

ZytoLight
FISH-Tissue Implementation Kit

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REF Z-2028-5 Σ 5

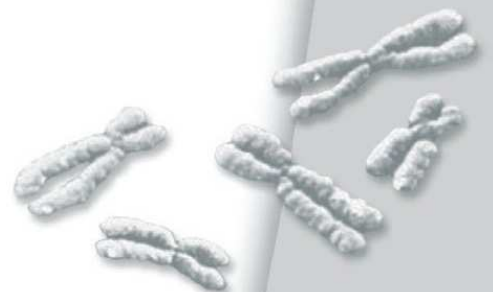
For fluorescence *in situ* hybridization (FISH) using any
ZytoLight FISH probe



IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



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1. Scope of Application

The ZytoLight FISH-Tissue 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).

Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Basic Principles

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

The ZytoLight FISH-Tissue Implementation Kit is to be used with any separately available ZytoLight FISH probe.

Duplex formation of the 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. 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 ZytoLight FISH-Tissue Implementation Kit

4.1 Components

The kit is made up of the following components:

| Code | Component | Quantity | | Container |
|------|--|-------------|--------|---------------------------|
| | | 20 ∇ | 5 | |
| PT1 | <u>Heat Pretreatment Solution Citric</u> | 500 ml | 150 ml | Screw-cap bottle (large) |
| ES1 | <u>Pepsin Solution</u> | 4 ml | 1 ml | Dropper bottle, white cap |
| WB1 | <u>Wash Buffer SSC</u> | 500 ml | 150 ml | Screw-cap bottle (large) |
| WB2 | <u>25x Wash Buffer A</u> | 2x 50 ml | 50 ml | Screw-cap bottle (medium) |
| MT7 | <u>DAPI/DuraTect-Solution</u> | 0.8 | 0.2 | Reaction vessel, blue lid |
| | Instruction manual | 1 | 1 | |

Z-2028-20 (20 tests): Components **(ES1)** and **(MT7)** are sufficient for 20 reactions. Component **(WB2)** is sufficient for 11x 3 staining jars of 70 ml each. Components **(PT1)** and **(WB1)** are sufficient for 7 staining jars of 70 ml each.

Z-2028-5 (5 tests): Components **(ES1)** and **(MT7)** are sufficient for 5 reactions. Component **(WB2)** is sufficient for 5x 3 staining jars of 70 ml each. Components **(PT1)** and **(WB1)** are sufficient for 2 staining jars of 70 ml each.

4.2 Storage and Shelf Life

The components of the kit must be stored at 2...8°C. The DAPI/DuraTect-Solution **(MT7)** must be stored protected from light.

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

4.3 Test Material

The ZytoLight FISH-Tissue Implementation Kit has been optimized for the 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 3-5 µm microtome sections
Draw up the sections onto silane-coated or adhesion slides (e.g. HistoBond®) and fix for 2-16 h at 50-60°C.

4.4 Additional Materials

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

- *ZytoLight FISH probe*
- *Water bath (37°C, 98°C)*
- *Xylene*
- *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*
- *Deionized or distilled water*
- *Drying block*
- *Coverslips (22 mm x 22 mm , 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 probe and DAPI/DuraTect-Solution (MT7) 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 the dark and/or using 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 separate denaturation are available on our homepage (www.zytovision.com)!

5. The ZytoLight FISH-Tissue 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 deionized or distilled water, respectively. These solutions can be stored in suitable containers and can be re-used (day 2).
- *Heat Pretreatment Solution Citric (PT1):* Warm to 98°C.
- *Wash Buffer SSC (WB1):* Bring to room temperature.

Day 2:

- *Preparation of 1x Wash Buffer A:* Dilute 1 part 25x Wash Buffer A (WB2) with 24 parts deionized or distilled water. Fill three staining jars with the 1x Wash Buffer A and pre-warm it to 37°C.
- *DAPI/DuraTect-Solution (MT7):* Bring to room temperature before use, protect from light.

5.2 Pretreatment (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 Heat Pretreatment Solution Citric (PT1) at 98°C

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

6. Transfer slides immediately to deionized or distilled water, wash for 2x 2 min and drain off or blot off the water
7. Apply (dropwise) Pepsin Solution (ES1) to the 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 5-15 min for

tissue samples and 0-3 min for cell samples. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.

8. Wash for 5 min in Wash Buffer SSC (WB1) and 1 min in deionized or distilled 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 ZytoLight FISH probe each 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 with rubber cement

3. Denature the slides at 75°C ($\pm 2^\circ\text{C}$) 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 (73-77°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, using 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%, and 100% ethanol, each for 1 min

Air dry the samples protected from light

5. Pipette 30 µl DAPI/DuraTect-Solution (MT7) 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/DuraTect-Solution (MT7) by gently pressing the slide between filter papers
7. Store the slide in the dark. For longer storage periods, this should take place at 2-8°C
8. Evaluation of the sample material is carried out by fluorescence microscopy. Filter sets for the following wavelength ranges are required:

| | | | |
|----------|---|--------------------|------------------|
| ZyBlue | – | excitation: 418 nm | emission: 467 nm |
| ZyGreen | – | excitation: 503 nm | emission: 528 nm |
| ZyGold | – | excitation: 532 nm | emission: 553 nm |
| ZyOrange | – | excitation: 547 nm | emission: 572 nm |
| ZyRed | – | excitation: 580 nm | emission: 599 nm |

6. Interpretation of Results

With the use of appropriate filter sets in interphases or metaphases 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 none 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 or metaphases. For a more detailed description of expected signal patterns, please refer to the respective probe manual.

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.

In order to judge the specificity of the signals, every hybridization should be accompanied by controls. 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 overlapping cells, in order to avoid false results, e.g. an amplification of genes. Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance equal to or less than the diameter of one signal, should be counted as one signal.

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

7. Literature

Kievits T, et al. (1990) *Cytogenet Cell Genet* **53**: 134-6.

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 deionized or distilled 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 correct | Check temperature; increase or decrease if necessary |
| | 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 |



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