In situ hybridization made easy

FISH OR CISH? TWO WAYS TO VISUALIZE YOUR RNA AND DNA TARGETS *IN SITU*.

In situ hybridization (ISH) is a powerful technique for localizing specific nucleic acid targets within fixed tissues and cells, allowing you to obtain temporal and spatial information about gene expression and genetic loci. While the basic workflow of ISH is similar to that of blot hybridizations—the nucleic acid probe is synthesized, labeled, purified, and annealed with the specific target—the difference is the greater amount of information gained by visualizing the results within the tissue.

Today there are two basic ways to visualize your RNA and DNA targets *in situ*—fluorescence (FISH) and chromogenic (CISH) detection. Characteristics inherent in each method of detection (Table 1) have made FISH and CISH useful for very distinct applications. While both use a labeled, target-specific probe that is hybridized with the sample, the instrumentation used to visualize the samples is different for each method. Here we highlight the differences and the advantages of each method.

Table 1—Inherent characteristics of ISH methods.

Technique	Instrument/ visualization method	Primary advantage	Primary application
FISH	Epifluorescence or confocal microscope	Visualization of multiple targets in the same sample	Gene expression and cytogenetics
CISH	Bright-field microscope	Ability to view the CISH signal and tissue morphology simultaneously	Molecular pathology diagnostics

FISH for your gene expression studies

"Our lab routinely uses FISH, and the FISH Tag™ Kits offer us an unparalleled means for in-house generation of high-quality FISH probes. Their cost-effectiveness and flexibility, coupled with their ease of use, make FISH Tag™ Kits the ideal tool for diagnostic and research cytogenetics."

—Will Westra, The Scripps Research Institute, San Diego, CA

Multiplex fluorescence *in situ* hybridization (FISH) exemplifies the elegance that only fluorescence-based strategies offer: the ability to assay multiple targets simultaneously and visualize co-localization within a single specimen. Using spectrally distinct fluorophore labels for each different hybridization probe, this approach gives you the power to resolve several genetic elements or multiple gene expression patterns in a single specimen, with multicolor visual display.[1]

FISH Tag™ Detection Kits provide all of the tools you need—enzymes, purification technology, and brilliant Alexa Fluor® dye labeling—for generating optimal FISH probes for multiplex assays in just two steps.[2] Nick translation (for DNA probes) or *in vitro* transcription (for RNA probes) is used to enzymatically incorporate amine-modified nucleotides—aminoallyl dUTP for DNA or aminoallyl UTP for RNA—followed by chemical labeling with amine-reactive Alexa Fluor® dyes. Compared to dye-labeled nucleotides, aminoallyl-modified nucleotides are consistently incorporated at high levels and covalently labeled using reliable succinimidyl ester coupling chemistry. The end result

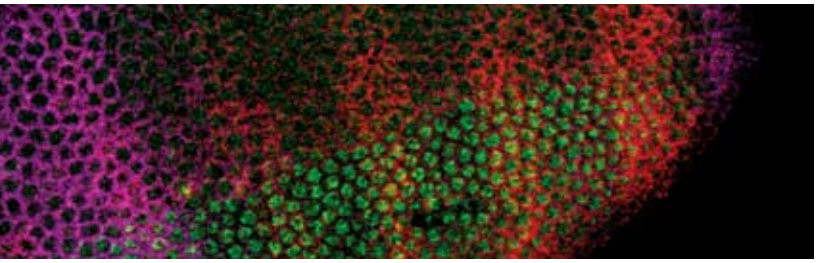

Figure 1—RNA targets labeled in a *Drosophila melanogaster* embryo. Simultaneous detection of expression of three genes in a whole mount *Drosophila melanogaster* embryo by fluorescence *in situ* hybridization (FISH) using the FISH Tag™ RNA Multicolor Kit. Green: *sog* (short gastrulation) labeled with Alexa Fluor® 488 dye; red: *ftz* (fushi tarazu) labeled with Alexa Fluor® 594 dye; magenta: *Kruppel* labeled with Alexa Fluor® 647 dye. The sample was mounted using *SlowFade®* Gold antifade reagent.

