Troubleshooting

Problem	Potential Cause(s)	Recommended Solution
No FISH signals are detected in the microscope.	• Excessive digestion of nuclei may lead to loss of FISH signals.	• Adjust pretreatment and protease conditions by reducing incubation time.
Diffuse signals.	• Over-digestion may lead to degradation of DNA.	• Adjust pretreatment and protease conditions by decreasing incubation time.
	• Antifade layer is too thick for focusing.	• Do not use too much DAPI/antifade. 15 µl per slide (24 x 60 mm² coverslip) are sufficient.
Hybridization signals become weak after a while.	• Immersion oil soaked in-between slide and coverslip.	• Replace coverslip and DAPI/antifade by removing coverslip, gently soaking the slide in 70 % ethanol and finally in 100 % ethanol for a few seconds. Air dry. Proceed with the counterstain protocol. Use 24 x 60 mm² coverslip even if only a small region is hybridized.
Some nuclei do not show the expected signal pattern.	• Part of the nucleus has been cut away.	• Tissue sections of 4 – 6 µm are advised.
	• Signals may appear in different planes of the nucleus.	• Focus through different planes.
Weak DAPI counterstain.	• DAPI concentration too low.	• Use DAPI/antifade of higher concentration. Please note: A high DAPI intensity may lead to crosstalk to AQUA filter or GREEN filter.
	• DAPI incubation time too short.	• Make sure that an incubation time of at least 10 min has been kept.
Weak unspecific background signal.	• Non-specific hybridization or cross-hybridization.	• Unspecific background can be reduced by increasing the stringency of the post-hybridization washings through higher temperature (74 °C for 4 min) or lower salt concentration (0.25x SSC).
High unspecific background.	• Remaining intercellular, cellular and cytoplasmic structures may impair the hybridization.	• Adjust pretreatment and protease conditions by increasing incubation time.
Poor morphology of nuclei: dark areas inside the nuclei, loss of nuclear borders.	• Over-digestion may lead to degradation of nuclei.	• Adjust pretreatment and protease conditions by reducing incubation time.
Tissue sections became detached.	• Pretreatment and protease conditions are too strong.	• Adjust pretreatment and protease conditions by reducing incubation time.
If the recommended measures do not solve the problem or your problem is not listed, please also refer to the troubleshooting in the pack insert of the respective MetaSystems DNA FISH probe or contact MetaSystems Probes.		

Customer Support

Please contact MetaSystems Probes GmbH in Germany or MetaSystems Group, Inc. in the USA by telephone or e-mail (contact details, see below). MetaSystems disclaims any proprietary interest in the marks and names of others.

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Symbols Used

Symbol	Description	Symbol	Description
⚠	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION	REF	Reference number
		LOT	Lot number
	Manufacturer		Maximum storage temperature
	Expiry date		Temperature limitation for storage. Lower and upper limits are indicated.

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MetaSystems Probes

TissueFISH Pretreatment Kit Part 1 of 2

MetaSystems' TissueFISH Pretreatment Protocol for Formalin-Fixed Paraffin-Embedded Tissue sections

REF D-0905-025-TF 01

LOT XXXXX

YYYY-MM

For laboratory use

	Components	Lot Number	Package Size	Storage Temperature
Part 1	Pretreatment Buffer	XXXXX	5x5ml	at RT *
	Protease Buffer	XXXXX	200ml	
Part 2	Protease Stock Solution	XXXXX	500µl	2 °C – 8 °C

* Some precipitations may occur at lower temperatures.
In this case please heat Protease Buffer to 25 °C and shake until the precipitate is disappearing.

Materials Provided

5 flasks of 10x Pretreatment Buffer (5 ml each), 1 flask of Protease Buffer (200 ml) and one vial of Protease Stock Solution (0.5 ml). Reagents are sufficient to run 5 procedures of 5 slides each.

Intended Use

MetaSystems' TissueFISH Pretreatment Kit is intended to be used for pretreatment of formalin-fixed paraffin-embedded (FFPE) tissue sections.

Safety Instructions

The TissueFISH PreTreatment Kit produced by MetaSystems Probes is for professional use and should only be used by qualified and trained personnel. In order to ensure safe operation and reproducible results please observe the safety notices and caution signs below.

