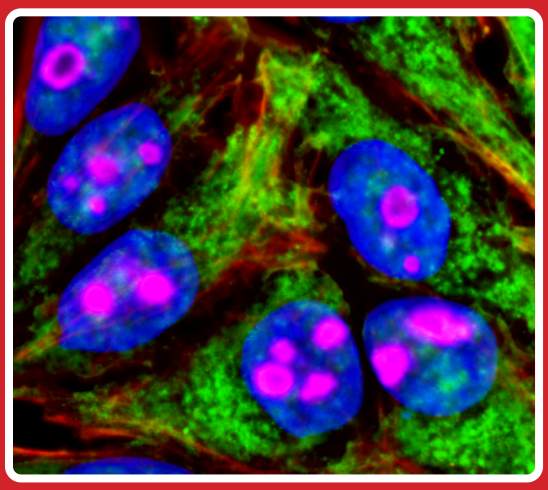


Tyramide Signal Amplification

Detect low abundance targets with CF® Dye tyramides

Advantages of Tyramide Signal Amplification:

- Detect low-abundance targets
- ICC, IHC and FISH compatible
- Sensitivity up to 100-fold that of conventional methods
- Similar workflow to conventional staining methods
- Uses less antibody
- Allows simplified primary antibody panel design for multiplexing

Tyramide signal amplification (TSA), sometimes called catalyzed reporter deposition (CARD), is a highly sensitive method enabling the detection of low-abundance targets in fluorescent immunocytochemistry (ICC), immunohistochemistry (IHC), and in situ hybridization (FISH) applications. For multiplex fluorescent IHC, TSA not only facilitates detection of low-abundance targets, but also simplifies antibody panel design since primary antibodies of choice may be used, irrespective of host species or isotype.

TSA involves horseradish peroxidase (HRP)-catalyzed deposition of tyramide on and near a target protein or nucleic acid sequence in situ. In the presence of low concentrations of H2O2, HRP is able to convert a labeled tyramide substrate into a highly reactive form that can covalently bind to tyrosine residues on proteins at or near the HRP. This generates high density tyramide labeling and is the reason for the exceptional sensitivity of this system. Tyramide can be labeled with a fluorophore or a hapten (such as biotin or DNP). Because the label is covalently linked to the sample, the antibodies can be stripped off without affecting signal. This allows multiple rounds of staining with antibodies from the same host species for multiplex detection.

Tyramide Signal Amplification Kits:

- Our kits provide all critical reagents for tyramide labeling
- Choose your tyramide: biotin tyramide or one of six CF® dye tyramides (either CF®488A, CF®543, CF®568, CF®594, CF®640R, or CF®680R)
- Choose your HRP conjugate: goat anti-mouse, goat anti-rabbit, or streptavidin-HRP
- The kits also contain Amplification Buffer, hydrogen peroxide, and BSA (for blocking buffer preparation)

Other Tyramide Products:

- We offer more than 20 standalone tyramide conjugates
- We also offer Ready-to-Use Tyramide Amplification Buffer

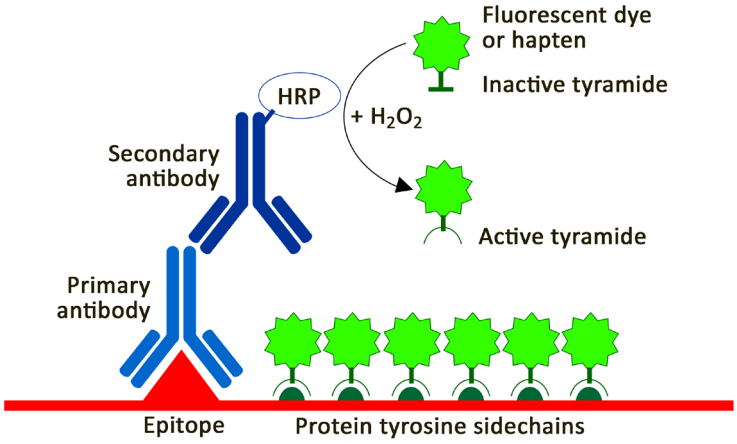
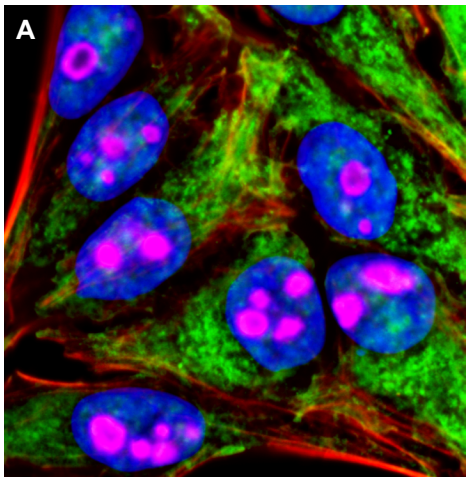


Figure 1. Illustration of the tyramide signal amplification system.