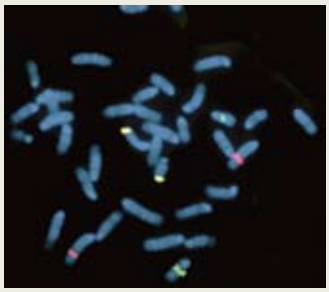

Figure 2—Mouse lymphocyte metaphase spread hybridized with locus-specific FISH probes labeled using FISH Tag™ Kits. Red: Chromosome 4 labeled with Alexa Fluor® 555 dye; yellow: Chromosome 10 labeled with Alexa Fluor® 647 dye (pseudocolored); green: Chromosome 16 labeled with Alexa Fluor® 488 dye. Image contributed by Will Westra, The Scripps Research Institute.

is a higher degree of labeling and improved signal-to-noise ratios in FISH applications. PureLink™ nucleic acid purification technology is included to rapidly and efficiently purify the labeled probe, providing high yields of DNA or RNA. *SlowFade®* Gold antifade reagent is included for superior photostability during imaging. And because there's no need for secondary detection, your results are immediate and visually distinct (Figures 1 and 2). FISH Tag™ Kits offer:

- a complete workflow solution for FISH applications
- exceptional signal intensity and photostability
- multiplexing capabilities with spectrally distinct dyes that allow you to view multiple targets simultaneously

In addition to the FISH Tag™ Kits, there are FISH labeling and detection options available for a variety of applications. For rare or very low-abundance targets, amplify the fluorescence signal of your FISH probes using the Alexa Fluor® dye tyramides in our TSA™ kits. Tyramide signal amplification (TSA) employs reliable horseradish peroxidase (HRP)–based signal amplification that is compatible with haptenylated probes such as those labeled with fluorescein, biotin, dinitrophenyl (DNP), or digoxigenin (Figure 3). In addition to the dramatic signal enhancement provided by TSA detection, dye tyramides are covalently deposited at the site of probe hybridization, providing excellent resolution and high signal-to-noise ratios. Alternatively, ARES™ DNA Labeling Kits provide two-step labeling technology in a basic kit format, without enzymes or a purification system. Protocols for nick translation and reverse transcription [2] are also included. In addition, the new ARES™ random priming protocol is optimized for generating bright FISH probes using this enzymatic labeling approach. ULYSIS™ Nucleic Acid Labeling Kits provide yet another option, offering direct, fast, and easy chemical labeling of nucleic acid probes. And ChromaTide® nucleotides include a variety of Alexa Fluor® and nonproprietary dye and haptenylated conjugates for synthesis of your probe for direct or indirect detection approaches. For a complete list of reagents and kits for your FISH applications, visit **probes.invitrogen.com**. →

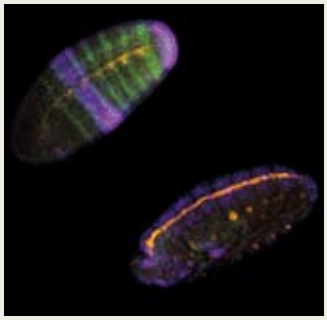

Figure 3—Advanced multiplexing capabilities achieved using a combination of FISH Tag™ and TSA™ Kits. *Drosophila melanogaster* embryos were hybridized with a green-fluorescent *ftz* probe (FISH Tag™ RNA Labeling Kit with Alexa Fluor® 488 dye), a far-red–fluorescent *Kruppel* probe (FISH Tag™ RNA Labeling Kit with Alexa Fluor® 647 dye), and a fluorescein UTP–labeled *Rhomboid* probe, followed by amplification of the probe signal with TSA™ technology using an anti-fluorescein/horseradish peroxidase (HRP) conjugate and Alexa Fluor® 555 tyramide (TSA™ Kit #40).

PRODUCT HIGHLIGHT

In situ hybridization system

In addition to reagents and kits for FISH and CISH, Invitrogen offers accessories and ancillary products such as the SPoT-Light® CISH™ Hybridizer, a hands-free denaturation and hybridization system. Note that the SPoT-Light® Hybridizer can be used with both FISH and CISH techniques.

