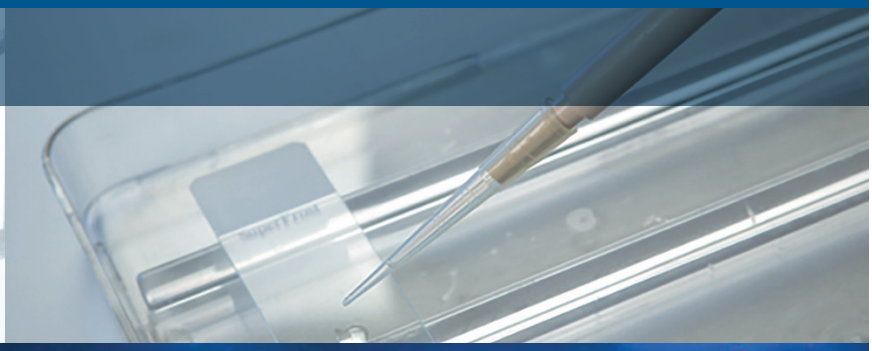
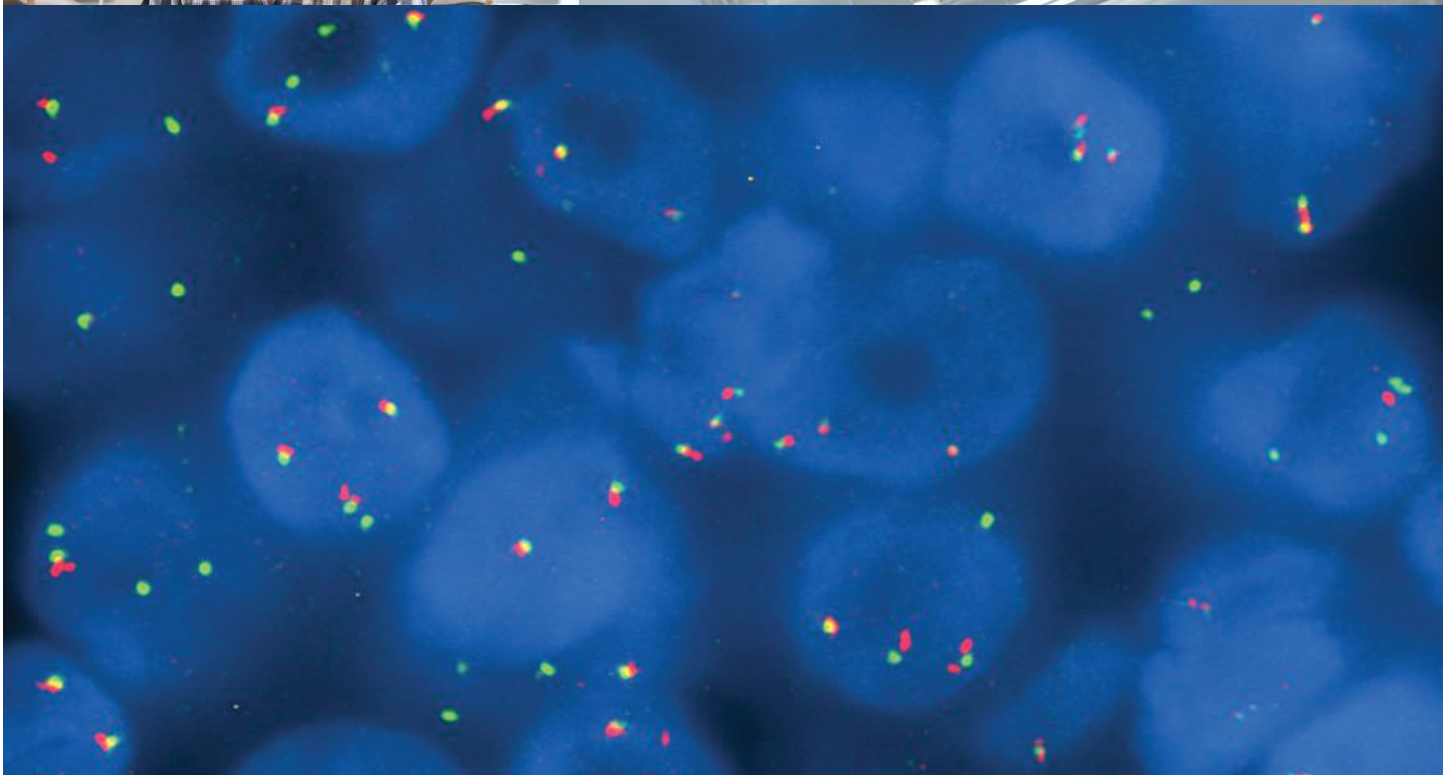
Agilent
Dako