⚠	**CAUTION: Hot water baths 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.

Solutions required and not supplied:

- ddH_2O
- Xylene or substitute
- Ethanol, 100 %
- 2x SSC, pH 7.0 – 7.5, room temperature (RT, ~18 – 22 °C)
- DNA FISH probe. We recommend using MetaSystems' XCyting DNA FISH Probes.
- 0.4x SSC, pH 7.0 – 7.5, 72 °C (± 1 °C)
- 2x SSC, 0.05 % Tween 20, pH 7.0, RT
- MetaSystems' DAPI/antifade, D-0902-500-DA (250 ng/ml)

Solutions to be prepared freshly and to be used only once:

Pretreatment Buffer:

1. Dilute 10x Pretreatment Buffer to 1x with ddH_2O, prior to use. (Add 45 ml ddH_2O to 1 flask of 10x Pretreatment Buffer)
2. Use a water bath to heat 50 ml 1x Pretreatment Buffer in a Coplin jar to 96 – 98 °C.

Protease Solution:

1. Pour 40 ml Protease Buffer into a Coplin jar at RT.
2. Add 80 µl of Protease Stock Solution just prior to use.

Procedure

Deparaffinization:

1. Heat FFPE slide on a hot plate to 60 °C for 2 min or until wax appears melted.
2. Immerse the still hot slide in xylene for 10 min at RT. Refresh xylene and repeat this step 1x.
3. Immerse slide in 100 % ethanol for 5 min at RT. Refresh 100 % ethanol and repeat this step 1x.
4. Slides can be dried at this point but should be further processed the same day.

Pretreatment:

1. Incubate slides in 1x Pretreatment Buffer (pH ~ 6.0, 96 – 98 °C). Please refer to Pretreatment Guidelines Chart below.
2. Wash slide for 5 min in 2x SSC at RT.
3. Immerse slide in Protease Solution at RT. Please refer to Pretreatment Guidelines Chart below.
4. Wash slide for 5 min in 2x SSC at RT.
5. Dehydrate slide for 2 min in 100 % ethanol and let air dry.
6. Apply 10 (7) µl of DNA FISH probe.
7. Cover with coverslip 22 x 22 (18 x 18) mm^2.
8. Seal with rubber cement.

Pretreatment Guidelines Chart		Protease Solution Incubation Time at RT		
		2.5 – 5 min	5 – 7.5 min	7.5 – 10 min
1x Pretreatment Buffer Incubation Time at ~96 – 98°C	20 min		liver, thyroid gland	brain, kidney, pancreas, stomach
	30 min	skin, bone marrow, breast, colon, lung, lymph node, uterine cervix, placenta, prostate, esophagus, tonsil	urinary bladder	spleen, testis
Parameters were optimized using "MetaSystems` XCyting DNA FISH Probes", tissue sections of 4 µm and 5 slides per Coplin jar. Pretreatment and protease conditions may differ depending on origin and condition of tissue and on upstream processing (e.g. tissue fixation, processing, embedding, cutting size). Prolonged pretreatment (> 30 min at 98 °C) and protease incubation (> 10 min at RT) may result in over-digestion. The degree of digestion will influence the quality of hybridization, signal to noise ratio and counterstaining. Tests are recommended to determine incubation times empirically.				

Denaturation and Hybridization:

1. Denature sample and probe simultaneously by heating slide on a hot plate at 75 °C (± 1 °C) for 5 min.
2. Incubate in a humidified chamber at 37 °C (± 1 °C) overnight.

Post-Hybridization Washing:

1. Remove coverslip and all traces of glue carefully.
   Optional: Immerse slide into 2x SSC at RT until coverslip floats off.
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 RT for 30 seconds.
4. Rinse briefly in distilled water to avoid crystal formation and let air dry.

Counterstain:

1. Apply 10 µl of the MetaSystems DAPI/antifade and overlay with a 24 x 32 mm^2 coverslip.
2. Allow penetration of DAPI/antifade for 10 min.
3. Proceed with microscopy and analysis or store slides at –20 °C. Hybridization signals are fine for at least six months.