CE
IVD

FlexISH®
Products for flexible FISH

Signal Interpretation Guide

FlexISH® ALK/ROS1 DistinguISH™ Probe

1 Probe · 2 Targets · 3 Answers
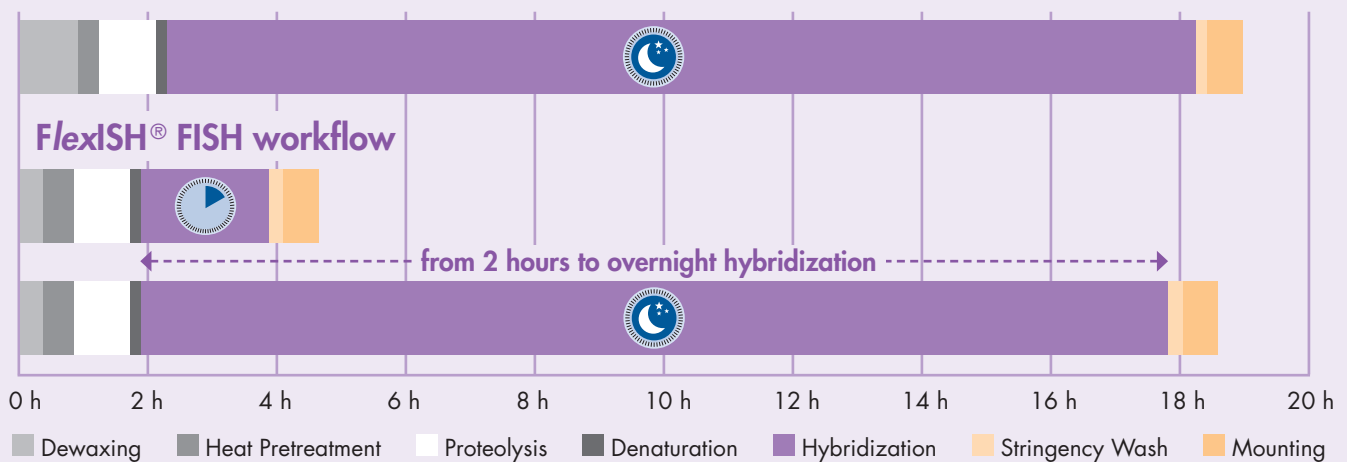
ZYTOVISION
Molecular diagnostics simplified

One Probe · Two Targets

The **F*lex*ISH® ALK/ROS1 DistinguISH Probe** is designed to simultaneously detect ALK and ROS1 rearrangements. Additionally, this innovative probe design enables the user to discriminate between possible aberrations affecting the chromosomal regions harboring either the ALK or the ROS1 gene. Rearrangements affecting the ALK or the ROS1 gene locus are frequently found in non-small cell lung cancer (NSCLC). The specific analysis of ROS1 and ALK rearrangements in NSCLC patients is a very effective and reliable tool for the diagnosis and selection of treatment with e.g. the tyrosine kinase inhibitor crizotinib[1, 3, 4].

F*lex*ISH® brings Flexibility to Your FISH

With the use of the **F*lex*ISH® ALK/ROS1 DistinguISH Probe** in combination with the **F*lex*ISH® Tissue Implementation Kit** reliable results can be obtained already within **4.5 hours**. The **F*lex*ISH® protocol** can also be incorporated into the routine workflow with overnight hybridization providing the highest flexibility[2].

Standard FISH workflow

F*lex*ISH® FISH workflow

from 2 hours to overnight hybridization

| 0 h | 2 h | 4 h | 6 h | 8 h | 10 h | 12 h | 14 h | 16 h | 18 h | 20 h |

■ Dewaxing ■ Heat Pretreatment □ Proteolysis ■ Denaturation ■ Hybridization ■ Stringency Wash ■ Mounting

- **F*lex*ISH®** maximizes your flexibility in terms of time and laboratory management. Hybridization time can be varied between **2 hours** and **overnight**.
- With a hybridization temperature of 37°C the **F*lex*ISH® protocol** is fully compatible with routine workflows in pathology laboratories.

FISH Protocols in Comparison

	ZytoLight®	Dako IQFISH	F*lex*ISH®
Pretreatment	127 min	108 min	103 min
Denaturation	10 min at 75°C	10 min at **66°C**	10 min at 75°C
Hybridization	overnight at 37°C	1-2 h at **45°C**	**flexible** between 2 h and overnight at **37°C**
Stringency Wash	5 min at 37°C 5 min at 37°C	10 min at **63°C** 2x 3 min at RT	10 min at **72°C** 3 min at RT
Dehydration & Mounting	33 min	36 min	33 min
Total Time	~ 19 h	~ 4 - 5 h	**~ 4.5 h - 19 h**

The indicated times include 15 min protease treatment, 15-30 min for the air drying steps, and 16 h for overnight incubation. All other times and temperatures are according to the respective instruction for use.