IQFISH
Panel for Lung Cancer

Fastest time to result
ROS1 - ALK - RET - MET





Agilent
Trusted Answers

Fastest Time To Result

The IQFISH panel for lung cancer is a set of oligonucleotide-based FISH probes, premixed with IQFISH Buffer, for the detection of rearrangements involving the ALK, ROS1 and RET genes, and the detection of MET gene amplification by fluorescence in situ hybridization (FISH).
These probes are for use on lung paraffin-embedded tissue sections.

These reagents combine two groundbreaking technologies:

Oligonucleotide-based SureFISH technology and formamide-free IQFISH.

SureFISH technology enables chromosomal aberration detection using a precise, synthetic oligonucleotide probe design process. IQFISH is a unique, ultra-fast hybridization technology that provides FISH results in less than 4 hours. Together these advancements provide the fastest time to result and reduce the cost of labor and assay repeats.

- Less time at the microscope: enabled by high signal-to-noise ratio and micro-gap probe design
- Shorter processing time: answers in 4 hours with a unique 90-minute hybridization
- Less failures: high quality histology workflow reduces assay failures

Genetic Aberrations in Lung Cancer

Recently, molecular characterization of non-small cell lung cancers has identified genetic aberrations that can be used to diagnose and effectively treat cancer. For example, approximately 5% of non-small cell lung cancers harbor a fusion of the anaplastic lymphoma kinase (ALK) gene at 2p23.2 with the echinoderm microtubule associated like 4 (EML4) gene (2p21) (1). Similarly, gene fusions involving the ROS1 and RET tyrosine kinases or amplification of the MET gene have been observed in 1-5% of lung cancer tumors (2-6).

