

Product Information

Tyramide Conjugates

Product List

Catalog No.	Conjugate	Ex/Em (nm)	MW
92170	CF@350	347/448	~614
92197	CF@405S	404/431	~689
96057	CF@405M	408/452	~621
92198	CF@405L	395/545	~1692
96053	CF@430	426/498	~707
92171	CF@488A	490/515	~666
92199	CF@514	516/548	~1337
96066	CF@532	527/558	~804
92172	CF@543	541/560	~1006
96077	CF@550R	551/577	~806
96021	CF@555	555/565	~1120
92173	CF@568	562/583	~833
96085	CF@583R	586/609	~892
92174	CF@594	593/614	~848
92194	CF@620R	617/639	~857
92175	CF@640R	642/662	~951
96022	CF@647	650/665	~1104
92195	CF@660R	663/682	~1007
92196	CF@680R	680/701	~1031
96052	CF@750*	755/779	~3040
96090	CF@754	748/793	~1000
96018	Fluorescein	492/514**	~495
96020	Cyanine 555	555/565	~851
92176	Biotin-XX	---	~590
96019	DNP	---	~416

* CF@750 Tyramide is not stable in TSA buffer, and should be added to the buffer immediately before performing the staining reaction.

** Ex/Em in pH 9 buffer

Size: 0.5 mg

Storage and Handling

Store tyramide conjugates at -20°C, protected from light. Product is stable for at least 12 months from date of receipt if stored as recommended. Stock solution of 1-10 mM may be prepared in DMSO and can be stored at ≤ -20°C for at least 12 months.

Product Description

Tyramide conjugates are used for tyramide signal amplification (TSA), which is a highly sensitive method enabling the detection of low-abundance targets in fluorescent immunocytochemistry (ICC), immunohistochemistry (IHC), and *in situ* hybridization (FISH) applications.

TSA uses horseradish peroxidase (HRP) to generate high-density labeling of a target protein or nucleic acid *in situ* (1-3). The target is labeled with HRP-conjugated detection reagent (e.g., antibody or streptavidin). The HRP-labeled sample is then incubated with labeled tyramide and hydrogen peroxide, which converts the labeled tyramide substrate into a highly reactive form. Multiple reactive tyramide molecules conjugate to tyrosine residues in the target to generate high density tyramide labeling. This leads to significant amplification of the signal at the target and is the reason for the exceptional sensitivity of this system.

Multiple TSA procedures can be performed sequentially to label different targets on the same sample by performing HRP quenching or antibody stripping after each tyramide reaction. The label that is covalently attached to the sample will remain.

For more details, please visit www.biotium.com/support/tech-tips/ to see our Tech Tip: Multi-Color Fluorescence Imaging Using Biotium's Tyramide Amplification Kits.

Biotium offers tyramide conjugates for 20 bright and photostable CF@ Dyes, as well as fluorescein, cyanine 555, biotin, and DNP. We also offer Tyramide Amplification Kits, which include HRP conjugates, tyramide stock solution, and blocking and reaction buffers (see Table 1.).

References

1) Journal of Immunological Methods. 125, 279(1989); 2) Journal of Immunological Methods. 137, 103(1991); 3) Cytometry A. 77A, 1020(2010).

Considerations for Tyramide Signal Amplification

TSA reactions using HRP-conjugated antibodies typically use tyramide concentrations of 1-5 μM in a reaction buffer containing 0.0015% hydrogen peroxide. Biotium offers Tyramide Amplification Buffer Plus (Cat. no. 22029), which is an optimized buffer that provides enhanced staining, and is supplied with a separate vial of hydrogen peroxide (see Related Products).

CF@750 is not stable in the oxidizing amplification buffer, particularly if stored premixed with buffer after one hour. However, CF@750 Tyramide may still be used if added to the amplification buffer just before use. CF@754 Tyramide was developed as a more stable alternative to CF@750 Tyramide and is better suited for automated staining workflows. CF@754 Tyramide may be added to the buffer in advance for several hours to overnight without degradation.

For best results, we recommend diluting tyramides in reaction buffer just before use. However, with the exception of CF@750 tyramide (see above), tyramides can be diluted in reaction buffer up to 24 hours before staining for use with automated staining instruments.

Tyramide Signal Amplification Workflow

The following is a general overview of a tyramide staining protocol. For detailed protocols, download the Tyramide Amplification Kit product information sheet on our website. Tyramide concentration or other staining conditions may require optimization.

