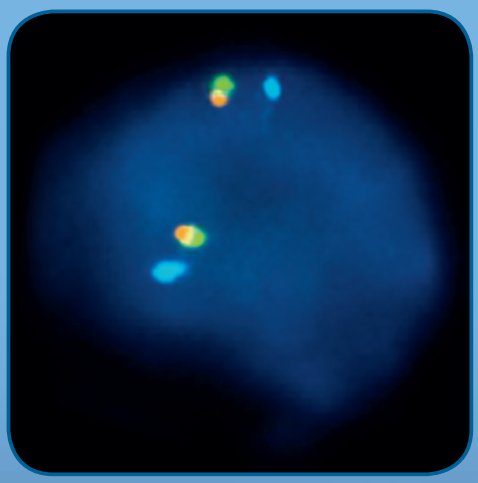
CE IVD

ZytoLight®

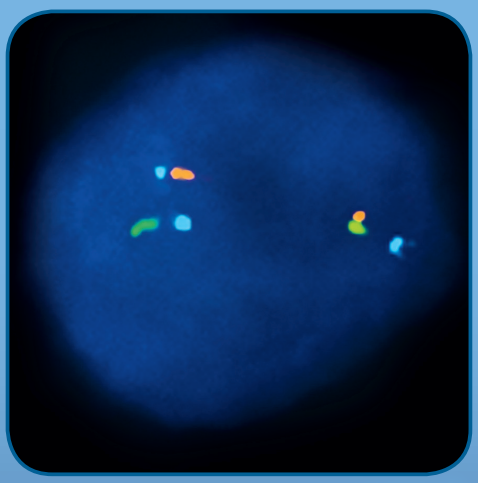
Products for FISH analysis

Signal Interpretation Guide

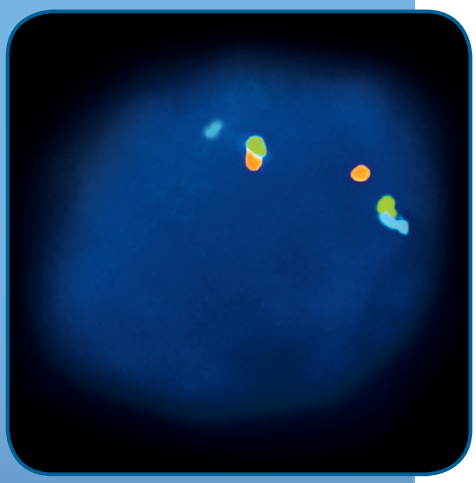
ZytoLight® SPEC ALK/EML4 TriCheck™ Probe



