CE IVD

ZytoPure® FISH Accessory Kit (20 tests)

Catalogue No.

- FA-Kit1-20 (20 tests)

Instructions for use

Intended use

The ZytoPure® FISH Accessory Kit is intended to be used for detection of DNA sequences in formalin-fixed, paraffin-embedded (FFPE) tissue specimens by fluorescence *in situ* hybridization (FISH) in combination with ZytoPure® FISH Probes. It is intended for use in *in vitro* diagnostic applications.

Summary and Description

Fluorescence *in situ* hybridization (FISH) allows specific detection of nucleic acid sequences directly in cells or tissue specimens. After pretreatment of cells or tissue specimens by heat and proteolytic digestion, a fluorophore-labeled DNA probe is applied to the specimen. Single stranded DNA in both probe and specimen is generated by denaturation at 75°C, and in a subsequent hybridization step at 37°C sequence specific duplexes are formed between the labeled probe DNA and the cellular target DNA. After removing excessive, non-bound probe through several washing steps, specific bound DNA probe can be detected by fluorescence microscopy. The microscope has to be equipped with filter sets suitable for the fluorescent dyes of the probe.

The ZytoPure® FISH Accessory Kit contains reagents necessary for performing FISH analysis and is intended to be used in combination with a ZytoPure® FISH Probe.

Reagents/Components provided

Component	Volume / Quantity	Vessel
FISH Pretreatment Buffer, Ready-to-use	500 ml	Screw-cap bottle (large)
Pepsin Solution, Ready-to-use	4 ml	Dropper bottle (white cap)
FISH Wash Buffer 20x	2 x 50 ml	Screw-cap bottle (medium)
DAPI/Antifade Solution, Ready-to-use	800 µl	Reaction vial (brown)
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Storage and handling

The kit should be stored at 2 - 8°C and is suitable for use until the expiry date indicated on the label. Do not use the kit after expiry date. DAPI/Antifade Solution must be protected from light while stored.

Precautions

- Use by qualified personnel only.
- Carefully read instructions for use prior to use.
- The product contains dangerous or potentially allergenic substances (in small volumes). Wear protective clothing to avoid contact of reagents and specimens with eyes, skin or mucous membranes.
- If reagents or specimens come in contact with sensitive areas, wash with large amounts of water.
- Disposal of reagents must be carried out in accordance with local regulations.
- A material safety data sheet (MSDS) is available upon request.

Materials required but not provided

- Xylene, ethanol 100%, deionized or distilled water
- Water bath, adjustable to 37°C and 98°C
- Staining jars
- Adjustable pipettes
- Rubber Cement (e.g. Zytomed Systems Cat. No. ZY-FX125 or ZY-FX50)
- Glass slides, coated, positively charged
- Coverslips (e.g. 22 mm x 22 mm, depending on tissue area to be hybridized)
- Hybridizer (e.g. Zytomed Systems ZytoBrite Hybridizer TDH-500)
- Fluorescence microscope equipped with appropriate filter sets (see instructions for use of the ZytoPure® FISH Probe for specifications)
- Immersion oil approved for fluorescence microscopy
- ZytoPure® FISH Probe

Important Notes

- The specimens must not dry out during hybridization and washing steps.
- All protocol steps involving ZytoPure® FISH probes and DAPI/Antifade solution should be performed under dimmed light.
- Store finished slides in the dark.
- Specifications for temperatures should be followed carefully. Any change of temperatures can negatively affect the quality of results.

Pre-analytic steps, preparation of specimens

- The ZytoPure® FISH Accessory Kit is optimised for use on formalin-fixed, paraffin-embedded tissue (FFPE). Fixation of the tissue should be performed at room temperature using 10% neutral buffered formalin for a sufficient period of time (e.g. 16 - 24 hours for tissue samples of 5 - 10 mm diameter). Pay attention to correct processing of the tissue sample including sufficient dehydration.
- Prepare sections of FFPE tissue with a thickness of 3 - 5 µm and mount them on coated, positively charged microscope slides.
- Mounted sections should be dried for 16 h minimum at 37°C in a drying oven before use in *in situ* hybridization procedures.
- We recommend always using fresh xylene/ethanol solutions for dewaxing. Do not use recycled solutions.
- We recommend optimising pepsin incubation time for every tissue type. As a guideline for tissue sections perform pepsin incubation for 5 - 15 min at 37°C. FFPE tissue stored for a long period of time (archive material) may require prolongation of pepsin incubation.

***In situ* hybridization procedure for FFPE tissue sections using the ZytoPure® FISH Accessory Kit**

Day 1

Preparatory steps

- Preparation of two ethanol series:
  - For dewaxing: 2 x 100%, 1x 90%, 1x 70% ethanol
  - For dehydration: 1x 70%, 1x 90%, 1x 100% ethanol
- Pre-warm „FISH Pretreatment Buffer“ to 98°C
- Prepare 1x „FISH Wash Buffer“ for one staining jar by mixing one part of 20 X FISH Wash Buffer with 19 parts of laboratory-grade water. E.g., add 95 ml of H_2O to 5 ml of 20 X FISH Wash Buffer.
- Bring ZytoPure® FISH Probe to room temperature

1) Dewaxing

• Incubate slide on hybridizer or heating plate This step does not replace the drying step (min. 16h at 37°C).	10 min	70°C
• Incubate slide in 100% xylene	2 x 10 min	RT
• Incubate slide in 2 x 100%, 1 x 90%, 1 x 70% ethanol	5 min each	RT
• Wash slide in H_2O_{dest}	2 x 2 min	RT