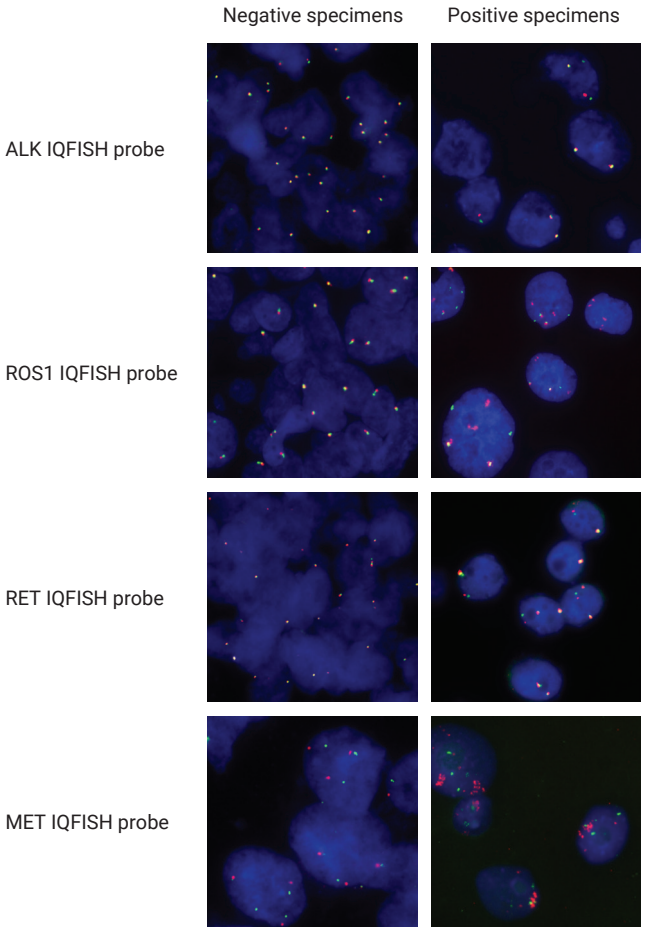


Figure 1. Sample hybridization images for IQFISH probes with both negative and positive specimens.

Fluorescence in situ hybridization (FISH) analysis is frequently performed to identify tumors carrying these chromosomal abnormalities. The ALK, ROS1, and RET break apart FISH probes enable detection of gene fusions through visible separation of orange-red and green fluorescent signals. The MET copy number probe reveals MET gene amplification by an increase in the number of MET signals (orange-red) compared to control probe (green) signals. Figure 1 shows hybridization images for negative and positive specimens, and Figure 2 shows probe maps and the typical signal pattern for each probe.

