IQFISH

Технические характеристики

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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).

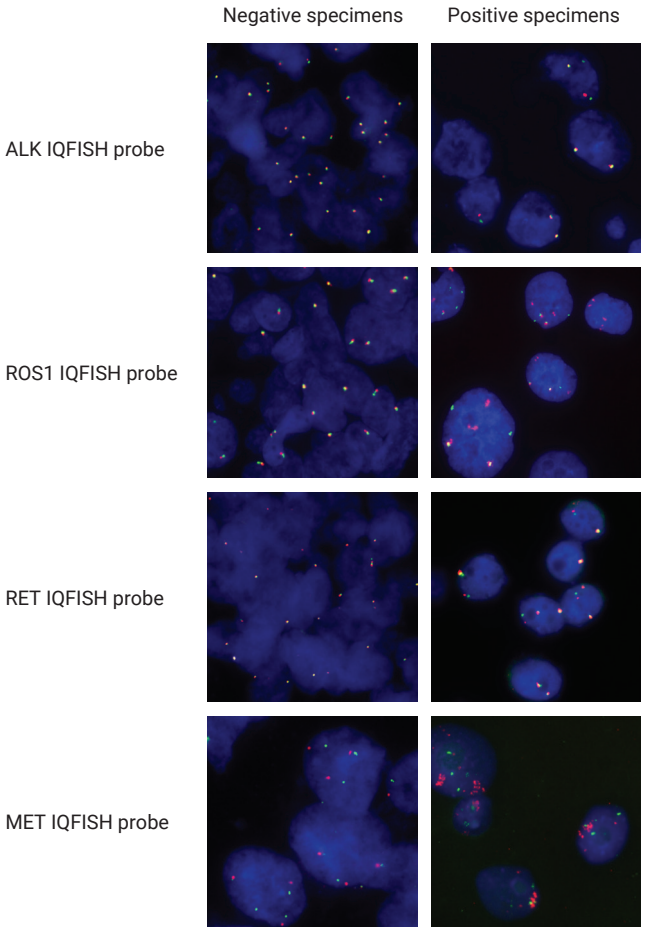


Figure 1. Sample hybridization images for IQFISH probes with both negative and positive specimens.

