



ZytoLight
FISH Implementation Kit

20 reactions

For fluorescence *in situ* hybridization (FISH) using a FISH probe
of ZytoVision

FOR RESEARCH USE ONLY

Product No.: Z-2028

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1. Scope of Application

The FISH Implementation Kit is designed to be used for the detection of human DNA sequences in either formalin-fixed, paraffin-embedded tissue or cell samples by fluorescence *in situ* hybridization (FISH) using a FISH probe of ZytoVision.

This product is designed for research purposes only and not for use in diagnostic applications.

2. Basic Principles

The presence of certain nucleic acid sequences in cells or tissue can be detected with *in situ* hybridization using tagged DNA probes. The hybridization results in duplex formation of sequences present in the test object and the specific gene probe.

The ZytoLight FISH Implementation Kit system is to be used with any, separately available ZytoLight FISH probe. FISH probes of ZytoVision can contain green-labeled polynucleotides (ZyGreen: excitation at 503 nm and emission at 528 nm, similar to FITC) and/or orange-labeled polynucleotides (ZyOrange: excitation at 547 nm and emission at 572 nm, similar to Rhodamine).

Duplex formation of fluorescence-labeled probes can be visualized using fluorescence microscopy, employing suitable filters.

3. Safety Precautions and Disposal

- ✓ Read the operating instructions prior to use!
- ✓ Do not use the reagents after the expiry date has been reached!
- ✓ Avoid any cross-contamination and micro-bacterial contamination of the reagents!
- ✓ Some of the system components contain substances (in low concentrations and volumes) that are harmful to health (SDS, PPD). Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses and lab garments)!

- ✓ If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
- ✓ Never pipet solutions with your mouth!
- ✓ The disposal of reagents must be carried out in accordance with local regulations!
- ✓ A material safety data sheet is available on request for the professional user!

4. The FISH Implementation Kit

4.1 Components

The kit is made up of the following components:

Code	Component	Quantity	Container
1	FISH Pretreatment Solution Citric I	500 ml	Screw-cap bottle (large)
2	Pepsin Solution	4 ml	Dropper bottle, white cap
3	2x SSC Solution	500 ml	Screw-cap bottle (large)
-	-	-	-
5	25x Wash Buffer A	2x 50 ml	Screw-cap bottle (large)
6	DAPI/Antifade-Solution	0.8 ml	Reaction vessel, blue lid
	Instructions	1	

A *ZytoLight* FISH probe is not included in the kit and can be ordered separately.

Components **(2)** and **(6)** are sufficient for approx. 20 reactions. Component **(5)** is sufficient for 10x 3 staining jars of 80ml each. Components **(1)** and **(3)** are sufficient for 6 staining jars of 80 ml each.

4.2 Storage and Shelf Life

The components of the FISH Implementation Kit must be stored at temperatures between 2°C and 8°C except the DAPI/Antifade-Solution (6), which must be stored at -20°C in the dark; a short-time storage at 4°C is possible.

If these storage conditions are followed, the FISH Implementation Kit will function, without loss of performance, at least until the expiry date printed on the label.

4.3 Test Material

The FISH Implementation Kit has been optimized for use with formalin-fixed, paraffin-embedded tissue and cell samples. When test material is used that has been fixed or embedded in a different manner (e.g. methanol-glacial-acetic-acid fixed cells or blood smears) the test protocol may need to be adapted by the user. Our specialists are available to support you whenever adjustments are necessary.

We recommend the following tissue preparation:

- ✓ Fixation in 10% neutrally buffered formalin for 24 h at RT

In order to achieve optimum and uniform fixation and paraffin embedding, the sample size should not exceed 0.5 cm³.

- ✓ Standard processing and paraffin embedding

Use premium quality paraffin. Infiltration and embedding should be carried out at temperatures lower than 65°C.

- ✓ Prepare 2-5 µm microtome sections

Draw up the sections onto silane-coated or adhesion slides (e.g. HistoBond®) and fix for 2-16 h at 50°C-60°C.