Normal ALK-EML4



ALK-EML4 Inversion



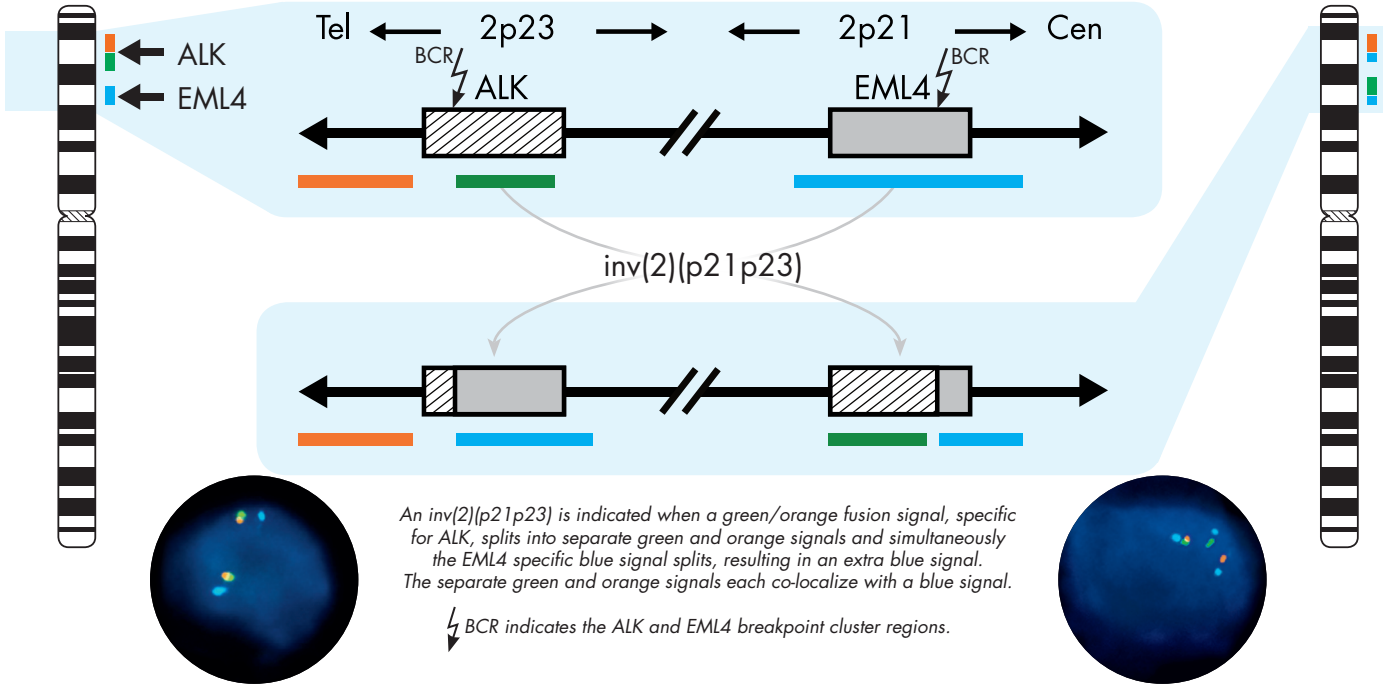
Non-EML4 ALK Translocation

ZYTOVISION
Molecular diagnostics simplified

Reliable Detection of ALK-EML4 Inversion

The **ZytoLight® SPEC ALK/EML4 TriCheck™ Probe** is designed to detect inversions [inv(2)(p21p23)] frequently observed in non-small cell lung cancer (NSCLC). These inversions are difficult to determine using conventional FISH probes, e.g. ALK dual color break apart, as the ALK and EML4 genes are mapped very close to each other. In case of an inversion, the gap between the orange and the green signal is often very small. Especially in these borderline cases, uncertainty in signal interpretation can be overcome by the ability to simultaneously check for the EML4 rearrangement status using the **SPEC ALK/EML4 TriCheck™ Probe**. Consequently, ZytoVision's **ZytoLight® SPEC ALK/EML4 TriCheck™ Probe** is the only FISH probe leading to a reliable discrimination of three different ALK gene conditions: non-rearranged, EML4-ALK inversion, non-EML4 ALK translocation!

ZytoLight® SPEC ALK/EML4 TriCheck™ Probe Design



An inv(2)(p21p23) is indicated when a green/orange fusion signal, specific for ALK, splits into separate green and orange signals and simultaneously the EML4 specific blue signal splits, resulting in an extra blue signal. The separate green and orange signals each co-localize with a blue signal.

BCR indicates the ALK and EML4 breakpoint cluster regions.

New ALK/EML4 TriCheck™ Evaluation Criteria

For the new evaluation criteria 374 lung adenocarcinomas were investigated.

ALK rearrangement was defined as:

- ALK break apart (one green/one orange) ≥ 1 - < 2 signal diameter with orange/aqua co-localization
- ALK break apart ≥ 2 signal diameter
- Isolated orange signals

Determine ALK status according to the new and simplified scoring guidelines shown in the scheme at the right published by Schildhaus HU, *et al.* 2016*.