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

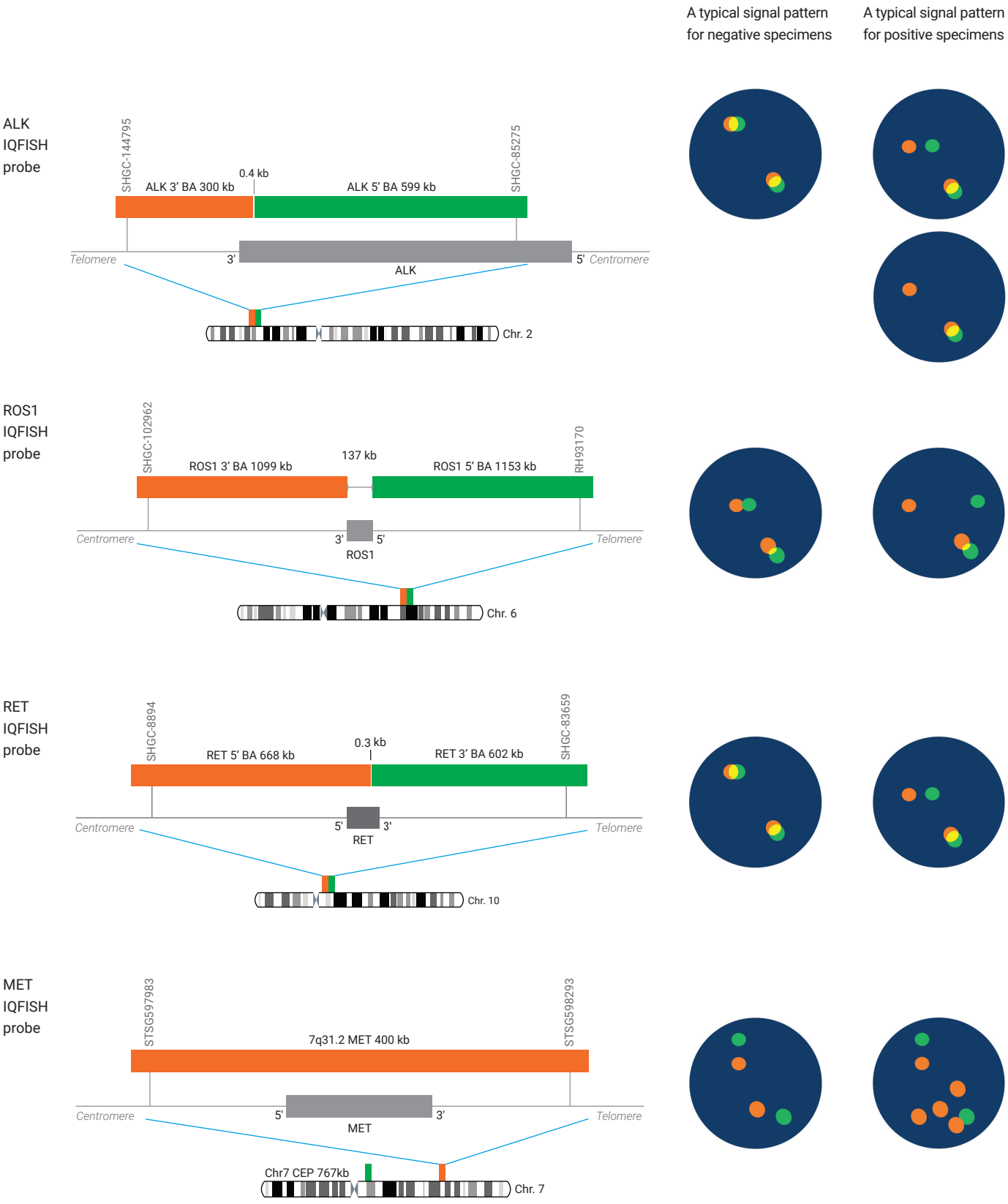


Figure 2. Probe map and typical signal pattern for ALK, ROS1, RET and MET IQFISH probes.

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.

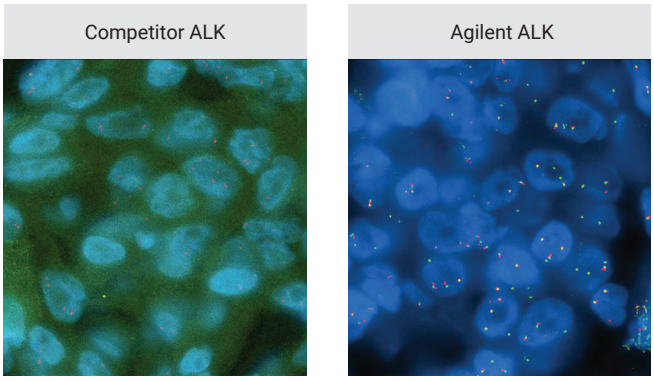


Figure 3. Comparison of competitor ALK (left) and Agilent IQFISH ALK (right) probes. Bright, crisp hybridization signals are produced with IQFISH probes that are free of repetitive DNA sequences and exposure to blocking agents known to suppress hybridization signal.

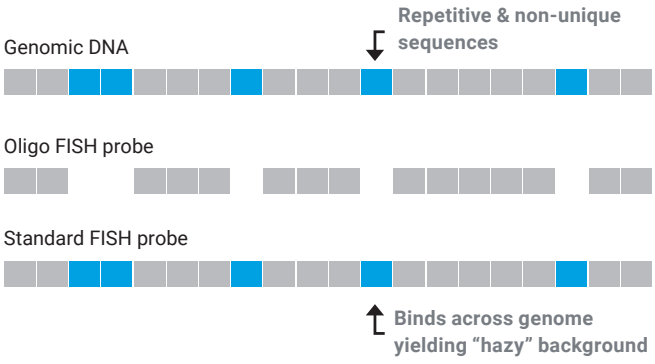


Figure 4. Oligonucleotide-based FISH (oligo FISH) probe design strategy. Repetitive elements are identified and removed during probe design process.

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.