4.4 Additional Materials

The following reagents and materials are not included in the kit:

- *ZytoLight FISH probe*
- *Xylene*
- *Water bath (37°C, 98°C)*
- *Hot plate*
- *Hybridization oven (heating oven)*
- *Staining jars, 50-80 ml*
- *Humidity chamber*
- *Pipet (10 µl, 30 µl)*
- *Adhesive pistol, including hot adhesive, or rubber cement (Fixogum)*
- *Ethanol 100%, denatured*
- *Distilled or deionized water*
- *Drying block*
- *Coverslips (22 mm x 22 mm and 24 mm x 60 mm)*
- *Fluorescence microscope*

4.5 Important Information

The following should be kept in mind:

- ✓ The tissue and cell sections must not be allowed to dry during the hybridization and washing steps!
- ✓ DNA-Probes and DAPI/Antifade-Solution (6) should not be exposed to light, especially strong light, for a longer period of time, i.e. all steps should be accomplished, where possible, in dark and/or lightproof containers!
- ✓ The temperature for denaturing and washing, described in the protocol, should be used as a guide. Dependent upon the age and the fixation step of the sample material, an increase or decrease in temperature of the denaturing or wash steps can lead to better hybridization results!
- ✓ This protocol is designed for the simultaneous denaturing of probe and sample. Protocols for individual denaturation are available on our homepage (www.zytovision.com)!

5. The FISH Implementation Kit Protocol

5.1 Preparatory Steps

- Day 1:

Preparation of two ethanol series (70%, 90% and 100% ethanol solutions): Dilute 7, 9, and 10 parts of 100% ethanol with 3, 1, and 0 parts of distilled or deionized water. These solutions can be stored in suitable containers and re-used (day 2).

FISH Pretreatment Solution Citric I (1): Warm to 98°C.

2x SSC Solution (3): Bring to room temperature.

- Day 2:

Preparation of 1x Wash Buffer A: Dilute 1 part 25x Wash Buffer A (5) with 24 parts distilled or deionized water. Fill three staining jars with the 1x Wash Buffer A and pre-warm it to 37°C.

DAPI/Antifade-Solution (6): Bring to room temperature before use, protect from light.

5.2 Pre-treatment (Dewax /Proteolysis) (day 1)

1. Incubate slides for 10 min at 70°C (e.g. on a hot plate)
2. Incubate slides for 2x 10 min in xylene
3. Incubate in 100%, 100%, 90% and 70% ethanol each for 5 min
4. Wash 2x 2 min in deionized or distilled water
5. Incubate for 15 min in pre-warmed FISH Pretreatment Solution Citric I (1) at 98°C

We recommend not to use more than six slides per staining jar.

6. Transfer slides immediately to deionized or distilled water and wash for 2x 2 min
7. Apply (dropwise) Pepsin Solution (2) to the air-dried tissue/cell section and incubate for 10 min at 37°C in a humidity chamber.

Depending on multiple factors, e.g., nature and duration of fixing, thickness of sections and nature of tissue/cells, different incubation times may be required. As an incubation guideline we recommend an incubation time of 10-30 minutes for tissue samples and 5-10 minutes for cell samples. As a general rule, we recommend that the optimum time for proteolysis will be ascertained in pre-tests.

8. Wash for 5 min in 2x SSC Solution (**3**) and 1 min in distilled or deionized water

9. Dehydration: in 70%, 90% and 100% ethanol each for 1 min

Air dry sections.

5.3 Denaturation and Hybridization (day 1)

1. Pipette 10 μ l of a ZytoLight FISH probe onto individual samples

A gentle warming of the probe, as well as using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure of the probe to light.

2. Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm). Seal the coverslip, e.g. with a layer of hot glue from an adhesive pistol or rubber cement

3. Denature the slides at 75°C (\pm 2°C) for 10 min, e.g. on a hot plate

Depending upon the age of the sample and variations in the fixation stage, it may be necessary to optimize the denaturing temperature (73°C-77°C).

