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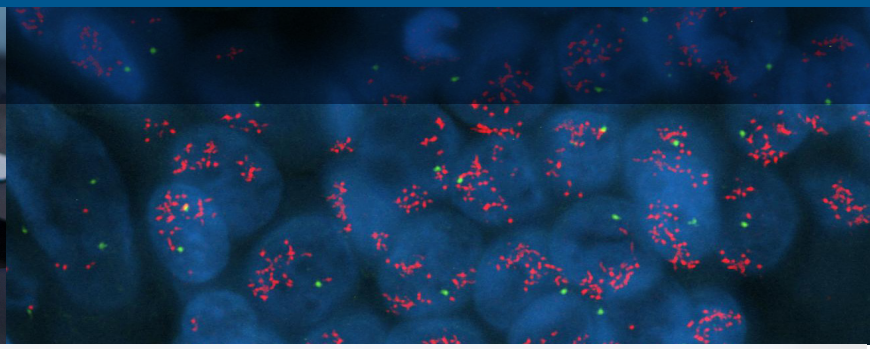
Dako

HER2 IQFISH pharmDx

Instant Quality FISH - The name says it all



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Our IQFISH hybridization buffer is revolutionizing cancer diagnostics

The cutting-edge IQFISH hybridization buffer reduces diagnostic test turnaround time from ~18 hours to less than 4 hours.

With it, pathology labs can perform DNA-based hybridization assays in much less time, with higher fluorescent signal intensity for better results compared to traditional FISH assays[1]. This represents a major advance for pathologists and oncologists, and ultimately for patients.

IQFISH hybridization buffer is formamide-free, making it a clear choice for your staff and the laboratory environment.

HER2 IQFISH pharmDx is the first FDA-approved product that uses the revolutionary, formamide-free IQFISH hybridization buffer. Top-quality hybridization, combined with diagnostic certainty - that is real progress.

HER2 IQFISH pharmDx is designed to quantitatively determine *HER2* gene amplification in breast cancer and metastatic gastric or gastroesophageal junction adenocarcinoma samples. *HER2* IQFISH pharmDx is indicated as an aid in the assessment of breast and gastric cancer patients for whom Herceptin (trastuzumab) treatment and for breast cancer patients for whom PERJETA (pertuzumab) or KADCYLA (ado-trastuzumab emtansine) is being considered[3].

"In summary, the 1-day HER2 IQFISH approach is a potent method for the determination of HER2 status offering some relevant advances further to the short incubation time, including enhanced tissue integrity and preserved fluorescent signal intensity when compared with the conventional HER2 FISH kit[2]."

– Hegyi et al. Appl mmunohistochem Mol Morphol, 2013.

IQFISH: Setting New Standards for Quality and Speed

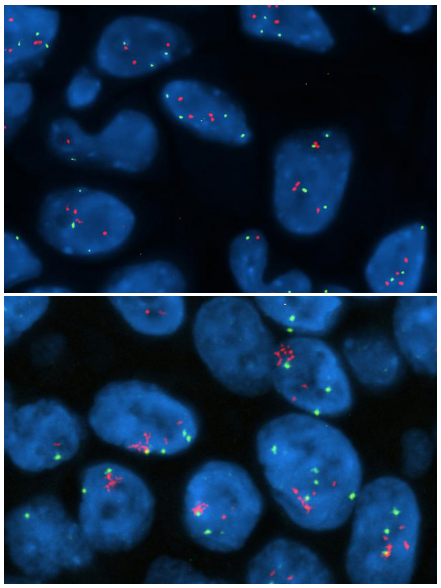

Figure 1. *HER2* IQFISH pharmDx stains of a *HER2* non-amplified (top) and a *HER2* amplified (bottom) breast tumor. See acknowledgement.

In cancer diagnostics, providing the right diagnosis to the right patient on time is everything

Quality

Enhanced visualization of morphology and bright signals help identify cancer cells more easily. Crisp, clear signals make counting accurate, easy and fast.

Fast

Hybridization takes only 1 to 2 hours with the IQFISH hybridization buffer. Enhanced staining quality and a total turnaround time of less than 4 hours from dewax to counting is the ultimate combination of quality and speed.