New Multiplex Probe Design to Simplify your FISH
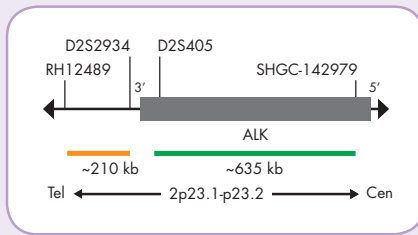
ALK Probe map (not to scale).
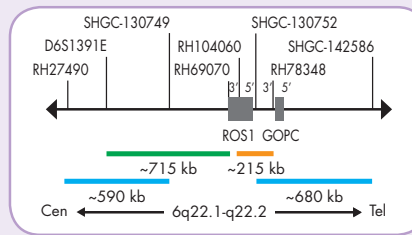
ROS1 Probe map (not to scale).

- With the use of the **F*lex*ISH® ALK/ROS1 DistinguISH Probe** two genetic targets can be detected simultaneously by performing just **ONE Assay**.
- Less patient material is necessary in order to get reliable results.

Evaluation Procedure

1. Localize the invasive component of a non-small cell lung cancer specimen on a corresponding H&E or IHC slide.
2. The area for counting should include clearly distinguishable and well distributed nuclei.
3. Count at least **50 consecutive and non-overlapping** intact nuclei in an area of a population of tumor cells in the invasive component of the tumor.
4. Determine the ALK and ROS1 status according to the **ALK/ROS1 Signal Interpretation Guide**.
   Rearranged if:
   - Distance between the separate green and the separate orange signal is ≥ **2 times** the estimated signal diameter
   - > **15 %** of neoplastic cells rearranged*
5. Report if ALK or ROS1 status is indeterminate due to e.g. artifacts, analytic testing failure, etc. or if ALK or ROS1 status is discordant with other histopathologic findings and repeat test with another specimen.

The validation of FISH probes is required for each type of tissue that is intended to be tested in clinical practice since different tissue types exhibit different cell types with different nuclei diameters which may result in different cut off values. 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.

The ALK/ROS1 DistinguISH™ Probe · One Probe · Three Answers

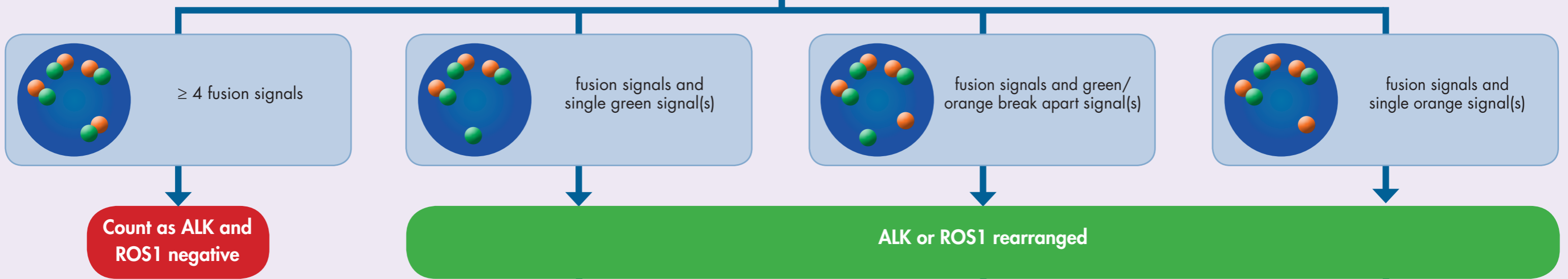## The only way to distinguish 3 different gene conditions!

(1) Non-rearranged — 2 ALK specific green/orange fusion signals, and 2 ROS1 specific green/orange/blue fusion signals.

(2) ALK positivity — Separate **orange** and **green** signal, **no co-localization** with **blue** signals.