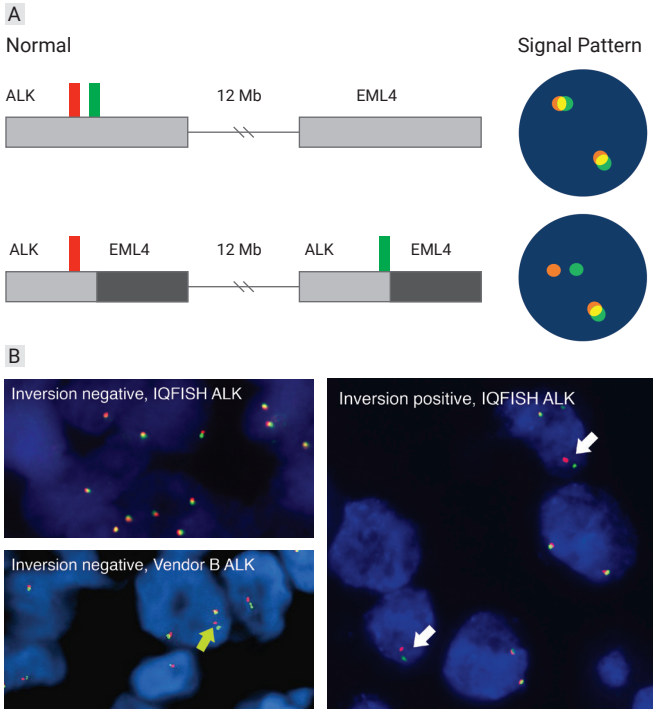


Figure 5. IQFISH ALK inversion detection with tighter co-localization.
Panel A. Illustration of an inversion event, and associated FISH signal pattern.
Panel B. Left: Inversion negative samples show co-localization of the orange-red and green signals. The IQFISH ALK probe (top left) shows tighter co-localized 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%.

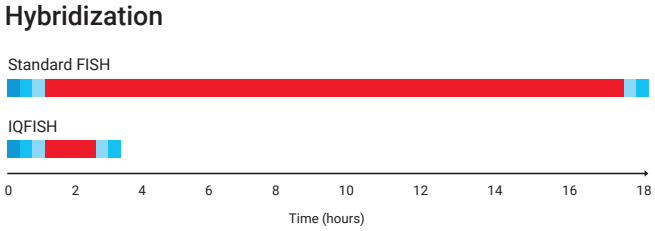


Figure 6. FISH processing time comparison.

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.

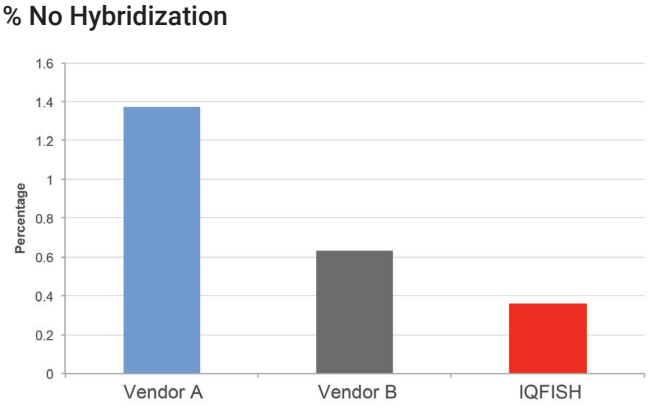


Figure 7. Comparison of ALK FISH Hybridization failures. Test failures (no hybridization signals) were compared between Agilent IQFISH and two other vendors. Sample size: Vendor A 73 tests; Vendor B 640 tests; Agilent IQFISH: 278 tests (Data provided by Propath, USCAP 2015. Agilent ALK probe and IQFISH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

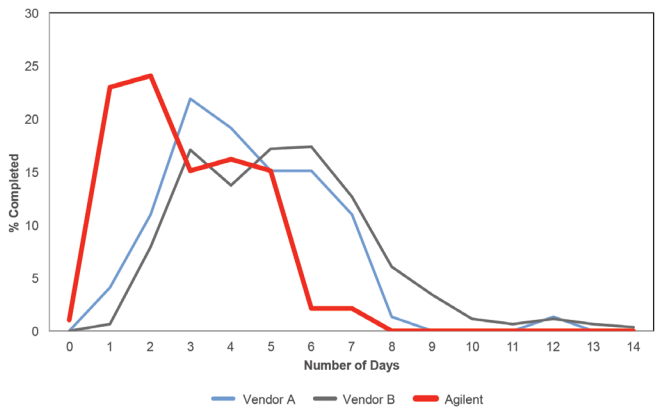


Figure 8. Histogram comparison of assay turnaround time. A 2-day savings in overall turnaround time was demonstrated in a clinical laboratory environment (Data provided by ProPath. Agilent ALK probe and IQFISH buffer were purchased separately, and mixed prior to use).

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.

Introduction

A number of gene rearrangements are associated with the different subtypes of Lymphoma (Figure 1). The IQFISH Lymphoma probe panel enables the identification of gene rearrangements through the use of break-apart and dual fusion probe methodologies.

