ZYTOVISION

F*ex*ISH
ALK/ROS1 DistinguISH Probe

REF Z-2203-50 Σ 5 (0.05 ml)

REF Z-2203-200 Σ 20 (0.2 ml)

For the qualitative detection of translocations involving the human ALK gene at 2p23.1-p23.2 and the human ROS1 gene at 6q22.1 by fluorescence *in situ* hybridization (FISH)

4250380P283RD

CE 0124

IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The F*ex*ISH ALK/ROS1 DistinguISH Probe (**PL161**) is intended to be used for the qualitative detection of translocations involving the human ALK gene at 2p23.1-p23.2 and the human ROS1 gene at 6q22.1 in formalin-fixed, paraffin-embedded specimens, such as non-small cell lung cancer (NSCLC), by fluorescence *in situ* hybridization (FISH). The probe is intended to be used in combination with the F*ex*ISH-Tissue Implementation Kit (Prod. No. Z-2182-5/-20).

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.

The probe is intended to be used as an aid to the differential diagnosis of NSCLC and therapeutic measures should not be initiated based on the test result alone.

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 F*ex*ISH ALK/ROS1 DistinguISH Probe is composed of:

- ZyGreen (excitation 503 nm/emission 528 nm) labeled polynucleotides (~10.0 ng/µl), which target sequences mapping in 2p23.1-p23.2* (chr2:29,460,144-30,095,822) proximal to the ALK breakpoint region and in 6q22.1* (chr6:116,912,298-117,627,255) proximal to the ROS1 breakpoint region (see Fig. 1).
- ZyOrange (excitation 547 nm/emission 572 nm) labeled polynucleotides (~2.5 ng/µl), which target sequences mapping in 2p23.2* (chr2:29,174,204-29,383,335) distal to the ALK breakpoint region and in 6q22.1* (chr6:117,659,135-117,871,701) distal to the ROS1 breakpoint region (see Fig. 1).
- ZyBlue (excitation 418 nm/emission 467 nm) labeled polynucleotides, (~70.0 ng/µl), which target sequences mapping in 6q22.1* (chr6:116,671,642-117,260,761) proximal to the ROS1 breakpoint region co-localizing with the green-labeled ROS1 polynucleotides and in 6q22.1-q22.2* (chr6:117,765,211-118,444,005) distal to the ROS1 breakpoint region co-localizing with the orange-labeled ROS1 polynucleotides (see Fig. 1.)
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

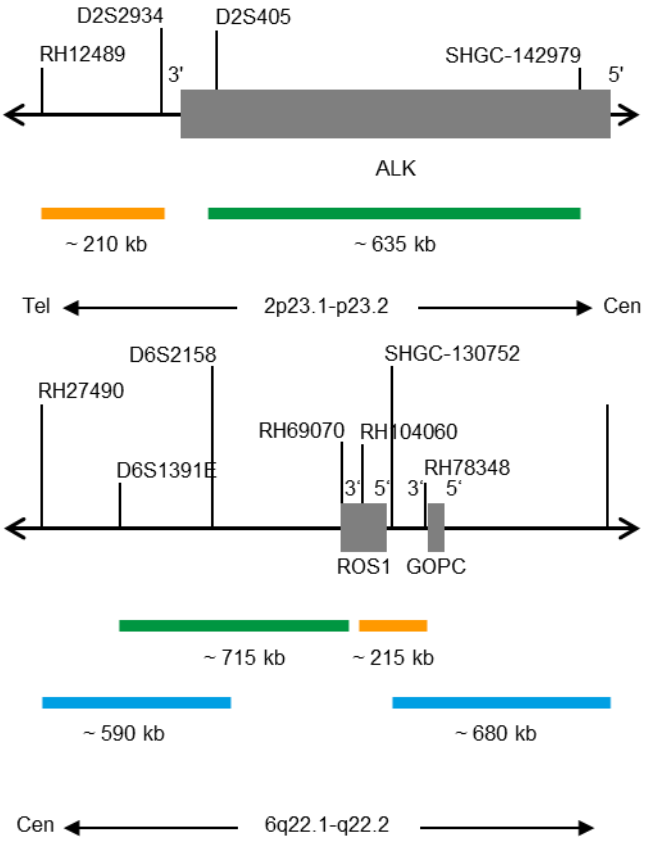

Fig. 1: Top: SPEC ALK Probe map, Bottom: SPEC ROS1 Probe map (not to scale)

The F*ex*ISH ALK/ROS1 DistinguISH Probe is available in two sizes:

- Z-2203-50: 0.05 ml (5 reactions of 10 µl each)
- Z-2203-200: 0.2 ml (20 reactions of 10 µl each)

4. Materials required but not provided

- F*ex*ISH-Tissue Implementation Kit (Prod. No. Z-2182-5/-20)
- Positive and negative control specimens
- Microscope slides, positively charged
- Water bath (37 °C, 98 °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
- Xylene

- 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 protected from light. Use 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 and potentially infectious. 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 probe 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:

The hazard-determining component is formamide.

Danger

H319	Causes serious eye irritation.
H351	Suspected of causing cancer.
H360FD	May damage fertility. May damage the unborn child.
H373	May cause damage to organs through prolonged or repeated exposure.
P201	Obtain special instructions before use.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.

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 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/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 probe should be used only for detecting loci described in chapter 3. "Reagents provided".
- The performance was validated using the procedures described in these instructions for use. 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.

The following fixatives are incompatible with FISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin

9. Preparation of specimens

Prepare specimens as described in the instructions for use of the F*ex*ISH-Tissue Implementation Kit.

10. Preparatory treatment of the device

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25 °C) before use, protect from light. Prior to opening the vial, mix by vortexing and spin down briefly.