(3) ROS1 positivity — Separate **orange** and **green** signal **co-localizing** with **blue** signals.
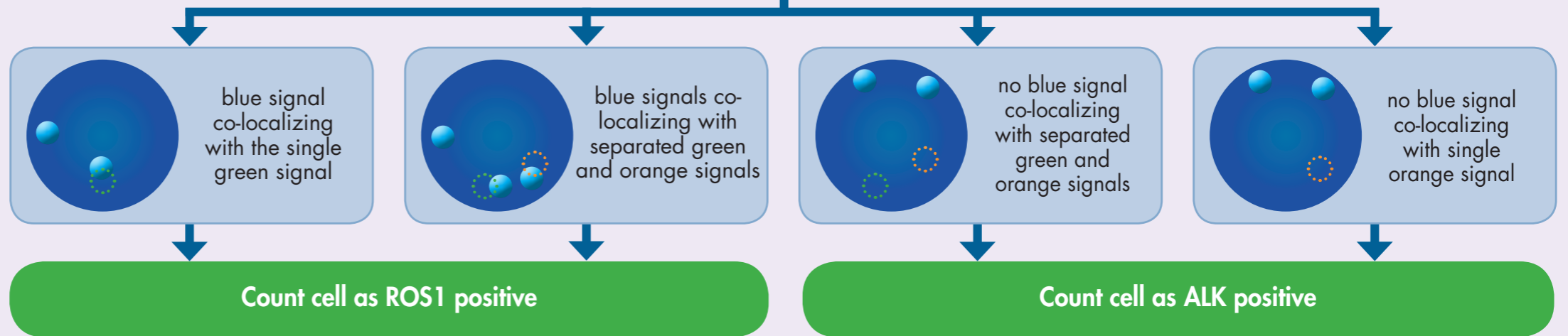
References
1. Bos M, et al. (2013) Lung Cancer 81: 142-3.
2. Brockhoff G, et al. (2016) Histopathology [Epub ahead of print].
3. Sasaki T, et al. (2010) Eur J Cancer 46: 1773-80.
4. Shaw AT, et al. (2014) N Engl J Med 371: 1963-71.

ALK/ROS1 Signal Interpretation Guide

Initial Screening using a Green/Orange Dual Bandpass Filter Set

≥ 4 fusion signals

fusion signals and single green signal(s)

fusion signals and green/orange break apart signal(s)

fusion signals and single orange signal(s)

Count as ALK and ROS1 negative

ALK or ROS1 rearranged

Check for blue/orange Co-Localization using a Blue Single Bandpass Filter Set

blue signal co-localizing with the single green signal

blue signals co-localizing with separated green and orange signals

no blue signal co-localizing with separated green and orange signals

no blue signal co-localizing with single orange signal

Count cell as ROS1 positive

Count cell as ALK positive

Merged Pictures for Clarification

blue co-localization with single green and/or orange signal confirms ROS1 positivity

no blue co-localization with single orange and/or green signal confirms ALK positivity

FlexISH®

Products for flexible FISH

FlexISH® ALK/ROS1 DistinguISH™ Probe

Prod. No.	Product	Label	Tests* (Volume)
Z-2203-50	FlexISH ALK/ROS1 DistinguISH Probe CE IVD	●/●/●	5 (50 µl)
Z-2203-200	FlexISH ALK/ROS1 DistinguISH Probe CE IVD	●/●/●	20 (200 µl)
Related Products			
Z-2182-5	FlexISH-Tissue Implementation Kit CE IVD		5
	Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; 5x FlexISH Wash Buffer, 150 ml; DAPI/DuraTect-Solution, 0.2 ml		
Z-2182-20	FlexISH-Tissue Implementation Kit CE IVD		20
	Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; 5x FlexISH Wash Buffer, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		

Using 10 µl probe solution per test. CE IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.

FlexISH®-Tissue Implementation Kit

FlexISH®-Tissue Implementation Kit contains all necessary reagents to perform successful and flexible FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- 5x FlexISH® Wash Buffer
- DAPI/DuraTect™-Solution

FlexISH® Fluorochromes

Two factors that mainly influence FISH analyses:
- **Fluorochromes of the FISH probes**
- **Appropriate filter sets**

Fluorochrome	Excitation	Emission	Equivalent to
● ZyBlue™	418 nm	467 nm	DEAC
● ZyGreen™	503 nm	528 nm	FITC
● ZyOrange™	547 nm	572 nm	Rhodamine

Recommended Filter Sets

All filter sets have a superior signal-to-noise ratio and need to be assembled in fluorescence filter holders specific for the respective microscope. Please contact info@zytovision.com for more information.

Prod. No	Product	Detected Fluorochrome
E-4030-1	DAPI Single Bandpass Filter Set v2	DAPI
E-4026-1	ZyBlue™ Single Bandpass Filter Set v2	●
E-4012-1	ZyGreen™ Single Bandpass Filter Set v2	●
E-4013-1	ZyOrange™ Single Bandpass Filter Set v2	●
E-4016-1	ZyGreen™/ZyOrange™ Dual Bandpass Filter Set v2	●/●

For more product information please contact info@zytovision.com or your local dealer.

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