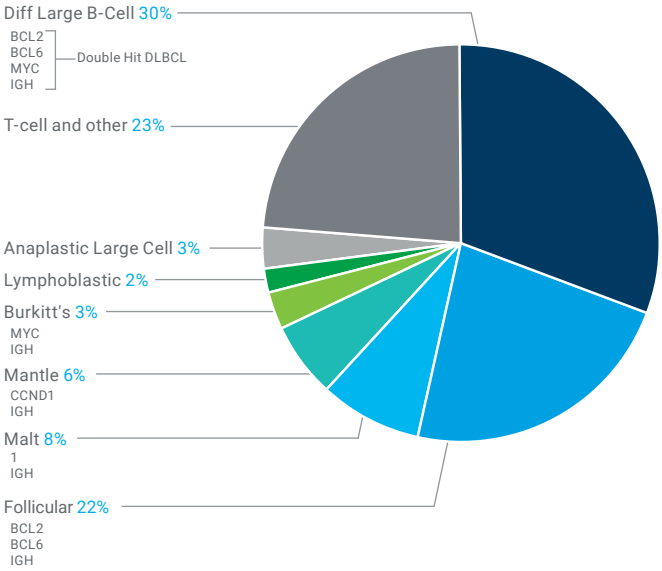


Figure 1. Distribution of Non-Hodgkin Lymphoma subtypes and targets.[1,2]

Fast, high-quality IQFISH for lymphoma

The IQFISH panel for lymphoma is a set of oligonucleotide-based ready-to-use FISH probes, premixed with IQFISH Buffer, for the detection of rearrangements involving the MYC, BCL2, BCL6, MALT1, CCND1 and IGH genes by fluorescence in situ hybridization (FISH). These probes are for use on formalin-fixed, paraffin-embedded (FFPE) tissue sections and are available in break-apart and dual-fusion methodologies.

Maximum information is obtained by utilizing two probe design methodologies:

1. Break-apart FISH probe design:

Break-apart (BA) probes consist of two child probes, designed to be on opposite sides of the translocation break point for a given gene, each labeled in a different color (Figure 2,8).

Break-apart probes generate signals in normal cells that are closely matched in size and co-localized (2 fusion).

Following a translocation, the signals are "broken apart" and no longer co-localize (for example: 1 red, 1 green, 1 fusion) (Figure 2). Hence, this design detects the break of a gene e.g. MYC. It is independent of its translocation partner and is well suited for when there are multiple potential partner genes.

2. Dual-fusion FISH probe design:

Dual-fusion (DF) probes consist of two child probes, each designed to target a given gene and each labeled in a different color (figure 3,8). These probes generate signals in normal cells that are closely matched in size and are separated by more than one signal distance (for example, 2 red, 2 green).

Following a translocation, typically the signals are co-localized, producing fusion signals (for example, 1 red, 1 green, 2 fusion, Figure 3,9). Hence, this design detects the fusion of two partner genes e.g. MYC and IGH involved in a translocation and is useful when confirming the presence of a specific fusion.

Signal patterns other than those described above may indicate variant translocations, deletions or other complex rearrangements resulting in atypical signal patterns.

The IQFISH probes and corresponding protocol were developed using lymph node tissue sections or extranodal lymphoid tissue sections (MALT1 probe only).

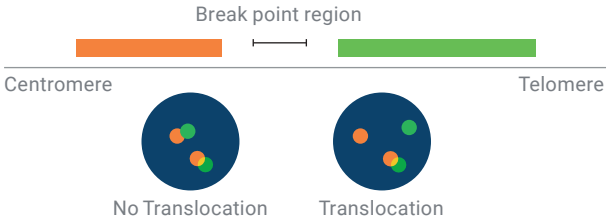


Figure 2. Break-apart FISH probe design and expected signal patterns.

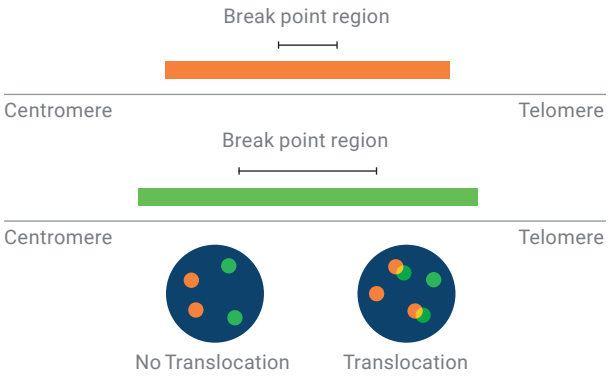


Figure 3. Dual-fusion probe design and expected signal patterns.

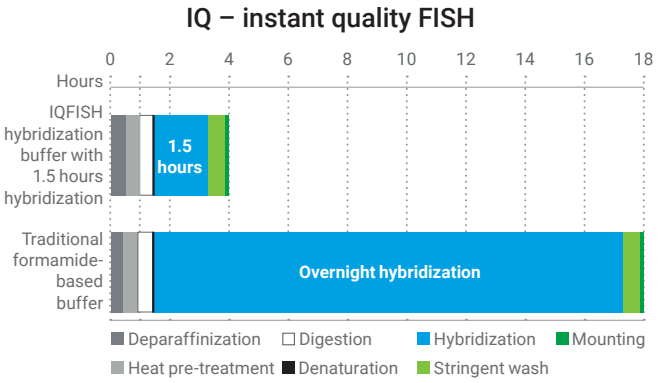
The assays combine two groundbreaking technologies: oligonucleotide- based SureFISH technology and formamide-free IQFISH. SureFISH technology enables chromosomal aberration detection using a precise, synthetic oligonucleotide probe design process. IQFISH is a unique, ultra-fast hybridization technology that provides FISH results in about 4 hours. Together these advancements provide fast, high-quality FISH results.