1. Fix, permeabilize, and block cell or tissue samples following general immunohistochemistry protocols.

Note: Inactivation of endogenous peroxidase activity may be required for some tissues or cell types. Endogenous biotin blocking may be required if using biotinylated probes.
2. Incubate samples with blocking buffer containing 10 mg/mL BSA and 0.5% Triton® X-100 for 1 hour at room temperature.

3. Dilute the primary antibodies using blocking buffer according to the manufacturer's guidelines. Incubate samples with primary antibodies at room temperature for 1 hour or 4°C overnight. Wash 3 x 5 minutes with PBS.
4. Optional: If you are using a biotinylated secondary antibody, incubate samples with secondary antibody in blocking buffer at the manufacturer's recommended concentration at room temperature for 1 hour, then wash as in step 3.
5. Incubate samples with 5 ug/mL HRP conjugate in blocking buffer at room temperature for 1 hour. Wash 3 x 5 minutes with PBS.
Note: HRP conjugates must be diluted in azide-free buffer.
6. Prepare working amplification buffer or use Tyramide Amplification Buffer Plus (Cat. no. 22029) or other commercially available amplification buffer containing hydrogen peroxide at a final concentration of 0.0015%. Follow manufacturer's instructions if using other commercially available amplification buffer. Dilute the tyramide conjugate in working amplification buffer at a final concentration of 2 uM.
Note: If using CF®750 Tyramide, we recommend adding the dye to the amplification buffer immediately before staining.
7. Incubate samples with amplification buffer containing tyramide conjugate for 10 minutes at room temperature, then rinse 3 times with PBS.
8. Mount samples with mounting medium and image fluorescence using the appropriate excitation and emission settings (see Product List).

Tyramide Amplification Kits

Biotium also offers Tyramide Amplification Kits which contain an antibody or streptavidin-HRP conjugate, one of six CF® Dye tyramides or biotin tyramide, and required buffers.

Table 1. Tyramide Amplification Kits

Tyramide Label	Ex/Em (nm)	Secondary Conjugate	Catalog No.
CF®488A	490/515	Goat anti-mouse HRP	33000
		Goat anti-rabbit HRP	33001
		Streptavidin HRP	33002
CF®543	541/560	Goat anti-mouse HRP	33003
		Goat anti-rabbit HRP	33004
		Streptavidin HRP	33005
CF®568	562/583	Goat anti-mouse HRP	33006
		Goat anti-rabbit HRP	33007
		Streptavidin HRP	33008
CF®594	593/614	Goat anti-mouse HRP	33009
		Goat anti-rabbit HRP	33010
		Streptavidin HRP	33011
CF®640R	642/662	Goat anti-mouse HRP	33012
		Goat anti-rabbit HRP	33013
		Streptavidin HRP	33014
CF®680R	680/701	Goat anti-mouse HRP	33015
		Goat anti-rabbit HRP	33016
		Streptavidin HRP	33017
Biotin-XX	---	Goat anti-mouse HRP	33018
		Goat anti-rabbit HRP	33019
		Streptavidin HRP	33020

Related Products

Catalog number	Product
20400	HRP Goat Anti-Mouse IgG (H+L)
20402	HRP Goat Anti-Rabbit IgG (H+L)
20474	HRP Goat Anti-Chicken IgG (H+L)
20470	HRP Goat Anti-Human IgG (H+L)
20406	HRP Goat Anti-Rat IgG (H+L)
20475	HRP Goat Anti-Llama IgG (H+L)
20839	HRP Chicken Anti-Goat IgG (H+L)
20404	HRP Donkey Anti-Mouse IgG (H+L)
20405	HRP Donkey Anti-Rabbit IgG (H+L)
20871	HRP Rabbit Anti-DNP
20864	HRP Goat Anti-DIG
29049	HRP Streptavidin
22027	Ready-to-Use Tyramide Amplification Buffer
22029	Tyramide Amplification Buffer Plus
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
92300-92302	Mix-n-Stain™ HRP Antibody Labeling Kit
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium without DAPI
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23016	EverBrite™ Hardset Mounting Medium with NucSpot® 640
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset Mounting Medium with DAPI
23019	EverBrite TrueBlack® Hardset Mounting Medium with NucSpot® 640
22013	Bovine Serum Albumin Fraction V
23005	CoverGrip™ Coverslip Sealant
90082	DMSO, Anhydrous

Visit www.biotium.com to view our full selection of products featuring bright and photostable CF® Dyes, including Mix-n-Stain™ Small Ligand Labeling Kits, primary and secondary antibodies, streptavidin, phalloidins, and much more.

CF Dye technology is covered by pending U.S. and international patents. Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.