro* diagnostic use.
  Outside the EU, MetaSystems' probe kits are for *research only*, unless otherwise stated.
- All probes by **MetaSystems** are for professional use and should only be used by qualified and trained personnel.
- Probes were produced and tested according to our quality system. 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 fumes; avoid skin contact! Wear gloves and a lab coat. In case of contact with skin or eyes, wash immediately with wate
⚠	**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 your institution's guidelines for hazardous waste disposal.

Storage

MetaSystems' probes should be stored at -20 °C. Please observe the expiry date indicated on the vials' labels. Reagents' exposure to light should be restricted to a minimum. Please store vials in the dark.

Quality Control and Product Labeling

Each vial of MetaSystems' probes and reagents is clearly labeled. You can find the following information on each label:

- Product description
- Catalogue number
- Lot number
- Storage temperature

Customer Support

If you have any difficulties in obtaining the desired results, please contact the **MetaSystems Probe Department**

by telephone : **+49 6205 39610**
by e-mail : **probes@metasystems.de**

Equipment Required but not Supplied

Solutions

- Water, double distilled
- 100 % Ethanol, denatured
- 1 N NaOH
- Tween™20 (Polyoxyethylenesorbitan-monolaurate syrup, e.g. Sigma P-1379)
- 20 x SSC (Saline-sodium Citrate Buffer: 3.0 M NaCl and 0.3 M $C_6H_5Na_3O_7$)

  Add 175.2g NaCL (Sodium chloride, M = 58,44g/mol) and 88.3 g $C_6H_5Na_3O_7$*$2H_2O$ (Citric Acid Trisodium Salt Dihydrate resp. tri-Sodium citrate Dihydrate, M = 294.10 g/mol) per liter of final volume to distilled water. We recommend using premade solutions available from different suppliers.
- DAPI/antifade: e.g. MetaSystems: D-0902-060-DA
  D-0902-120-DA

Laboratory Equipment

- Water bath with accurate temperature control
- Freezer (-20 °C)
- pH meter, calibrated
- Timer
- Microcentrifuge
- Immersion oil, recommended by the microscope manufacturer (fluorescence grade)
- Cover slips (glass): 22 x 22 mm² and 24 x 32 mm²
- Forceps
- Gloves
- Humidified chamber 37 °C
- Fluorescence Microscope with suitable filters (see below)
- Variable micropipettes with volumes ranging from 1 µl to 1 ml, calibrated
- Coplin jars
- Thermometer
- Rubber Cement
- Imaging system, e. g. **Isis** (**MetaSystems**)

Fluorescence Microscope Recommendation

For optimal visualization of the probe we recommend

- Fluorescence illumination: 100 watt mercury lamp illuminators (HBO) or metalhalide fluorescence illumination systems, like EXFO XCITE (EXFO, Canada), LEJ HXP120 (LEJ, Germany, also available from CARL ZEISS or LEICA), or similar.
- Objectives: objectives x 63 or x 100 suitable for epi-fluorescent illumination.

Fluorescence Filters Recommendations

- for **24XCyte**, **21XMouse** and **22XRat** probe kits: for all multi-color probe kits band path filters with narrow band characteristic should be employed to minimize (avoid) spectral cross-talking between fluorochromes. These filter sets by CHROMA are recommended by MetaSystems:

Label	Filter	Recommended for
DAPI (counterstain)	SP-100	24XCyte, 21XMouse, 22XRat
aqua	31036v2	
green	MF-101	
orange	31003	
red	SP-107 (SP-103v1)	
near infrared	SP-104v2	

Imaging System Recommendations

MetaSystems recommends the **Isis** imaging system. For mFISH/**mBAND** probe kits, the color karyotyping and mFISH/**mBAND** software upgrades are required. Please note, that the **mBAND** technique and the **XCyte** probe kits are proprietary to **MetaSystems** and maybe cannot be analyzed using other imaging systems.

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 Finished Slides

Hybridized FISH slides can be analyzed for at least two weeks if stored in the dark at temperatures below +4 °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.

Protocol for 24XCyte, 21XMouse, XRat and XCyte

Slide Denaturation

Solutions required:

- 0.1 x SSC, pH 7.0 - 7.5, room temperature
- 0.1 x SSC, pH 7.0 - 7.5, 4 °C
- 2 x SSC, pH 7,0 - 7.5, 70 °C (+/- 1 °C)
- 2 x SSC, pH 7,0 -7.5, 4 °C
- NaOH 0.07 mol/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 2 x SSC to 70 °C (+/- 1 °C) in a water bath.
3. Put slides into 2 x SSC at 70 °C (+/- 1 °C) and incubate slide **for 30min.**
4. Remove coplin jar from water bath, let cool **for 20 min**.
5. Transfer slide to 0.1 x SSC at room temperature **for 1 min**.
6. Denature slide in 0.07 N NaOH at room temperature **for 1 min**.
7. Put slide into 0.1 x SSC 4°C **for 1 min**.
8. Put slide into 2 x SSC, 4°C **for 1 min**.
1. Transfer to a coplin jar with 70% ethanol **for 1 min**.
2. Subsequently, transfer to a coplin jar with 95 % and 100 % ethanol, incubate **for 1 min each**.
3. Let air dry.