Probe Maps

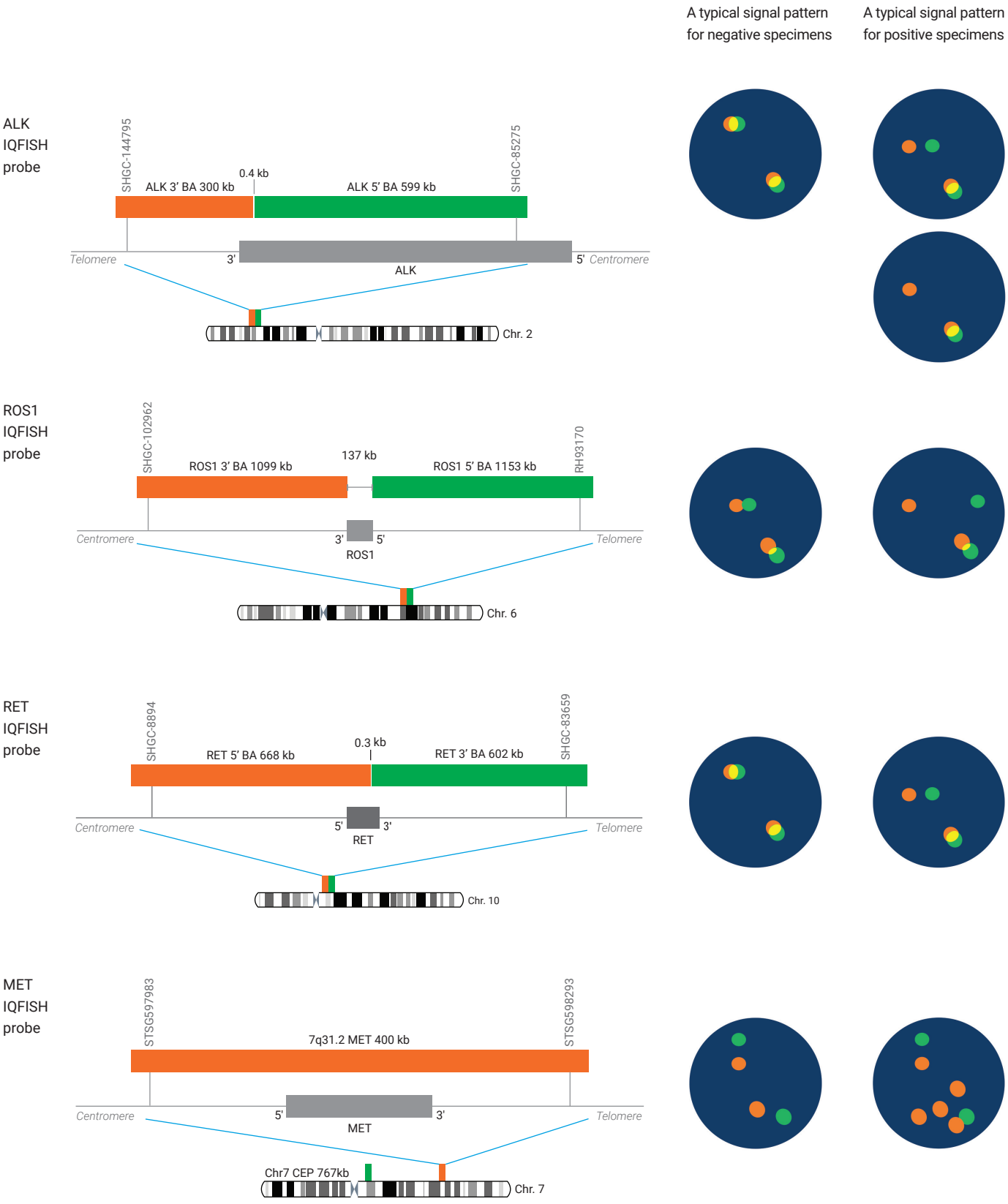


Figure 2. Probe map and typical signal pattern for ALK, ROS1, RET and MET IQFISH probes.

Higher Signal-to-Noise Ratios

IQFISH probes are designed *in silico* and chemically synthesized using Agilent's high-fidelity, oligonucleotide library synthesis (OLS) technology.

FISH probes from other vendors are purified from bacterial library clones harboring human genomic DNA fragments; therefore, they include repetitive sequences that can bind non-specifically throughout the nuclei, producing a hazy background. Consequently, BAC-based probes usually come premixed with Cot-1 DNA, which blocks the background signal from repeated sequences; however, it also suppresses the overall hybridization signal.

Agilent IQFISH probes provide higher signal-to-noise ratios (Figure 3). During probe design, all repetitive elements are removed (Figure 4). This increases signal specificity and decreases hybridization background. It also eliminates the need for a blocking agent and associated signal suppression. The combined effect is a high signal-to-noise ratio and easy visualization.

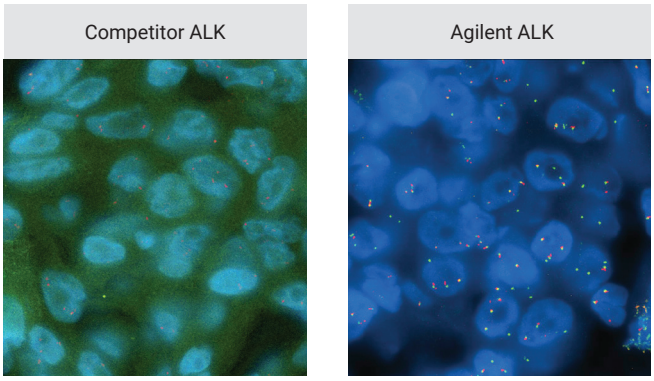


Figure 3. Comparison of competitor ALK (left) and Agilent IQFISH ALK (right) probes. Bright, crisp hybridization signals are produced with IQFISH probes that are free of repetitive DNA sequences and exposure to blocking agents known to suppress hybridization signal.

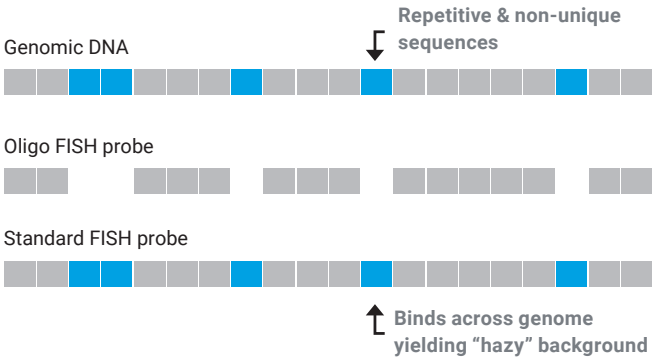


Figure 4. Oligonucleotide-based FISH (oligo FISH) probe design strategy. Repetitive elements are identified and removed during probe design process.