IQFISH assays combine oligonucleotide-based SureFISH technology and formamide-free IQFISH buffer.

- **Faster time to results:**
  Unique 90-minute hybridization enables answers in 4 hours compared to 16+ hours for traditional formamide-based FISH assays.
- **Less time at the microscope:**
  Repeat and blocker-free design provides high signal-to-noise ratio, brighter signals and lower hybridization background, making quicker evaluations possible.
- **Robust protocol:**
  Streamlined FFPE protocol utilizing the Histology FISH Accessory Kit enables high-quality reproducible results with few failures.
- **Non-toxic, formamide-free IQFISH hybridization buffer:**
  Technicians are not exposed to the teratogenic effects of formamide. IQFISH probes can safely be applied to slides on the bench or on Hybridizer.

Faster time to results

Each of the IQFISH probes come Ready-to-Use 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 thereby providing a more IHC-like turn-around- time (TAT).
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%.



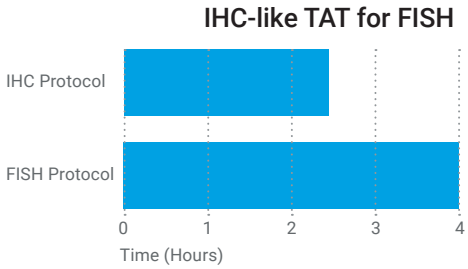


Figure 4. The IQFISH Fast Hybridization Buffer reduces hybridization time to 90 minutes compared to overnight hybridization with traditional formamide-based buffer.

Brighter signals and lower hybridization background due to repeat- and blocker-free design

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

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

Agilent IQFISH probes address this by targeting only the unique sequences. During probe design, oligos are specifically placed in gene-specific regions while avoiding repetitive sequence. This increases signal brightness and specificity while decreasing hybridization background.
It also eliminates the need for blocking agent and associated signal suppression. The combined effect is a high signal-to-noise ratio and easy visualization.

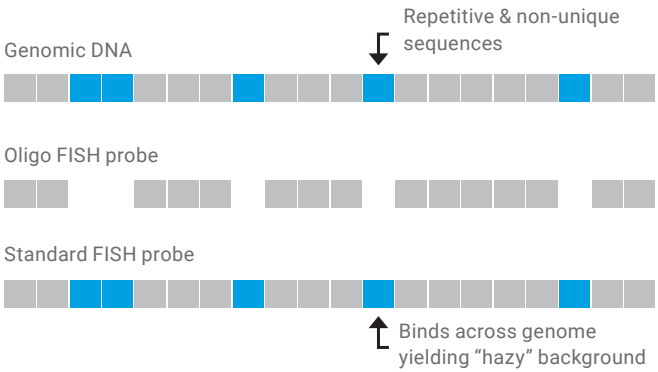


Figure 5. Oliogonucleotide-based FISH (oligo FISH) probe design strategy. Repetitive elements are identified and removed during probe design process.

Overcoming diagnostic challenges with IQFISH

MYC breakpoints heterogeneity

Detection of MYC translocations by FISH is frequently performed in pathology laboratories as part of the diagnostic work-up for suspected Burkitt and Diffuse Large B-cell Lymphomas[2]. However, designing a probe that covers all relevant MYC breakpoints is a challenge as breakpoints are widely dispersed on the 8q24 locus[3,4]. With some probe designs there is a risk that far 5' (centromeric) and far 3' (telomeric) breakpoints may be missed, resulting in an inaccurate risk stratification for those patients[5].

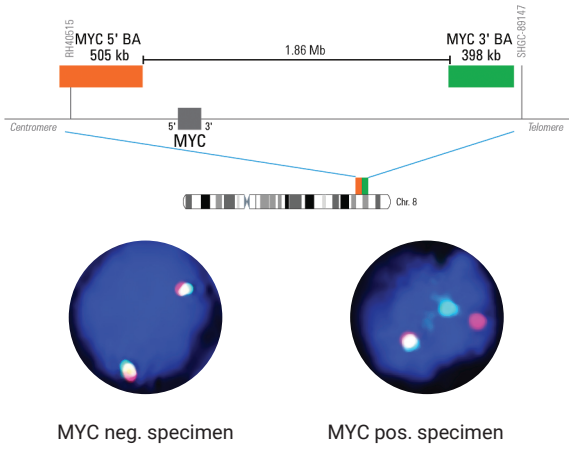


Figure 6. MYC IQFISH Break-Apart Probe map capturing breakpoints with IGH as well as non-IGH partners.