Probe Denaturation and Hybridization

Note: Start the probe denaturation during pretreatment and denaturation of chromosome slides. Time the procedure so that the prepared slide has just dried when the probe prehybridization is due.

Procedure:

1. Prepare probe cocktail according to the intended hybridization area: 7 µl for a 18 x 18 mm^2 cover slip, 10 µl for a 22 x 22 mm^2 cover slip, or 12 µl for a 24 x 24 mm^2 cover slip.
2. Denature probe by incubating at 75 °C (+/- 1ºC) **for 5 min**.

   Note: If you have a PCR machine, you could use it for probe denaturation and prehybridization. (program: 75 °C for 5 min, 10 °C for 30 s, 37 °C for 30 min).
3. Put on ice briefly.
4. Incubate at 37 °C (+/- 1 ºC) **for 30 min**.
5. Spin briefly to collect probe cocktail .
6. Pipette denatured and prehybridized probe cocktail onto the denatured chromosome preparation.
7. Overlay with cover slip.
8. Seal with rubber cement.
9. Incubate 1 - 2 days in a humidified chamber at 37 °C (+/- 1 ºC).

Posthybridization Washing

Solutions required:

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

Procedure:

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

Counterstain

Solutions required:

- MetaSystems' *DAPI/antifade* (■■■■■) (Ref.-No. D-0902-060-DA or D-0902-120-DA) or DAPI/antifade (250 ng/ml)

Note: DAPI has to be applied at low concentration to avoid crosstalking to DEAC filter or to wide FITC filter. Some antifading reagents do not work well for all fluorchromes.

Procedure:

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

Labeling Scheme

24XCyte

Chr.	aqua	green	orange	red	nir*
1					
2					
3					
4					
5					
6					
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8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

21XMouse

Chr.	aqua	green	orange	red	nir*
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
X					
Y					

22 XRat

Chr.	aqua	green	orange	red	nir*
1					
2					
3					
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11					
12					
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14					
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X					
Y					

nir = near infrared

Troubleshooting

Several factors may influence the results. Do not focus only on one potential for the source of trouble, reflect on the whole process: hybridization procedure, microscopy, and image processing.

Problem	Potential Cause(s)	Action
• No FISH signals can be detected in a FISH microscopes	• Reflected light shutter closed / stop slider in light path. • Fluorescent lamp is off. • Wrong fluorescence filter in light path. • Objective out off position. • Phototube 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 soak in-between slide and coverslip.	• Replace coverslip and DAPI/antifade. Use 24 x 60 mm² coverslip even if only a small region is hybridized.
• Diffuse signals	• Preparation not adquately illuminated. • Focus plane cannot be adjusted poperly. • Layer is too thick for focussing.	• Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp. • Use enough immersion oil. Do not mix up different immersion oils. Use immersion oil suitable for fluorescence. • Do not use too much DAPI/antifade. 15 to 20 µl per slide (24mm x 60 mm cover slip) are sufficient.
Weak signals	• Chromosome slide preparation too old • Denaturation of chromosomes not adequate.	• Slides should not be older than two weeks. We recommend slide preparation one day before hybridization • Aging, baking or further fixation may inhibit the hybridization and is not recommended.
Weak DEAC signals	• DAPI intensity is too high resulting in crosstalk to DEAC filter.	• Use DAPI/antifade of low concentration.
Weak FITC signals	• pH value of washing solutions to low. • DAPI intensity is too high resulting in crosstalk to FITC filter.	• Ensure that pH value is between 7.0 and 7.5 of solutions. FITC fluorophores are very sensible to pH below 7. • Use DAPI/antifade of low concentration.
High unspecific background	• Remaining cytoplasmic proteins of the cells may impair the hybridization.	• Pretreat slides with Pepsin.
HIgh diffuse background in green color channel.	• pH value of washing solutions to low. • DAPI intensity is too high resulting in crosstalk to FITC filter.	• Ensure that pH value is between 7.0 and 7.5 of solutions. FITC fluorophores are sensitive to pH below 7. • Reduce DAPI/antifade concentration.
If the recommended measures do not solve the problem, or your problem is not listed, please contact MetaSystems.		

Trademarks

Cy™ is a trademark of GE Healthcare, UK Limited
TexasRed® is a registered trademark of Molecular Probes, Inc.
Tween™ is a trademark of ICI America, Inc.

MetaSystems disclaims any proprietary interest in the marks and names of others.

Patent Information

The **mBAND** procedure is subject of German patent No. DE 198 06 303 C2 ; U.S. patent 7,050,911, and European patent No. 0 957 176 B1 owned by MetaSystems GmbH.

References

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

Symbols Used

Symbol	Description
IVD	This symbol marks a product as a “In Vitro Diagnostic Medical Device”.
	Next to this symbol you will find the detailed address and contact details of **MetaSystems** (manufacturer).
	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION.
	This symbol refers to additional important product information.
REF	This symbol points out the order no. of this MetaSystems Laboratory Manual