Unique Micro-gap Design

Agilent's oligonucleotide-based SureFISH technology permits probe placement with base-pair-level precision. This enables a unique "micro-gap" design for the ALK and RET IQFISH probes. Whereas the spacing between child probes with BAC-based probes is typically 100-300 kb, the spacing between Agilent's child probes is only about 0.4 kb.
As a result, Agilent's "micro-gap design" provides tighter co-localization of orange-red and green signals in nuclei without the inversion, so that cases with the inversion are easier and faster to analyze (Figure 5).

This design methodology is beneficial because the ALK-EML4 fusion is the result of an inversion between genes that are separated by approximately 12 Mb.
Similarly, RET fusions with KIF5B are the result of an 11 Mb inversion. Detecting such intra-chromosomal inversions can be challenging due to limited signal separation compared with the signal separations seen in a translocation to a different chromosome.

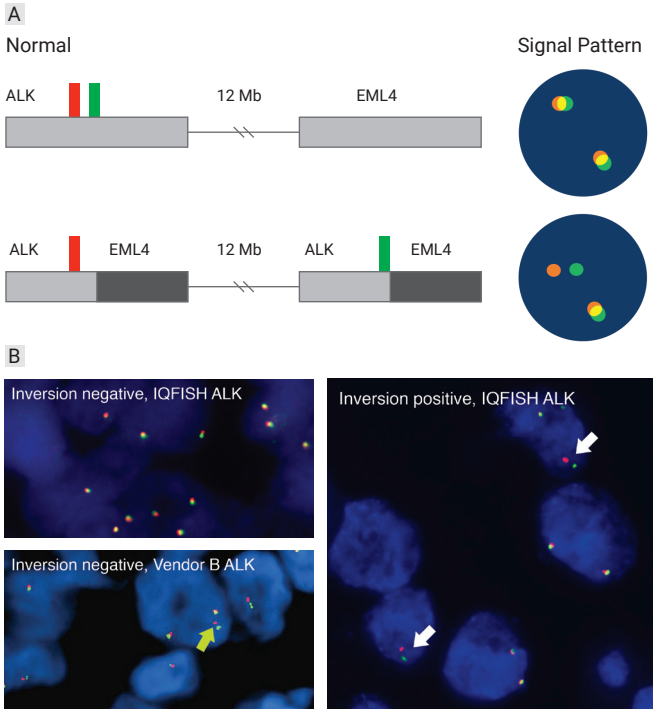


Figure 5. IQFISH ALK inversion detection with tighter co-localization.
Panel A. Illustration of an inversion event, and associated FISH signal pattern.
Panel B. Left: Inversion negative samples show co-localization of the orange-red and green signals. The IQFISH ALK probe (top left) shows tighter co-localized signals than the ALK probe from vendor B (bottom left). This enables easier detection of inversion events. Right: Inversion positive samples show separation of the orange-red and green signals. The relatively short separation distance (white arrow) is due to the small genomic region involved.

Faster Hybridization

Each of the IQFISH probes come supplied in Agilent's IQFISH Fast Hybridization Buffer. This unique buffer reduces the hybridization time from 16 hours to just 90 minutes by improving hybridization kinetics (Figure 6).
For most laboratories, this means that FISH testing can now be done five days a week, rather than four days, improving operational efficiency by up to 25%.