A cell or tissue sample is labeled with primary and secondary antibody using conventional methods. The horseradish peroxidase, conjugated to the secondary antibody, catalyzes the conversion of labeled tyramide into a reactive radical. The tyramide radical then covalently binds to nearby tyrosine residues, providing high-density labeling.

biotium



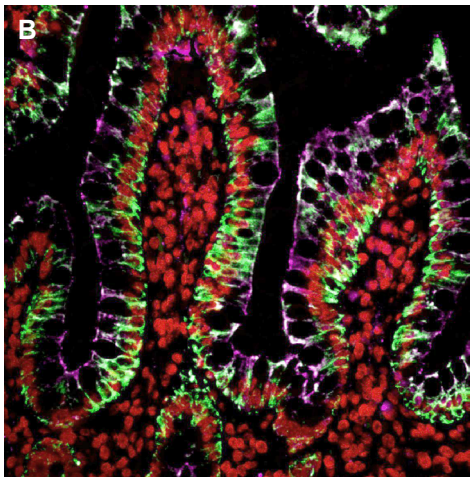


Figure 2. Multiplex staining of cells and tissue sections with tyramides.

A. Sequential labeling of formaldehyde-fixed HeLa cells with two Tyramide Amplification Kits. Mitochondria (green) visualized with rabbit anti-COX IV primary antibody and Tyramide Amplification Kit with HRP goat anti-rabbit IgG and CF®488A-tyramide, followed by peroxidase quenching. Nucleoli (magenta) visualized with mouse anti-cyclin B1 primary antibody and Tyramide Amplification Kit with HRP goat anti-mouse IgG and CF®568-tyramide. Actin (red) detected with CF®640R-phalloidin, and cell nuclei (blue) stained with DAPI.

B. Multiplex tyramide labeling of FFPE tissues. CF®488A-tyramide labeling pan-CK (green); Cy®3-tyramide labeling histone H1 (red); CF®640R-tyramide labeling ZO1 (magenta). Primary antibodies were from mouse, and secondary antibody was HRP-conjugated goat anti-mouse. Each labeling was performed sequentially, with antibody removal by microwave treatment.

Tyramide Signal Amplification Kits

Tyramide Label	Ex/Em	Secondary conjugate	Cat. #
CF®488A	490/515 nm	Goat anti-mouse HRP	33000
		Goat anti-rabbit HRP	33001
		Streptavidin HRP	33002
CF®543	541/560 nm	Goat anti-mouse HRP	33003
		Goat anti-rabbit HRP	33004
		Streptavidin HRP	33005
CF®568	562/583 nm	Goat anti-mouse HRP	33006
		Goat anti-rabbit HRP	33007
		Streptavidin HRP	33008
CF®594	593/614 nm	Goat anti-mouse HRP	33009
		Goat anti-rabbit HRP	33010
		Streptavidin HRP	33011
CF®640R	642/662 nm	Goat anti-mouse HRP	33012
		Goat anti-rabbit HRP	33013
		Streptavidin HRP	33014
CF®680R	680/701 nm	Goat anti-mouse HRP	33015
		Goat anti-rabbit HRP	33016
		Streptavidin HRP	33017
Biotin-XX	N/A	Goat anti-mouse HRP	33018
		Goat anti-rabbit HRP	33019
		Streptavidin HRP	33020

Amplification Buffer

Cat. #	Product Name
22027	Ready-to-Use Tyramide Amplification Buffer, 1X

Standalone Dye & Hapten Labeled Tyramides

Tyramide Label	Ex/Em	Size	Cat. #
CF®350	347/448 nm	0.5 mg	92170
CF®405L	395/545 nm	0.5 mg	92198
CF®405S	404/431 nm	0.5 mg	92197
CF®405M	408/452 nm	0.5 mg	96057
CF®430	426/498 nm	0.5 mg	96053
CF®488A	490/515 nm	0.5 mg	92171
FITC	492/514 nm	0.5 mg	96018
CF®514	516/548 nm	0.5 mg	92199
CF®532	527/558 nm	0.5 mg	96066
CF®543	541/560 nm	0.5 mg	92172
CF®555	555/565 nm	0.5 mg	96021
Cyanine 555 (Cy®3)	555/565 nm	0.5 mg	96020
CF®568	562/583 nm	0.5 mg	92173
CF®594	593/614 nm	0.5 mg	92174
CF®620R	617/639 nm	0.5 mg	92194
CF®640R	642/662 nm	0.5 mg	92175
CF®647	650/665 nm	0.5 mg	96022
CF®660R	663/682 nm	0.5 mg	92195
CF®680R	680/701 nm	0.5 mg	92196
CF®750	755/777 nm	0.5 mg	96052
Biotin-XX	N/A	0.5 mg	92176
DNP	N/A	0.5 mg	96019

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