2) Tissue pretreatment, proteolysis

• Incubate slide in “FISH Pretreatment Buffer”	15 min	98°C
• Wash slide in H_2O_{dest}	2 x 2 min	RT
• Apply „Pepsin Solution“, incubate Optimal time of proteolysis should be determined in preliminary tests, guideline for FFPE tissue samples is 5 - 15 min.	5 - 15 min	37°C
• Wash slide in 1x „FISH Wash Buffer“	5 min	RT
• Wash slide in H_2O_{dest}	1 min	RT
• Dehydrate slide in 70%, 90%, 100% ethanol	1 min each	RT
• Air dry slide		

3) Denaturation and hybridization (protected from light)

• Apply ready-to-use ZytoPure® FISH Probe to tissue specimen by pipetting (use 10 µl probe for 22 mm x 22 mm cover slip)		
• Cover specimen with cover slip, seal with rubber cement		
• Denaturation in hybridizer Note: Provide humid atmosphere in hybridizer chamber.	10 min	75°C
• Hybridization in hybridizer Note: Specimens must not dry out during hybridization step, provide humid atmosphere in hybridizer chamber.	16-24 h	37°C

Day 2

Preparatory steps

- Prepare 1x „FISH Wash Buffer“, sufficient for 3 staining jars
- Pre-warm 3 staining jars with 1x „FISH Wash Buffer“ to 37°C
- Bring „DAPI/Antifade-Solution“ to room temperature (protected from light)

4) Post-hybridization (under dimmed light)

• Carefully remove rubber cement sealing		
• Incubate slide in 1x „FISH Wash Buffer“ for removal of cover slip	1 - 3 min	37°C
• Wash slide in 1x „FISH Wash Buffer“	2 x 5 min	37°C
• Dehydrate slide in 70%, 90%, 100% ethanol	1 min each	RT
• Air dry slide		

5) Nuclear counterstaining (under dimmed light)

• Apply „DAPI/Antifade-Solution“ (use ~15 µl for 22 mm x 22 mm cover slip)		
• Cover slide with cover slip, avoid air bubbles		
• Incubate (protected from light)	15 min	RT
• Remove excess „DAPI/Antifade-Solution“ by gently pressing the slide between filter paper		

6) Analysis

- Analyse specimen using a fluorescence microscope equipped with suitable filter sets

7) Storage of slide

- Store slide at 2 - 8°C protected from light

Quality controls

- We recommend carrying out internal and external controls with every hybridization run to monitor the performance of reagents used and for correct interpretation of results.
- Non-neoplastic cells/nuclei can serve as an internal control. Occasional loss of signals can occur and is due to truncation artifacts in FFPE tissue sections.
- Formalin-fixed, paraffin-embedded cells from cell cultures with known gene status can be used as external controls (e.g. Zytomed Systems Cell Control Array Receptor Cat. No. MB-CC REZ for ERBB2/CEP17 ZytoPure® FISH Probe).

Limitations of the procedure

- Interpretation of results should only be done by qualified personnel familiar with the method and probe signal patterns under the supervision of a pathologist. Modifications of the protocol may lead to divergent results. They are the responsibility of the user and are to be validated for correct outcome.
- Unsuitable pre-analytic parameters, like prolonged ischemia, over- or underfixation, insufficient dehydration or embedding of tissue specimens can negatively affect the results.
- Tissue specific problems, like strong autofluorescence, can negatively affect the results.

Troubleshooting

Listed below are some of the most common sources of error. For problems that occur which are not listed here please contact Zytomed Systems` technical support (email: international@zytomed-systems.de) or your local distributor.

Missing or weak signals

Possible cause	Measures
Washing of specimen (day 2) too stringent	Check for correct washing temperature and time
	Check for correct dilution of 20x FISH Wash Buffer
Insufficient tissue pretreatment	Adjust pepsin incubation time
Inappropriate filter sets	Use filter sets with appropriate Ex./Em. specifications, see “Specifications” of the probe

Strong background and/or unspecific signals

Possible cause	Measures
Insufficient washing of specimen (day 2)	Check for correct washing temperature and time
	Check for correct dilution of 20x FISH Wash Buffer
Autofluorescence of specimens	Possible cause: inappropriate glass slides, specimen contains dyes for marking orientation of resected tissue, use of inappropriate pens for marking glass slides
Insufficient dewaxing of specimen	Use ZytoPure® FISH Accessory Kit (Cat. No. FA-Kit1-20) protocol, use fresh dewaxing solutions

Impaired tissue morphology

Possible cause	Measures
Pepsin incubation too long	Reduce pepsin incubation time, test different pepsin incubation times
Insufficient fixation of tissue sample	Use 10% neutral buffered formalin solution

Bibliography

- Liehr T (editor) : Fluorescence In Situ Hybridisation (FISH) Application Guide, Springer Protocols, Springer, 2010
- Beatty B, Mai S, Squire J (editors): FISH, A Practical Approach No. 260, Oxford University Press, 2002

Explanations of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue		Verwendbar bis Use By Utiliser jusque		Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation
LOT	Chargenbezeichnung Batch Code Code du lot		Lagerungstemperatur Temperature Limitation Limites de température	RUO	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement
IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro		Achtung/Gefahr Warning/Danger Attention/Danger		Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH Anhaltinerstraße 16 14163 Berlin, Germany Tel: (+49) 30-804 984 990 www.zytomed-systems.de
	Achtung/Gefahr Warning/Danger Attention/Danger		Achtung Warning Attention	Gefahr Danger Danger	

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