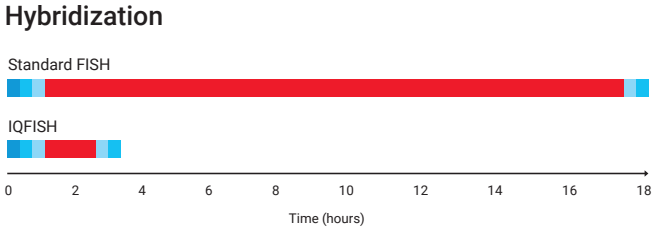


Figure 6. FISH processing time comparison.

Lower Assay Repeat Rate

IQFISH Probes are designed for use with the Dako Histology FISH Accessory Kit (PN K5799). This streamlined workflow features ready-to-use reagents and has only approximately 1-hour of hands on time. The IQFISH workflow not only yields better signal-to-noise ratios, but also results in fewer hybridization failures (Figures 3, 7).

The low failure rate originates from the robust protocol, which includes a unique high temperature pretreatment step and optimized pepsin digestion (Figure 9).
This optimized protocol — which works well across multiple different tissue types — provides reproducible results, leading to fewer assay repeats.

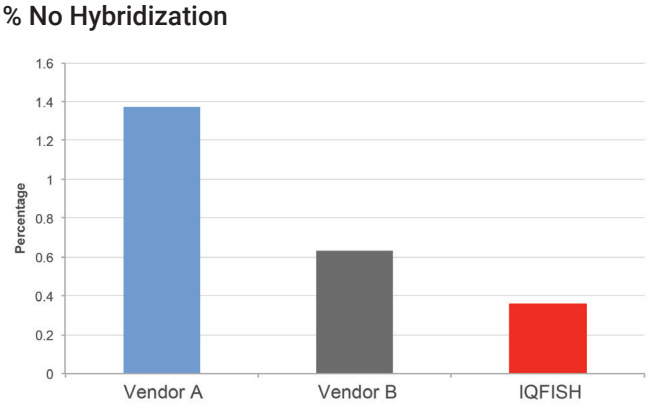


Figure 7. Comparison of ALK FISH Hybridization failures. Test failures (no hybridization signals) were compared between Agilent IQFISH and two other vendors. Sample size: Vendor A 73 tests; Vendor B 640 tests; Agilent IQFISH: 278 tests (Data provided by Propath, USCAP 2015. Agilent ALK probe and IQFISH buffer were purchased separately, and mixed prior to use).

Fastest Time to Result

When using IQFISH probes, clinical laboratories can expect a significant reduction in assay turnaround time.
Together the shorter hybridization step, shorter analysis time, and the reduced need to repeat assays can decrease overall turnaround time by as much as two days (Figure 8).

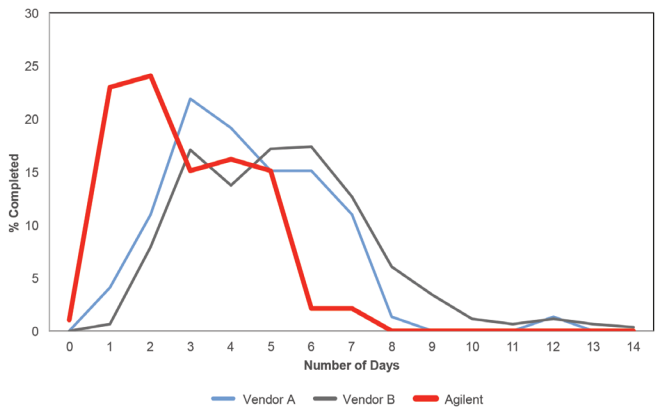


Figure 8. Histogram comparison of assay turnaround time. A 2-day savings in overall turnaround time was demonstrated in a clinical laboratory environment (Data provided by ProPath. Agilent ALK probe and IQFISH buffer were purchased separately, and mixed prior to use).