11. Assay procedure

Specimen pretreatment
Perform specimen pretreatment (dewaxing, proteolysis) according to the instructions for use of the F*ex*ISH-Tissue Implementation Kit.

Denaturation and hybridization

1. Pipette 10 µl of the probe onto each pretreated specimen.
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) for sealing.

3. Place slides on a hot plate or hybridizer and denature specimens for 10 min at 75 °C.
4. Perform hybridization for 2 h up to 16 h (i.e. overnight) at 37 °C by either transferring the slides to a hybridizer or to a humidity chamber and a hybridization oven.

It is essential that specimens do not dry out during the hybridization step.

Post-hybridization
Perform post-hybridization processing (washing, counter-staining, fluorescence microscopy) according to the instructions for use of the F*ex*ISH-Tissue Implementation Kit.

12. Interpretation of results

With the use of appropriate filter sets, the hybridization signals of the probe appear green (proximal to the ALK and ROS1 breakpoint region), orange (distal to the ALK and ROS1 breakpoint region), and blue (proximal and distal to the ROS1 breakpoint region).

Normal situation: In interphases of normal cells or cells without a rearrangement involving the ALK or ROS1 gene region, four green/orange fusion signals appear when using an appropriate dual bandpass filter set, and two blue signals appear when using an appropriate single bandpass filter set (see Fig. 2).

Aberrant situation: **:** One ALK gene region affected by a translocation is indicated by one separate green signal and one separate orange signal not co-localizing with blue signals. Loss of one green signal resulting in an isolated orange signal is the result of a deletion of 5'-ALK sequences. One ROS1 gene region affected by a translocation is indicated by one separate green signal and one separate orange signal, each co-localizing with a blue signal. Loss of one orange signal resulting in an isolated green signal co-localizing with a blue signal is the result of a deletion distal to the ROS1 breakpoint region or due to an unbalanced translocation affecting this chromosomal region (see Fig. 2).

Overlapping green and orange signals may appear as yellow signals.

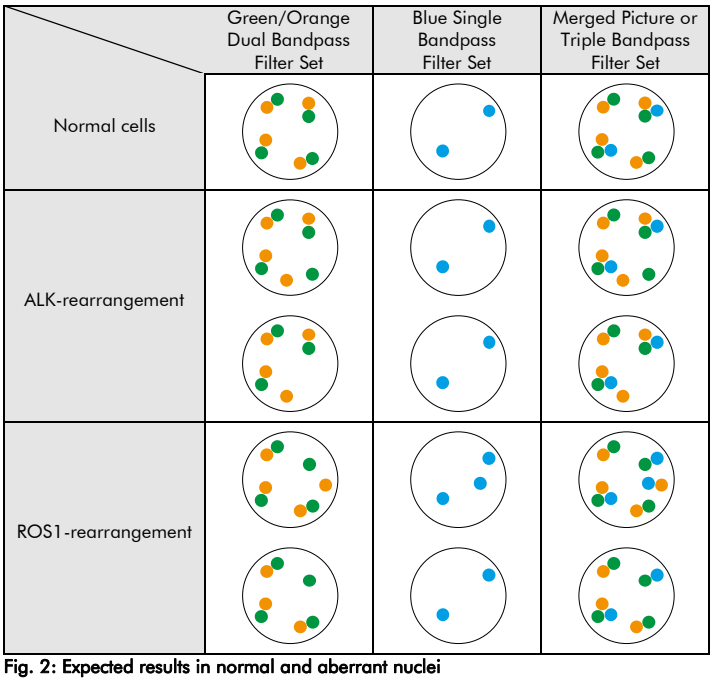

Fig. 2: Expected results in normal and aberrant nuclei

Genomic aberrations due to small deletions, duplications or inversions might result in inconspicuous signal patterns.
Other signal patterns than those described above may be observed in some abnormal samples. These unexpected signal patterns should be further investigated.

Please note:

- 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 ≤ 1 signal diameter, should be counted as one signal.
- Do not evaluate overlapping nuclei.
- Do not count over-digested nuclei (recognized by dark areas visible inside of the nuclei).
- Do not count nuclei with strong auto-fluorescence, which hinders signal recognition.
- A negative or unspecific result can be caused by multiple factors (see chapter 16 "Troubleshooting").
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

13. 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, e.g., fibroblasts.

External control: Validated positive and negative control specimens.

14. Performance characteristics

14.1 Analytical performance

The performance was evaluated according to the instructions for use of the F*ex*ISH-Tissue Implementation Kit.

Analytical sensitivity:	100% (95% CI 98.5 – 100.0)
Analytical specificity:	100% (95% CI 97.0 – 100.0)

14.2 Clinical performance

Diagnostic sensitivity:	100% (95% CI 87.7 - 100.0) vs ALK FISH 100% (95% CI 59.0 - 100.0)vs. ROS1 FISH
Diagnostic specificity:	100% (95% CI 87.7 - 100.0) vs. ALK FISH 100% (95% CI 59.0 - 100.0) vs. ROS1 FISH

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
Specimen has not been properly fixed	Optimize fixing time and fixative
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
Incomplete dewaxing	Use fresh solutions; check duration of dewaxing
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
Specimen has not been properly fixed	Optimize fixing time and fixative
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, decrease if necessary
Insufficient drying before probe application	Extend air-drying

Overlapping nuclei

Possible cause	Action
Inappropriate thickness of tissue sections	Prepare 2-4 µm microtome sections

Specimen floats off the slide

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time

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

- Ginestet F, *et al.* (2018) *Clin Lung Cancer 19*: e647-e653.
- 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

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.
Please contact helptech@zytovision.com
For the summary of safety and performance, please refer to www.zytovision.com.

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