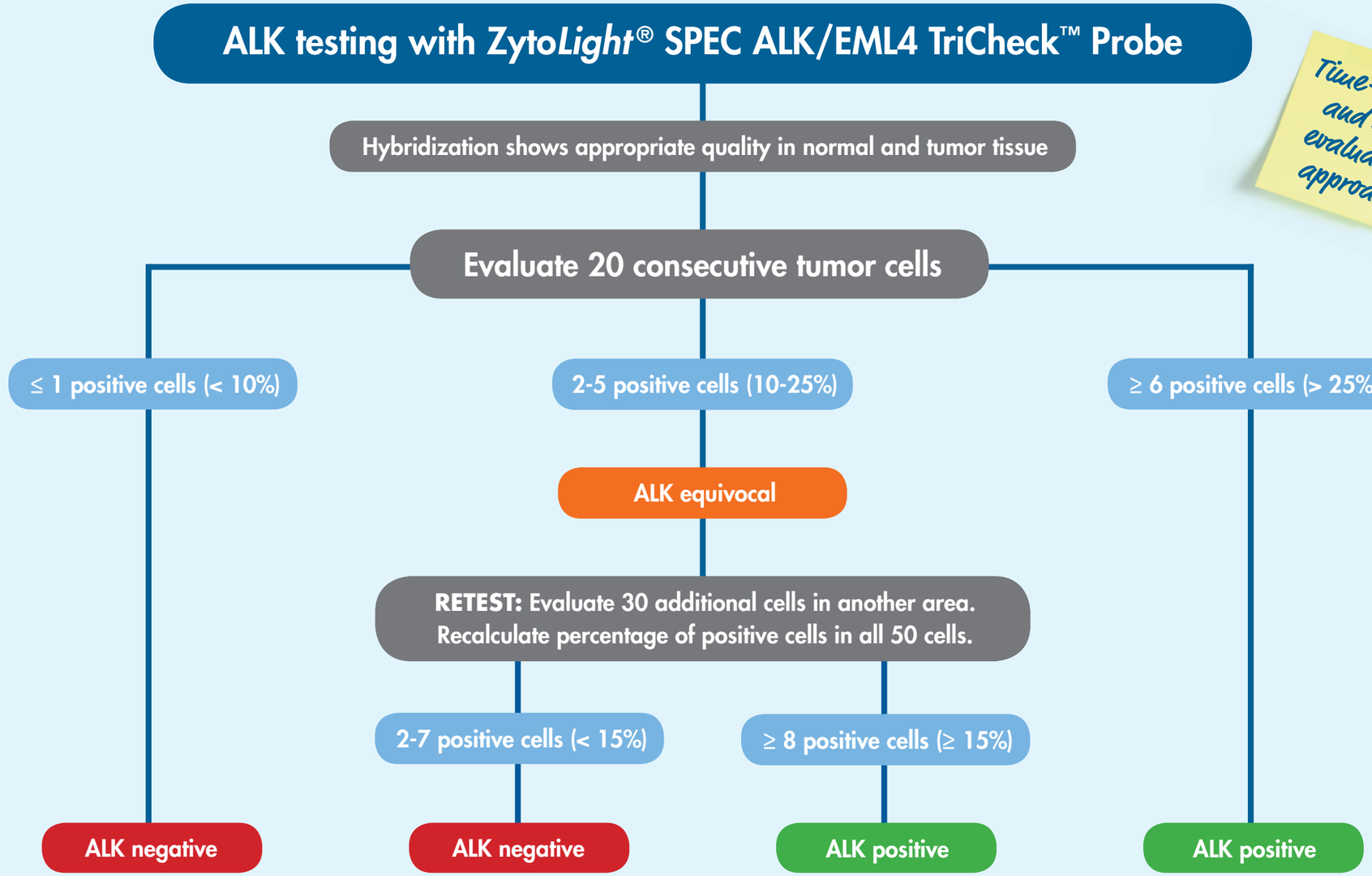
New Evaluation Procedure

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 just **20 consecutive and non-overlapping cells** in an area of a population of tumor cells in the invasive component of the tumor.
4. Determine the ALK status according to the **new ALK Signal Interpretation Guide** and the **new Scoring Guidelines Scheme**.
5. Report if ALK status is indeterminate due to e.g. artifacts, analytic testing failure, etc. or if ALK 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.

