ZYTOVISION

Zyto*Light*
FISH-Cytology Implementation Kit

REF Z-2099-20 Σ 20

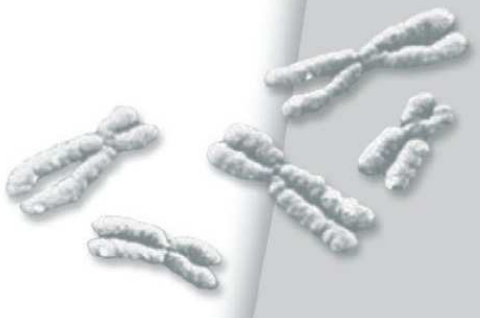
For fluorescence *in situ* hybridization (FISH) on cytology specimens using any Zyto*Light* FISH probe

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



As of: July 7, 2010 (4.5)

Contents

1. Scope of Application

The ZytoLight FISH-Cytology Implementation Kit is designed to be used for the detection of human DNA sequences in cytology specimens 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 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-Cytology 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-Cytology Implementation Kit

4.1 Components

The kit is made up of the following components:

Code	Component	Quantity	Container
		Σ 20	
ES2	Cytology Pepsin Solution	4 ml	Dropper bottle, transparent cap
WB5	20x Wash Buffer TBS	50 ml	Screw-cap bottle
PT4	10x $MgCl_2$	50 ml	Screw-cap bottle
PT5	10x PBS	50 ml	Screw-cap bottle
WB7	Cytology Stringency Wash Buffer SSC	500 ml	Screw-cap bottle (large)
WB8	Cytology Wash Buffer SSC	500 ml	Screw-cap bottle (large)
MT1	DAPI/Antifade-Solution	0.8	Reaction vessel, blue lid
	Instruction manual	1	

Components **(ES2)** and **(MT1)** are sufficient for 20 reactions. Components **(PT4)**, **(PT5)**, **(WB7)**, and **(WB8)** are sufficient for 7 staining jars of 70 ml each. Component **(WB5)** is sufficient for 14 staining jars of 70 ml each.

4.2 Storage and Shelf Life

The components of the kit must be stored at 2...8°C except for the DAPI/Antifade-Solution **(MT1)**, which must be stored at -16...-22°C in the dark; a short-time storage at 2...8°C is possible.

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 Zyto*Light* FISH-Cytology Implementation Kit has been optimized for use on metaphase and interphase cells from peripheral blood, cultures, or direct preparations prepared by standard cytogenetic methods, see e.g.: Beatty B, Mai S, Squire J (eds.): FISH A Practical Approach, *Oxford University Press* (2002). Our specialists are available to support you whenever adjustments are necessary.

We recommend the following specimen preparation:

✓ Incubate specimens overnight (12-16 h) at 37°C for aging

Alternatively, aging of specimens can be accomplished by incubation of slides for 2 min in 2x SSC at 73±1°C immediately prior to proteolysis.

4.4 Additional Materials

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

- *ZytoLight FISH probe*
- *Water bath (72±1°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)*
- *Formaldehyde, neutrally buffered*
- *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 cytology specimens must not be allowed to dry during the hybridization and washing steps!
- ✓ 1% Formaldehyde solution should be freshly prepared prior to use and should be discarded afterwards. Unused solutions can be stored at 2…8°C for up to 6 months.
- ✓ DNA probe and DAPI/Antifade-Solution **(MT1)** 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 and times for the stringency wash, described in the protocol, should be followed accurately since incorrect washing conditions can lead to no or weak signals.
- ✓ 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-Cytology Implementation Kit Protocol

5.1 Preparatory Steps

Day 1:

- *Preparation of an 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 re-used.
- *Preparation of 1x Wash Buffer TBS:* Dilute 1 part 20x Wash Buffer TBS **(WB5)** with 19 parts deionized or distilled water.
- *Preparation of 1% Formaldehyde solution:* For 100 ml 1% Formaldehyde solution mix either 2.7 ml of 37% neutrally buffered formaldehyde or 10 ml of 10% neutrally buffered formaldehyde with 10 ml of 10x $MgCl_2$ **(PT4)** and 10 ml of 10x PBS **(PT5)** and adjust volume to 100 ml with deionized or distilled water. Mix thoroughly.

Day 2:

- *Cytology Stringency Wash Buffer SSC* **(WB7)**: Prewarm to 72±1°C.
- *Cytology Wash Buffer SSC* **(WB8)**: Bring to room temperature.
- *DAPI/Antifade-Solution* **(MT1)**: Bring to room temperature before use, protect from light.

5.2 Pretreatment (Proteolysis/Post-Fixation) [day 1]

1. Apply (dropwise) Cytology Pepsin Solution **(ES2)** to the cytology specimen and incubate for 10 min at 37°C in a humidity chamber

> *Depending on multiple factors, e.g. nature and duration of fixing as well as nature of cells, different incubation times may be required. We recommend an incubation time of 5-15 min for cytology specimens. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.*

2. Incubate slides for 5 min in 1x Wash Buffer TBS

3. Incubate slides for 5 min in 1% Formaldehyde solution

4. Incubate slides for 5 min in 1x Wash Buffer TBS

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

Air dry specimens.

5.3 Denaturation and Hybridization [day 1]

1. Pipette 10 µl Zyto*Light* 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 72±1°C for 2 min, e.g. on a hot plate

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 cytology specimens 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. Carefully remove the coverslip

3. Wash, using Cytology Stringency Wash Buffer SSC **(WB7)** for 2 min at 72±1°C

The Cytology Stringency Wash Buffer SSC should be pre-warmed to 72±1°C. Check with a thermometer if necessary.

We recommend not to use more than four slides per staining jar. When necessary use blank slides to adjust number to four.

4. Wash, using Cytology Wash Buffer SSC **(WB8)** for 1 min at room temperature

The Cytology Wash Buffer SSC should be pre-warmed to room temperature. Check with a thermometer if necessary.

We recommend not to use more than four slides per staining jar. When necessary use blank slides to adjust number to four.

Air dry the specimens protected from light.

5. Pipette 30 µl DAPI/Antifade-Solution **(MT1)** 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 **(MT1)** 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. Evaluate the sample material by fluorescence microscopy using appropriate filter sets.

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

Barch MJ, Knutsen T, Spurbeck JL (eds.): The AGT cytogenetics laboratory manual, *Lippincott-Raven*, Philadelphia (1997) ISBN 0 397 51651 7.

Beatty B, Mai S, Squire J (eds.): FISH A Practical Approach, *Oxford University Press* (2002) ISBN 0 19 963884 5.

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 sample has not been properly fixed	Optimization of fixing method
	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; increase or decrease if necessary
	Stringency wash conditions incorrect	Check wash temperature and time and adjust if necessary
	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
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 specimens between the individual incubation steps	Prevent dehydration
	Washing temperature following hybridization too low	Check temperature; increase if necessary
Poor nuclei morphology or weak nuclei staining	Proteolytic pretreatment too strong	Shortening of incubation time
	Denaturation temperature not correct	Check temperature; decrease if necessary

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