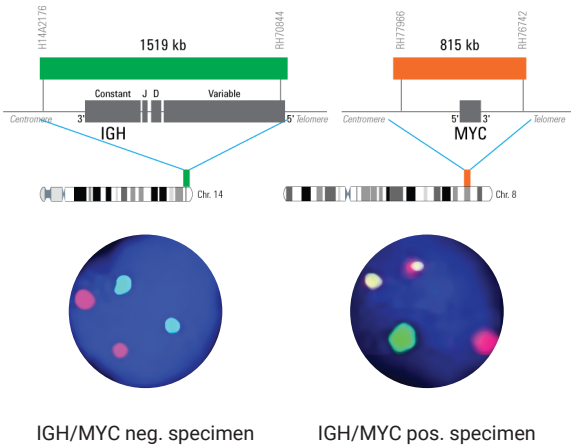


Figure 7. IGH/MYC Dual Fusion Probe map capturing MYC breakpoints with the IGH partner.

To address this, the Agilent MYC IQFISH BA probe set is designed with a wider span (1.86 MB) between the orange-red and the green probes (Figure 6). This separation allows the probe to capture a broad range of breakpoints, including those with non-IGH partners such as IGK and IGL, while still maintaining the expected orange-red/green signal co-localization on non-rearranged samples.

Faster results in "double hit" cases

The unique 90-minute hybridization enables answers in 4 hours compared to 16+ hours for traditional formamide-based FISH assays. The faster time to results is especially beneficial in "double hit" cases - a sub-set of diffuse large B-cell lymphoma (DLBCL) with the presence of MYC translocations associated with BCL2 and/or BCL6 translocations. These patients have a poor prognosis and may benefit from more aggressive therapies that should be initiated as early as possible after diagnosis.

The faster IQFISH protocol, the shorter reading time due to brighter signals combined with a well-preserved tissue morphology and the higher share of evaluable cases may lead to a faster average response time; thus reducing the time to start an appropriate treatment of "double hits" cases[6].