* Schildhaus HU, *et al.* (2016) Validation of a simplified approach to detect ALK translocations in lung cancer samples by FISH. Mod Pathol 29 (2s): 482A.

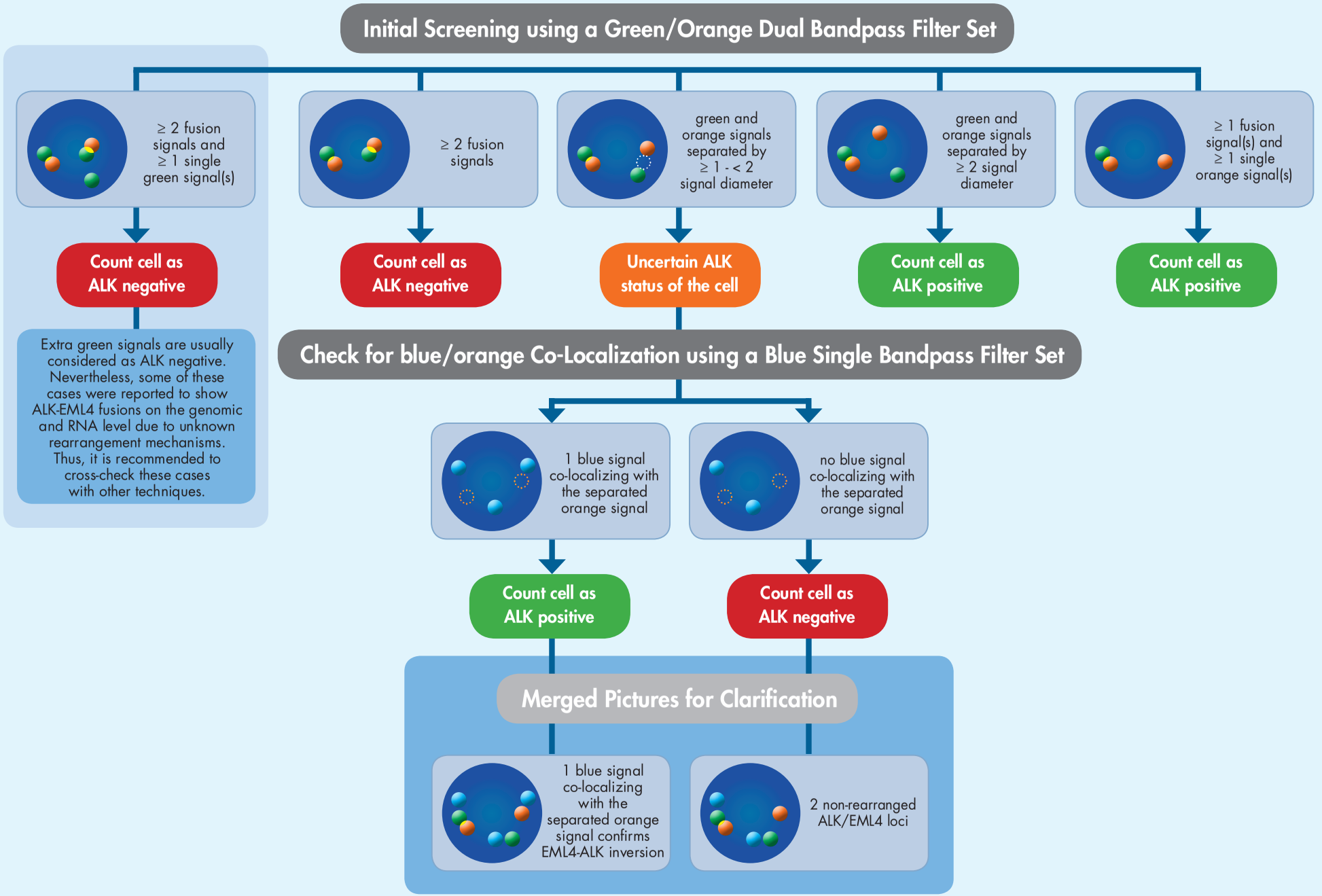
New Scoring Guidelines for a simplified ALK FISH Analysis published by Schildhaus HU, *et al.* 2016