IQFISH FFPE Workflow

1 Dewax

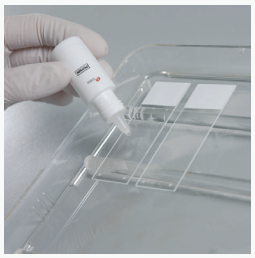
Substitute Xylene/ Xylene for 5 minutes x2; EtOH 96% 2 minutes x2; EtOH 70% 2 minutes x2; wash buffer for 2 minutes

2 Pretreatment

Heat slides immersed in pretreatment buffer to **>95°C** for 10 minutes using a microwave; cool for 15 minutes

3 Pepsin

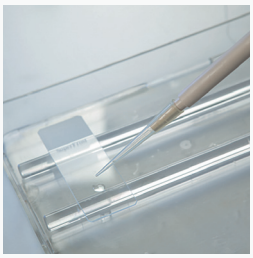
Apply ready-to-use pepsin; incubate at **37°C for 7 minutes**; tap off excess pepsin, wash buffer **3 minutes x2**



4 Probe addition

Ensure probe is completely thawed and **vortex vial** for **15 seconds**

Apply 10 µl probe; add coverslip and seal

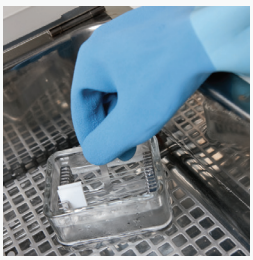


5 Hybridization

Co-denature at **80°C for 10 minutes**; hybridize at 45°C for 90 minutes

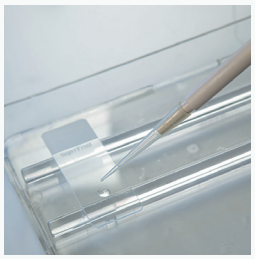
6 Wash

Place in stringent wash buffer at **63°C for 10 minutes**; wash buffer at 25°C for 3 minutes x2; dehydrate in EtOH series: 70%, 85%, 96% for 2 minutes each; air dry



7 Mount

Apply **15 µl** of mounting medium; coverslip



8 Scope

View using a **Cy3**/FITC filter (Chroma# 59009)

Figure 9. IQFISH FFPE workflow with Dako Histology Accessory Kit. Key steps are highlighted in blue.

Ordering Information

The ALK, ROS1, RET and MET IQFISH probes are available in 20-test and 120-test (6 packs of 20) kit sizes.
The orange-red child probe (CY3) and green child probe (FITC) are premixed and diluted in IQFISH Fast Hybridization Buffer. Each vial provides 200 µl of probe mix, which is enough for 20 tests (10 µl per test). Visualization with a CY3/FITC dual filter (Chroma #59009) is recommended.

	Part Number	Production Description	Volume
CE	G111600-8	ALK IQFISH Break-Apart Probe	20 tests
CE	G111601-8	ROS1 IQFISH Break-Apart Probe	20 tests
CE	G111602-8	RET IQFISH Break-Apart Probe	20 tests
CE	**G111603-8**	MET IQFISH Probe with CEP7	20 tests
CE	G211600-8	ALK IQFISH Break-Apart Probe, 6 packs	6x20 tests
CE	G211601-8	ROS1 IQFISH Break-Apart Probe, 6 packs	6x20 tests
CE	G211602-8	RET IQFISH Break-Apart Probe, 6 packs	6x20 tests
CE	G211603-8	MET IQFISH Probe with CEP7, 6 packs	6x20 tests
CE	K5799	Dako Histology FISH Accessory Kit	20 tests

References

1. Soda M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature (2007) 448:561–66.

2. Rikova K, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell (2007) 131:1190–203.

3. Takeuchi K, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med. (2012) 18:378–81.

4. Shaw AT, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med. (2014) 371:1963–71.

5. Cappuzzo F, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol. (2009) 27:1667–7.

6. Go H, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol. (2010) 5:305–13.

IQFISH Probes are CE marked under the European In Vitro Diagnostic Directive (99/79/EC). These products are not approved for sale in the U.S.

This information is subject to change without notice.

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