IQFISH Probe Maps

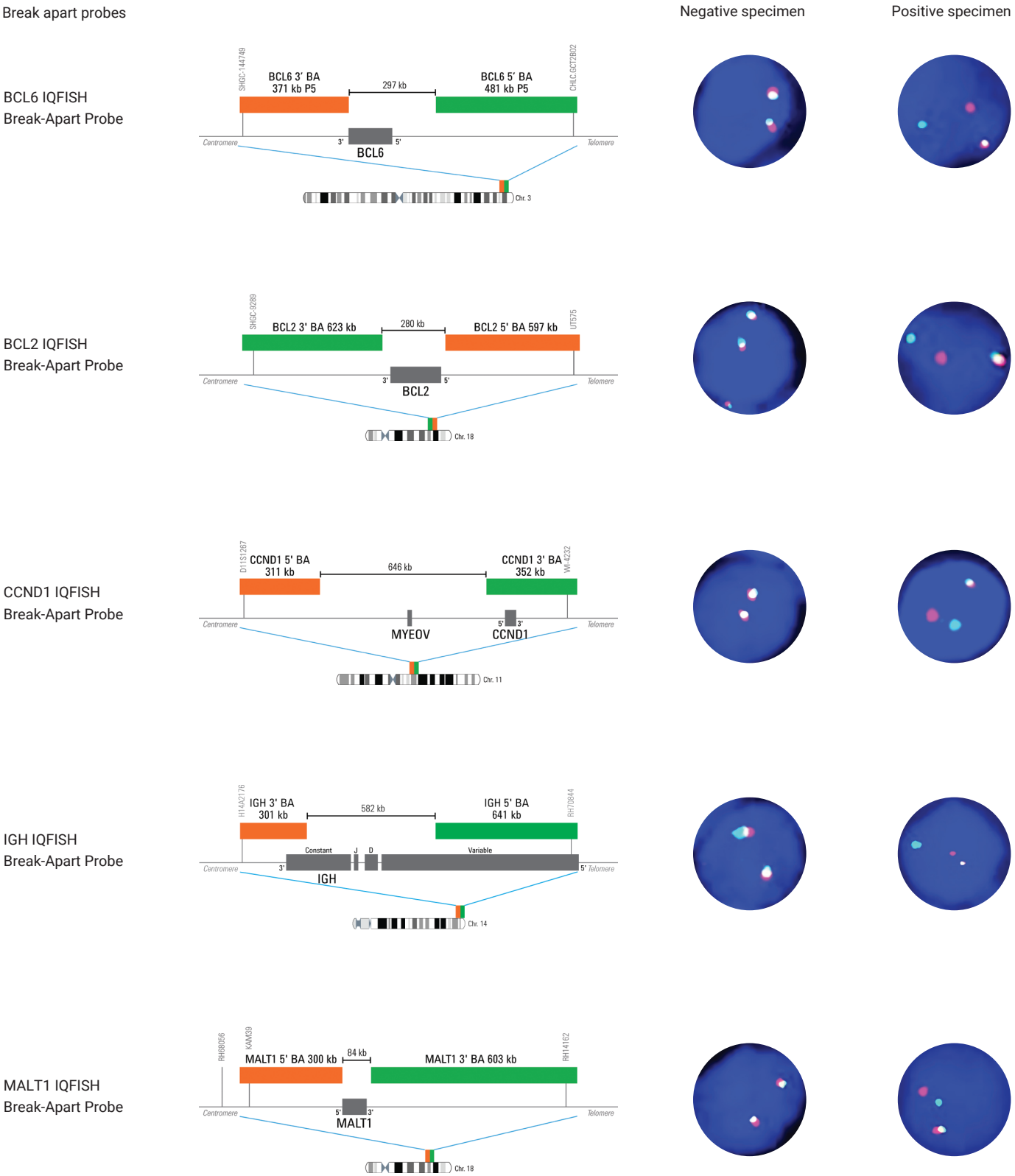


Figure 8. IQFISH Break-Apart Probe maps and images.

IQFISH Probe Maps cont.