Accurate

The robust assay delivers accurate results with high concordance to other FDA-approved FISH assays[3].

Formamide-free

Eliminate the exposure of lab personnel to formamide.

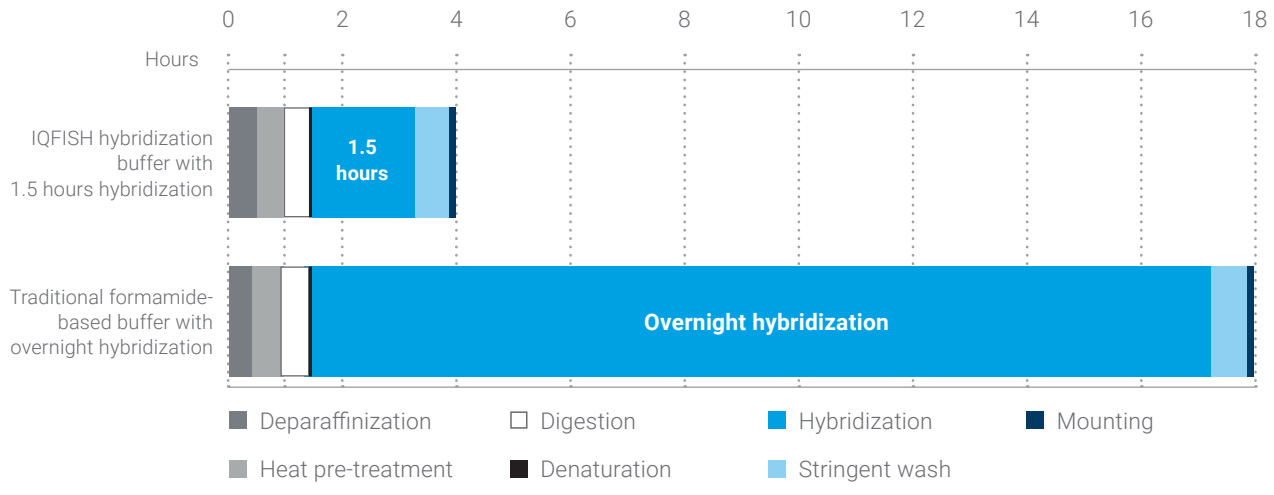

Figure 2. Timeline for IQFISH procedure vs. traditional FISH

IQFISH: Save time, reduce costs and increase quality

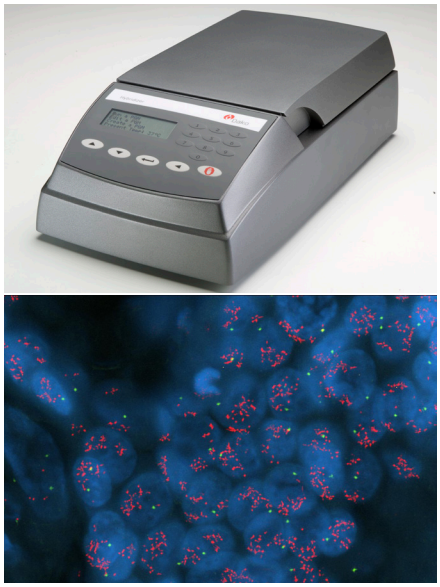

Figure 3. *HER2 IQFISH pharmDx™ stain of a HER2 amplified breast tumor. See acknowledgement.*

Superior performance of *HER2* IQFISH pharmDx compared to a traditional HER2 FISH assay

A study was conducted to compare two FDA-approved *HER2* kits on almost 1500 patient samples tested over an eight-month period by ProPath Services[6]. The results showed that *HER2* IQFISH pharmDx:

Saves Time

Less hands-on time and reduced risk of errors

All assay reagents in the *HER2* IQFISH pharmDx kit are provided in RTU format or in 20x liquid concentrates that simply need to be diluted with DI water, eliminating the need to weigh powders and adjust pH of solutions. The assay's fast turnaround time enables same day results for *HER2* FISH testing which can lead to increased laboratory operational efficiency.