Product	Quantity	Cat. no.
SPoT-Light® CISH™ Hybridizer (120 VAC, 50–60 Hz)	1 each	76-2000
SPoT-Light® CISH™ Hybridizer (240 VAC, 50–60 Hz)	1 each	76-2001
Humidity Control Cards	10 per pack	76-2002

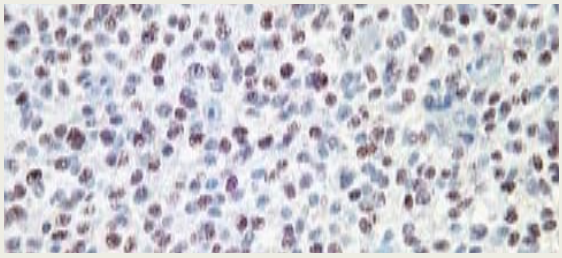

Figure 4—Gene amplification was detected in glioblastoma tissue using a SPoT-Light® EGFR CISH™ probe and CISH™ Polymer Detection Kit (20× magnification).

CISH for a more complete diagnostic picture

"CISH will be very popular with pathologists: you get all the advantages of IHC, including less complex staining protocols, short procedure times, and low-cost permanent slides that can be scored with a routine light microscope, shown to colleagues, and brought to tumor boards."

—Dr. Jeffrey S. Ross, Albany Medical College, Albany, NY

Chromogenic *in situ* hybridization allows detection of gene amplification, gene deletion, chromosome translocations, and chromosome number using conventional peroxidase or alkaline phosphatase reactions under a bright-field microscope on formalin-fixed, paraffin-embedded (FFPE) tissues. Tissue morphology and gene aberrations can thus be viewed simultaneously, and because of its similarities to immunohistochemistry (IHC) techniques, CISH can be easily adapted for use in histology labs. This increases the amount of information available to physicians, helping them make the best therapeutic decisions in patient care.

Invitrogen offers a number of Zymed® SPoT-Light® CISH™ probes and detection kits with potential diagnostic utility in routine histology labs. Probes and detection kits validated for research use include amplification probes (Figure 4), deletion probes, centromeric probes, and translocation probe pairs (Table 2). Learn more at www.invitrogen.com/pathology. ■

References

1. Kosman, D. et al. (2004) *Science* 305:846.
2. Cox, W.G. and Singer, V.L. (2004) *Biotechniques* 36:114–122.

Table 2—SPoT-Light® products for CISH.

Product name	Quantity	Cat. no.	Companion CISH™ Detection Kit	Cat. no.
Amplification probes				
HER2	20 tests	84-0100*†	CISH™ Polymer Kit	84-9246
Cyclin D1	20 tests	84-1900		
EGFr	20 tests	84-1300		
N-Myc	20 tests	84-0400		
Topoll α	20 tests	84-0600		
HER2 CISH Kit	20 tests	84-0146‡	NA	NA
Deletion probes				
19q	20 tests	84-2600	CISH™ Polymer Kit	84-9246
4q12 (PDGFRA)	20 tests	84-3100		
Centromeric probes				
Chromosome 17 Centromeric	20 tests	84-0500	CISH™ Centromere Detection Kit	84-9248
Translocation probe pairs				
BCR/ABL	20 tests	84-1400	CISH™ Bone Marrow/Blood Smear Kit	84-9214
EWS	20 tests	84-0300	CISH™ Translocation Detection Kit	84-9288
SYT	20 tests	84-2500		

Products are for *in vitro* diagnostic use unless otherwise noted.
* Available in the U.S. only. † Analyte specific reagent; analytical and performance characteristics are not established. ‡ Available outside the U.S. only.
NA = not applicable.

Product	Quantity	Cat. no.
FISH Tag™ DNA Green Kit *with Alexa Fluor® 488 dye*	10 rxns	F32947
FISH Tag™ DNA Orange Kit *with Alexa Fluor® 555 dye*	10 rxns	F32948
FISH Tag™ DNA Red Kit *with Alexa Fluor® 594 dye*	10 rxns	F32949
FISH Tag™ DNA Far Red Kit *with Alexa Fluor® 647 dye*	10 rxns	F32950
FISH Tag™ DNA Multicolor Kit *Alexa Fluor® dye combination*	10 rxns	F32951
FISH Tag™ RNA Green Kit *with Alexa Fluor® 488 dye*	10 rxns	F32952
FISH Tag™ RNA Orange Kit *with Alexa Fluor® 555 dye*	10 rxns	F32953
FISH Tag™ RNA Red Kit *with Alexa Fluor® 594 dye*	10 rxns	F32954
FISH Tag™ RNA Far Red Kit *with Alexa Fluor® 647 dye*	10 rxns	F32955
FISH Tag™ RNA Multicolor Kit *Alexa Fluor® dye combination*	10 rxns	F32956