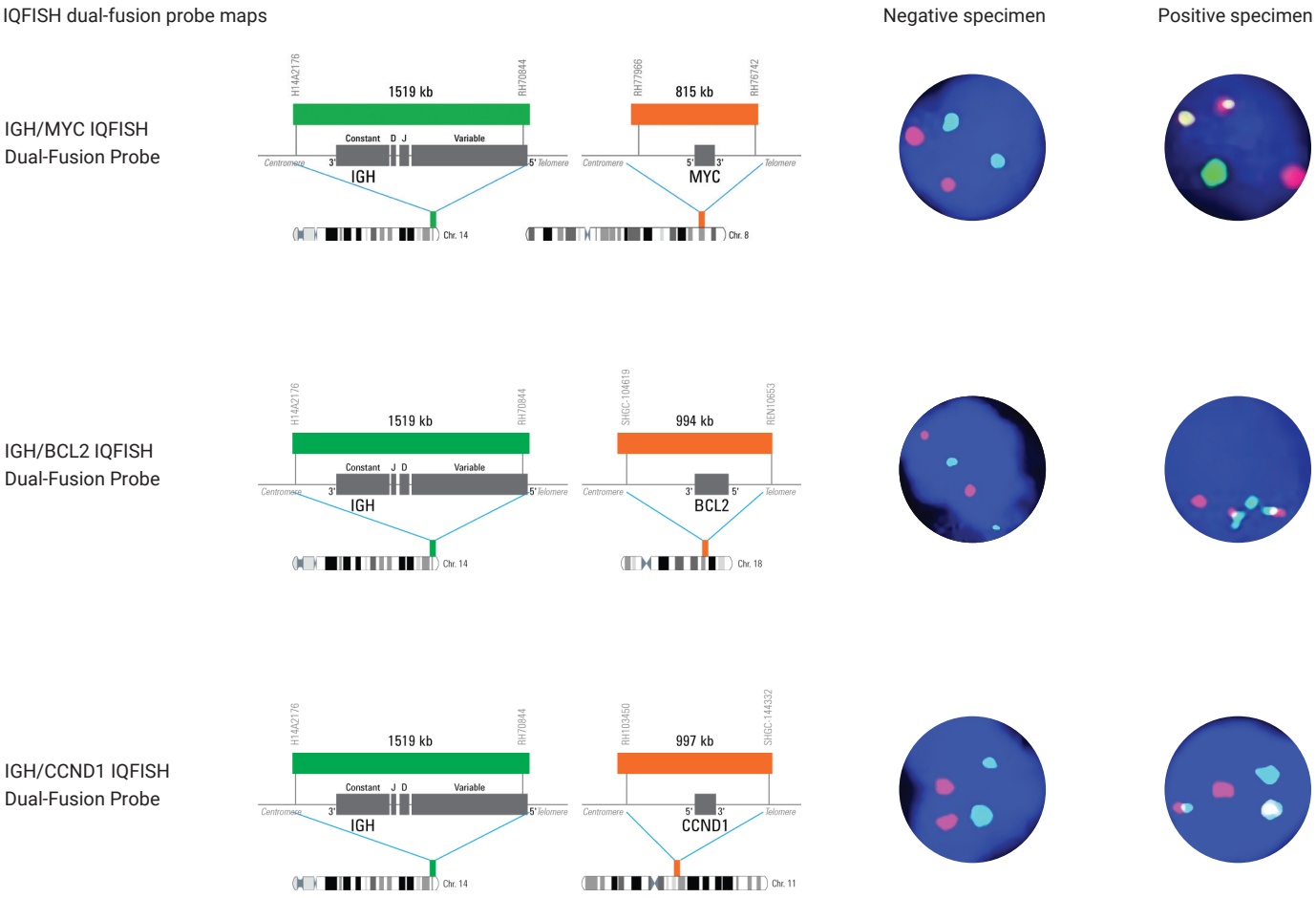


Figure 9. IQFISH Dual-Fusion Probe maps and images.

Robust FFPE FISH protocol

The robust protocol provides high-quality reproducible results and few failures

Oligo-based IQFISH probes are designed for use with the Dako Histology FISH Accessory Kit (K579911-2). 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.[6]

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

Ordering Information

The IQFISH lymphoma probes are available in 20-test 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).

	Production Name	Part Number	Volume
CE	MALTI IQFISH Break-Apart Probe	G111619-2	20 tests
CE	BCL2 IQFISH Break-Apart Probe	G111620-2	20 tests
CE	BCL6 IQFISH Break-Apart Probe	G111621-2	20 tests
CE	CCND1 IQFISH Break-Apart Probe	**G111622-2**	20 tests
CE	MYC IQFISH Break-Apart Probe	G111623-2	20 tests
CE	IGH IQFISH Break-Apart Probe	G111624-2	20 tests
CE	IGH/CCND1 IQFISH Dual Fusion Probe	G111625-2	20 tests
CE	IGH/MYC/IQFISH Dual Fusion Probe	G111626-2	20 tests
CE	IGH/BLC2 IQFISH Dual Fusion Probe	G111627-2	20 tests
CE	Histology FISH Accessory Kit	K579911-2	20 tests
CE	Hybridizer (200-240 V)	S245130-2	
CE	Hybridizer Humidity Control Strips	S245230-2	20 strips

For In Vitro Diagnostic Use

Filter recommendations

Filters are individually designed for specific fluorochromes and for each microscope. For the interpretation of IQFISH staining assays, the following combination of filters should be used:

Specific DAPI filter

High-quality Cy3/FITC double filter e.g. Chroma #59009

(alternatively specific Cy3 and FITC single filters).

Fluorochrome	Excitation Wavelength	Emission Wavelength
FITC	495 nm	517 nm
Cy3	547 nm	565 nm

References

1. Lymphoma statistics from around the world.
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Trusted Answers

IQFISH FFPE Workflow

1 Dewax

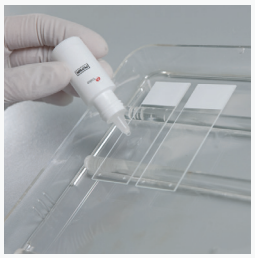
Substitute Xylene/ Xylene for 5 minutes x2; EtOH 96% 2 minutes x2; EtOH 70% 2 minutes x2; wash buffer for 2 minutes at room temperature

2 Pretreatment

Heat slides immersed in pretreatment buffer to **>95°C** for 10 minutes using a microwave; cool for 15 minutes; wash buffer **3 minutes x2** at room temperature

3 Pepsin

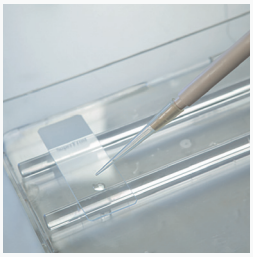
Dry slide around tissue with lintless wipe; Apply ready-to-use pepsin; incubate at **37°C for 30 minutes**; tap off excess pepsin, wash buffer **3 minutes x2** at room temperature



4 Probe addition

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

Apply 10 µl probe; add coverslip and seal

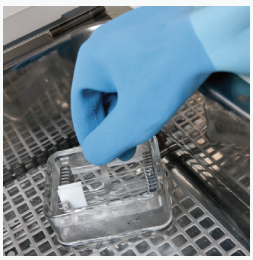


5 Hybridization

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

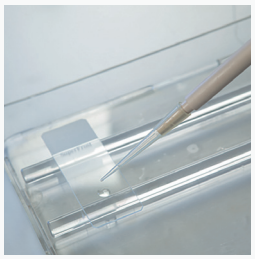
6 Wash

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

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