The *HER2* IQFISH pharmDx assay also has fewer hands-on steps prior to hybridization, which further reduces the overall assay turnaround time when compared to another *HER2* FISH assays[6]. Time savings are also realized during the analysis and enumeration due to the quality of the slides processed with the *HER2* IQFISH pharmDx kit.

Reduces costs

Less repeat testing

Repeat testing due to assay failure can compromise laboratory productivity and have a negative impact to patient case management. During an eight-month period, ProPath reported a decrease in repeat testing from 2.7% to 0.4% after implementing the *HER2* IQFISH pharmDx assay[6].

The decrease in repeat rate translated into a significant savings for the laboratory. Less time spent on the assay steps and sample interpretation also offers advantages for reducing costs for the laboratory[6].

"In our opinion, the improved signal to background ratio generated by the HER2 IQFISH pharmDx assay allowed for a more accurate determination of copy numbers for both HER2 and the control probe. The HER2 IQFISH pharmDx assay also required significantly less time to score[6]."

– **Cohen and Alsobrook, 2014.**

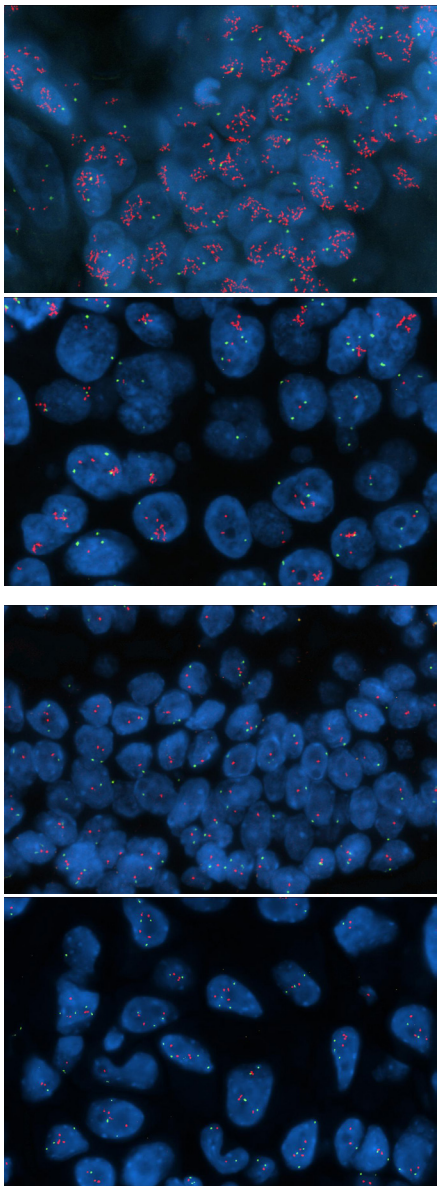

Figure 5. *HER2* IQFISH pharmDx stains of a *HER2* amplified (top) and a *HER2* non-amplified (bottom) breast tumor. See acknowledgment.

Increases quality

Intense signals and low background

The *HER2* BAC probe and CEN-17 PNA probes comprise a robust assay that correlates well in internal and external validation studies[3,6]. A challenge for FISH assays is suppressing background hybridization signals without compromising specific signals. A blocking reagent is not required in assays that utilize IQFISH hybridization buffer due to the unique hybridization mechanism[1] which contributes to the high signal-to-noise ratio that is observed on *HER2* IQFISH pharmDx slides.

The *HER2* IQFISH pharmDx assay preserves nuclear morphology, which is essential in orienting the FISH slide to an H&E or IHC slide and ensuring that signals are being enumerated in the tumor area. The intense pinpoint hybridization signals and low background contribute to efficient sample analysis.

HER2 IQFISH pharmDx

- Quantitatively determines *HER2* gene amplification
- Identifies patients with breast or gastric cancer likely to benefit from Herceptin® (trastuzumab) treatment
- Identifies breast cancer patients likely to benefit from PERJETA (pertuzumab) or KADCYLA (ado-trastuzumab emtansine) treatment

"IQFISH provided excellent quality signals without any background staining. As a rule, DAPI stain and red and green signals were brighter with IQFISH than with FISH."

– **Franchet et al. Histopathol, 2013.**

"The highly concordant data support IQFISH as a useful alternative to FISH, allowing reliable assessment of HER2 status. Use of this method could lead to reporting of HER2 status to the oncologist within a day[5]."

