ZytoLight

FISH-Cytology Implementation Kit

REF Z-2099-20 Σ 20

For use in fluorescence *in situ* hybridization (FISH) procedures

4250380N727X

CE IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended use

The ZytoLight FISH-Cytology Implementation Kit is intended to be used in combination with ZytoLight FISH probes on cytology specimens by fluorescence *in situ* hybridization (FISH).

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

2. 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.

3. Reagents provided

The ZytoLight FISH-Cytology Implementation Kit is available in one size and 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.

4. Materials required but not provided

- ZytoLight 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

5. Storage and handling

Store at 2-8 °C in an upright position. Additionally, the DAPI/DuraTect-Solution (**MT7**) must be stored protected from light. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and precautions

- Read the instructions 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. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.

- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- 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!

Special labelling of ES2:

EUH210	Safety data sheet available on request. < 20 % of the mixture consists of ingredient(s) of unknown acute toxicity (inhalation).

Hazard and precautionary statements for PT4, PT5, WB5, 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 water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Hazards and precautionary statements for MT7:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated 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/human geneticist to be familiar with the ISH 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/human geneticist 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 fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.
- The performance was validated using the procedures described in the instruction for use of the respective ZytoVision probe and implementation kit. Modifications to these procedures might alter the performance and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

8. Interfering substances

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

9. 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.

10. 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 11. "Assay procedure". Components (**PT4**) and (**PT5**) may form precipitates at 2-8°C. If necessary warm up to 37°C for 10 min until precipitates have fully dissolved prior to use. All other kit reagents are ready-to-use. No reconstitution, mixing, or dilution is required.

11. Assay procedure

11.1 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 10% 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.
- *ZytoLight FISH Probe*: Bring to RT before use, protect from light.

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.

   ***ES2** may form precipitates, which do not affect the quality.*

   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.

11.2 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
ZyGreen 2.0	493 nm	518 nm
ZyGold	532 nm	553 nm
ZyOrange	547 nm	572 nm
ZyRed	580 nm	599 nm

12. 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.

13. Recommended quality control procedures

Refer to the instructions for use of the respective ZytoVision probe.

14. Performance characteristics

Refer to the instructions for use of the respective ZytoVision probe.

15. Disposal

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

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to www.zytovision.com for more information.

Weak signals or no signals at all

Possible cause	Action
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
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*

Cross hybridization signals; noisy background

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time
Slides cooled to room temperature before hybridization	Transfer the slides quickly to 37 °C

Morphology degraded

Possible cause	Action
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, 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

17. 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.

18. Revision

Revision	Description of the change
1.2.1	11. Assay procedure ZyGreen 2.0 added

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
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