Time-saving and safe evaluation approach

Conclusion

- The new ZytoLight ® SPEC ALK/EML4 TriCheck™ Probe scoring guidelines resulted in a sensitivity and specificity of 100% compared to the Vysis LSI ALK Dual Color Break Apart Probe.
- 99% of all lung cancer specimens needed an analysis of as little as 20 tumor cells.
- Expenditure of time per case was halved.
- For cases with low tumor cell content reliable results can be obtained.


Initial Screening using a Green/Orange Dual Bandpass Filter Set
≥ 2 fusion signals and ≥ 1 single green signal(s)
Count cell as ALK negative
Extra green signals are usually considered as ALK negative. Nevertheless, some of these cases were reported to show ALK-EML4 fusions on the genomic and RNA level due to unknown rearrangement mechanisms. Thus, it is recommended to cross-check these cases with other techniques.
≥ 2 fusion signals
Count cell as ALK negative
green and orange signals separated by ≥ 1 - < 2 signal diameter
Uncertain ALK status of the cell
green and orange signals separated by ≥ 2 signal diameter
Count cell as ALK positive
≥ 1 fusion signal(s) and ≥ 1 single orange signal(s)
Count cell as ALK positive
Check for blue/orange Co-Localization using a Blue Single Bandpass Filter Set
1 blue signal co-localizing with the separated orange signal
Count cell as ALK positive
no blue signal co-localizing with the separated orange signal
Count cell as ALK negative
Merged Pictures for Clarification
1 blue signal co-localizing with the separated orange signal confirms EML4-ALK inversion
2 non-rearranged ALK/EML4 loci

ZytoLight® Products for FISH analysis

ZytoLight® SPEC ALK/EML4 TriCheck™ Probe

Prod. No.	Product	Label	Tests* (Volume)
Z-2117-50	ZytoLight SPEC ALK/EML4 TriCheck Probe CE IVD	●/●/●	5 (50 µl)
Z-2117-200	ZytoLight SPEC ALK/EML4 TriCheck Probe CE IVD	●/●/●	20 (200 µl)
Related Products			
Z-2028-5	ZytoLight FISH-Tissue Implementation Kit CE IVD Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 210 ml; 25x Wash Buffer A, 50 ml; DAPI/DuraTect-Solution, 0.2 ml		5
Z-2028-20	ZytoLight FISH-Tissue Implementation Kit CE IVD Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; Wash Buffer SSC, 560 ml; 25x Wash Buffer A, 100 ml; DAPI/DuraTect-Solution, 0.8 ml		20
Z-2099-20	ZytoLight FISH-Cytology Implementation Kit CE IVD Incl. Cytology Pepsin Solution, 4 ml; 20x Wash Buffer TBS, 50 ml; 10x $MgCl_2$, 50 ml; 10x PBS, 50 ml; Cytology Stringency Wash Buffer SSC, 500 ml; Cytology Wash Buffer SSC, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		20

** 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.*

ZytoLight® FISH-Tissue Implementation Kit

ZytoLight® FISH-Tissue Implementation Kit contains all neccessary reagents to perform user-friendly and successful FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- Wash Buffer SSC
- 25x Wash Buffer A
- DAPI/DuraTect™-Solution

ZytoLight® 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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