ZYTOVISION

Zyto*Light*
FISH-Cytology Implementation Kit

REF Z-2099-20 Σ 20

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

CE IVD

In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The Zyto*Light* FISH-Cytology Implementation Kit is intended to be used in combination with Zyto*Light* FISH probes for the detection of genetic aberrations, e.g., translocations, deletions, amplifications, and chromosomal aneuploidies, in cytology specimens by fluorescence *in situ* hybridization (FISH).

Interpretation of the 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. Clinical relevance

Genetic aberrations, e.g., translocations, deletions and/or amplifications, are associated with various human neoplasms. Chromosomal aneuplodies are observed in many congenital disorders.

3. Test principle

The fluorescence *in situ* hybridization (FISH) technique allows for the detection and visualization of specific nucleic acid sequences in cell preparations. Fluorescently-labeled DNA fragments, so called FISH probes, and their complementary target DNA strands in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. After counterstaining the DNA with DAPI, hybridized probe fragments are visualized using a fluorescence microscope equipped with excitation and emission filters specific for the fluorochromes with which the FISH probe fragments have been directly labeled.

4. Reagents provided

The Zyto*Light* FISH-Cytology Implementation Kit is composed of:

Code	Component	Quantity Σ 20	Container
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)
MT7	DAPI/DuraTect-Solution	0.8 ml	Reaction vessel, blue lid
	Instructions for use	1	

Z-2099-20 (20 tests): Components **ES2** and **MT7** 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.

5. Materials required but not provided

- Zyto*Light* FISH probe
- Positive and negative control specimens
- Microscope slides, uncoated
- Water bath (70°C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable pipettes (10 µl, 25 µl)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- 37% formaldehyde, acid-free, or 10% formalin, neutrally buffered
- 2x Saline-Sodium Citrate (SSC), e.g., from 20x SSC Solution (Prod. No. WB-0003-50)
- Deionized or distilled water
- Coverslips (22 mm x 22 mm, 24 mm x 60 mm)
- Rubber cement, e.g., Fixogum Rubber Cement (Prod. No. E-4005-50/-125) or similar
- Adequately maintained fluorescence microscope (400-1000x)
- Immersion oil approved for fluorescence microscopy
- Appropriate filter sets

6. Storage and handling

The components of the kit must be stored at 2-8°C. Additionally, the DAPI/DuraTect-Solution (**MT7**) must be stored protected from light. Return components to storage conditions immediately after use. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label. Do not use reagents beyond expiry date indicated on the label.

7. Warnings and precautions

- Read the instruction for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- This product contains substances (in low concentrations and volumes) that are harmful to health and potentially infectious. 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!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents.

- The specimens must not be allowed to dry during the hybridization and washing steps!
- 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!

Hazard and precautionary statements for PT4, PT5, WB7, and WB8:

The hazard determining component is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/ face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Hazard and precautionary statements for WB5:

The hazard determining components are:
- 2-amino-2-(hydroxymethyl)propane-1,3-diolhydrochloride
- 2-amino-2-(hydroxymethyl)-1,3-propanediol
- a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Warning

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the FISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper freezing, thawing, washing, drying, heating, or contamination with other specimens or fluids may produce artefacts or false results.
- The performance was validated using the procedures described in this instruction for use. Modifications to these procedures might alter the performance and have to be validated by the user.

9. Interfering substances

Red blood cells present in the specimen might exhibit autofluorescence which hinders signal recognition.

10. Preparation of specimens

Incubate slides for 2 min in a 2x SSC solution at 73°C immediately prior to proteolysis for aging.

Alternatively, aging of specimens can be accomplished by incubation of specimens overnight (12-16 h) at 37°C.

11. Preparatory treatment of the device

20x Wash Buffer TBS **(WB5),** 10x $MgCl_2$ **(PT4),** and 10x PBS **(PT5)** are to be pretreated according to the instructions in 12. "Assay procedure". All other kit reagents are ready-to-use. No reconstitution, mixing, or dilution is required.

12. Assay procedure

12.1 Day 1

Preparatory steps

- *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% acid-free formaldehyde or 25 ml of neutrally buffered formalin (4% 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.
- *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 can be re-used.

Pretreatment (Proteolysis/Post-Fixation)

(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.

Denaturation and hybridization

(1) Pipette 10 µl of the Zyto*Light* FISH Probe onto each pretreated specimen.

Avoid long exposure of the probe to light.

(2) Cover specimens with a 22 mm x 22 mm coverslip (avoid trapped bubbles) and seal the coverslip.

We recommend using rubber cement (e.g., Fixogum Rubber Cement) for sealing.

(3) Place slides on a hot plate or hybridizer and denature specimens for 5 min at 72°C.

(4) Transfer the slides 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.

12.2 Day 2

Preparatory steps

- Cytology Stringency Wash Buffer SSC **(WB7)**: Prewarm to 70°C.
- Cytology Wash Buffer SSC **(WB8)**: Bring to room temperature.
- DAPI/DuraTect-Solution **(MT7)**: Bring to room temperature before use, protect from light.

Post-hybridization and detection

(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 70°C.

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

We recommend to use 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.

(5) Air dry the samples protected from light.
(6) Pipette 25 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.

(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:

Fluorescent dye	Excitation	Emission
ZyBlue	418 nm	467 nm
ZyGreen	503 nm	528 nm
ZyGold	532 nm	553 nm
ZyOrange	547 nm	572 nm
ZyRed	580 nm	599 nm

13. 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 more details on the interpretation of results, please refer to the respective probe manual.

14. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by internal and external controls. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

Internal control: Non-neoplastic cells within the specimen that exhibit normal signal pattern.

External control: Validated positive and negative control specimens.

15. Performance characteristics

Refer to the instruction for use of the respective probe.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

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

Weak signals or no signals at all

Possible cause	Action
No target sequences available	Use appropriate controls
Proteolysis, denaturation, hybridization, or stringency wash temperature incorrect	Check temperature of all technical devices used, using a calibrated thermometer
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
Probe evaporation	When using a hybridizer, the use of the wet stripes/water filled tanks is mandatory. When using a hybridization oven, the use of a humidity chamber is required. In addition, the coverslip should be sealed completely, e.g., with Fixogum, to prevent drying-out of the sample during hybridization
Too low concentrated stringency wash buffer	Check concentration of stringency wash buffer
Old dehydration solutions	Prepare fresh dehydration solutions
Fluorescence microscope adjusted wrongly	Adjust correctly
Inappropriate filter sets used	Use filter sets appropriate for the fluochromes of the probe. *Triple-bandpass filter sets provide less light compared to single or dual-bandpass filter sets. Consequently, the signals may appear fainter using these triple-bandpass filter sets*
Photo-damage of the probes/fluorophores	Accomplish hybridization and washing steps in the dark

Cross hybridization signals; noisy background

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time
Probe volume per area too high	Reduce probe volume per specimen/area, distribute probe dropwise to avoid local concentration
Slides cooled to room temperature before hybridization	Transfer the slides quickly to 37°C
Too high concentrated stringency wash buffer	Check concentration of stringency wash buffer
Washing temperature following hybridization too low	Check temperature; increase if necessary
Dehydration of specimens between the individual incubation steps	Prevent dehydration by sealing the slides and performing incubation in a humid environment

Morphology degraded

Possible cause	Action
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
Insufficient drying before probe application	Extend air-drying

Weak counterstain

Possible cause	Action
Low concentrated DAPI solution	Use DAPI/DuraTect-Solution (ultra) (Prod. No. MT-0008-0.8) instead
DAPI incubation time too short	Adjust DAPI incubation time

18. 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.

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