– **Franchet et al. Histopathol, 2013.**

Outstanding concordance to traditional FISH

Concordance to *HER2* FISH pharmDx of 98.6% for gastric cancer and 99.2% for breast cancer[3]. Concordance to PathVysion® of 100% for breast cancer [4].

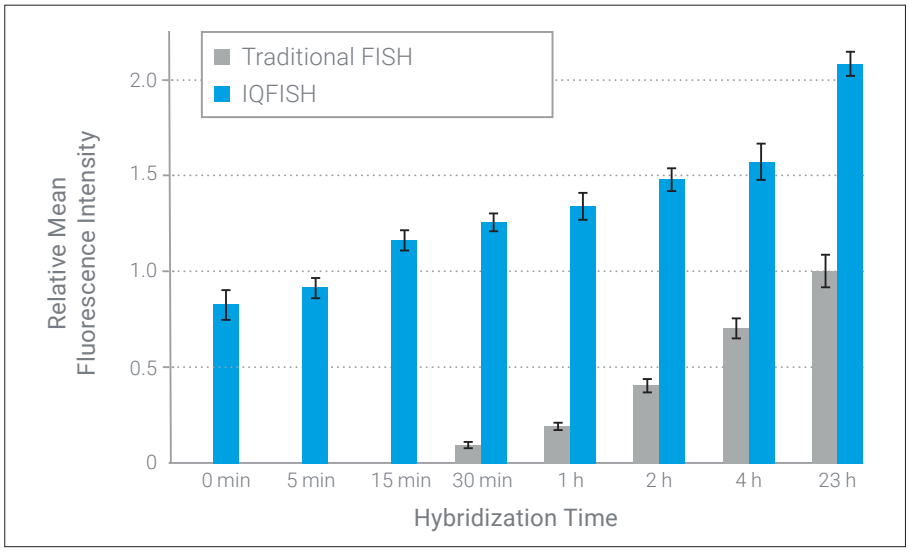

Figure 4. IQFISH hybridization results in increased signal intensity.

Brighter signals within 1 hour[1]

A time-chase comparison of HER2 DNA FISH signal intensities using IQFISH hybridization buffer and formamide buffer was performed on FFPE breast carcinoma tissue sections. Optimal signal intensity using IQFISH buffer is obtained with 1 to 2 hours of hybridization. Identical probe concentration was used in the two buffers. The blackbars represent the 95% confidence interval (n = 45 signals).

HER2 IQFISH pharmDx kit includes:

- Dual-target probe mix (*HER2*/CEN-17)
- Pre-treatment solution
- Pepsin, ready-to-use
- Pepsin diluent
- Stringent wash buffer
- Wash buffer
- Fluorescence mounting medium, with DAPI
- Coverslip sealant

Product Name	Targets	Indication	Code
HER2 IQFISH pharmDx	*HER2*/CEN-17	Breast/Gastric	K5731

Acknowledgement

Special thanks to ProPath Services for supplying the stains presented in this brochure. The presented FISH images have not been edited or manipulated and are published in their original format as provided by ProPath Services, not in any way affiliated Agilent Technologies.

References

1. Matthiesen SH and Hansen CM. Fast and non-toxic in situ hybridization without blocking of repetitive sequences. PloS One 2012;7: e40675.
2. Hegyi K, Lonborg C, Monus A, Mehes G. One-day FISH approach for the high-speed determination of HER2 gene copy status in breast carcinoma. Appl Immunohistochem Mol Morphol 2013;21:567-71.
3. Internal validation studies as referenced in the package insert for the *HER2* IQFISH pharmDx kit.
4. Rüschoff J. *HER2* IQFISH pharmDx in-situ hybridization results in 3½ hours - performance evaluation and clinical perspectives. Symposia at ECP 2012. https://www.agilent.com/en/video/intra-laboratory-comparison-study
5. Franchet C, Filleron T, Cayre A, Mounie E, Penault-Llorca F, Jacquemier J, et al. Instant-quality fluorescence in-situ hybridization as a new tool for HER2 testing in breast cancer: a comparative study. Histopathol 2014;64:274-83.
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