4. Transfer the slide to a humidity chamber and hybridize overnight at 37°C (e.g. in a hybridization oven)

It is essential that the tissue/cell samples do not dry out during the hybridization step.

5.4 Post-Hybridization and Detection (day 2)

1. Carefully remove the rubber cement or glue

2. Remove the coverslip by submerging in 1x Wash Buffer A at 37°C for 1-3 min

3. Wash in 1x Wash Buffer A for 2x 5 min at 37°C

The 1x Wash Buffer A should be pre-warmed. Check with a thermometer if necessary.

- 4.** Incubate the slides in 70%, 90% und 100% ethanol, each for 1 min

Air dry the samples protected from light

Alternatively, wash the samples in distilled or deionized water for 5 min at room temperature and allow to air dry.

- 5.** Pipette 30 μ l DAPI/Antifade-Solution (6) onto the slides. Avoiding trapped bubbles, cover the samples with a coverslip (24 mm x 60 mm). Incubate in the dark for 15 min

Using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure to light.

- 6.** Carefully remove excess DAPI/Antifade-Solution (6) by gently pressing the slide between filter papers

- 7.** Store the slide in the dark. For longer storage periods, this should take place at 4°C

- 8.** Evaluate the sample material by fluorescence microscopy. Filter sets for the following wavelength ranges are required: ZyGreen-labeled probe: excitation at 503 nm and emission at 528 nm, similar to FITC; ZyOrange-labeled probe: excitation at 547 nm and emission at 572 nm, similar to Rhodamine.

6. Interpretation of Results

With the use of appropriate filter sets, the hybridization signals of ZyGreen-labeled probes appear green; the hybridization signals of ZyOrange-labeled probes appear orange. In order to judge the specificity of the signals, every hybridization should be accompanied with controls. In interphases of normal cells or cells without aberrations of chromosomes two signals per probe/fluorescence label appear except for probes targeting X and/or Y chromosomes resulting in one to two signals per probe/fluorescence label depending on the gender. In cells with chromosomal aberrations, a different signal pattern can be visible in interphases, i.e. increased chromosomal region copy number.

The polynucleotides contained in FISH probes can function in themselves, as an internal control that a successful hybridization has occurred, as well as proving the integrity of the cellular DNA.

Additionally, we recommend using at least one control sample in which the copy number of chromosomal regions targeted by the FISH probe is known.

Care should be taken not to evaluate cells lying on each other, in order to avoid false results, e.g. an amplification of genes.

A negative or unspecific result can be caused by multiple factors (see point 8).

7. Literature

Kievits T, et al. (1990) *Cytogenet Cell Genet* **53**: 134-136.

Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992); ISBN 0 19 963327 4.

8. Problems and Possible Causes

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Problem	Possible cause	Action
Streaks on the slide after stopping the pepsin treatment	Precipitation	Wash section immediately in distilled or deionized water
Weak signal or no signal at all	No target sequences available	Use controls
	Cell or tissue sample has not been properly fixed	Optimization of fixing time
	Proteolytic pretreatment not carried out properly	Optimization of incubation time
	Denaturing temperature not properly	Check temperature if necessary increase or decrease
	Hybridization temperature not correct	Check temperature
	Fluorescence microscope wrongly adjusted	Change adjustment; check appropriate filter sets
	Too strong beam of light while handling probes/slides	Accomplish hybridization and washing steps in the dark
Uneven and in some parts only very light staining	Incomplete dewaxing	Use fresh solutions; check length of dewaxing times
Cross hybridization signals; strong background staining	Probe volume per area too high	Reduce probe volume per section/area, distribute probe dropwise to avoid local concentration
	Proteolytic pretreatment too strong	Optimization of incubation time
	Dehydration of sections between the individual incubation steps	Prevent dehydration
	Washing temperature following hybridization too low	Check temperature
Section floats off the slide	Proteolytic pretreatment too strong	Shortening of incubation time
	Unsuitable slide coating	Use appropriate slides