

Product Information

Tyramide Conjugates

See [product page](#) for a full list of product names, unit sizes, and catalog numbers.

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. Prepare the stock with DMF or DMSO. The stock solution can be stored at 4°C for at least 12 months.

Color and Form: Lyophilized solid

Product Technical Information

See [product page](#) for spectral properties and other dye-specific technical information. See our [Spectra Viewer](#) to view and download the dye excitation and emission spectra.

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).

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 see our [Tech Tip: Multi-Color Fluorescence Imaging Using Biotium's Tyramide Amplification Kits](#).

Biotium offers tyramide conjugates for a wide selection of 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 Related Products).

For researchers seeking near-infrared detection, our near-IR CF® Dye Tyramide conjugates are next-generation long wavelength dyes that represent a true breakthrough in the field. Their novel molecular engineering makes them exceptionally bright and photostable, overcoming the excessive dye aggregation and poor stability issues common to other commercially available near-IR dyes. Additionally, they possess a unique structural features that render them more water soluble than other near-IR dyes without introducing excessive charge.

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).

For best results, we recommend diluting tyramides in reaction buffer just before use. However, with the exception of CF®750 tyramide, 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 3 x 5 minutes with PBS.

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 by adding hydrogen peroxide to Tyramide Amplification Buffer Plus (Cat. No. 22029) at a final concentration of 0.0015%, or follow manufacturer's instructions if using another commercially available amplification buffer.

Note: If using CF®750 Tyramide, we recommend adding the dye to the amplification buffer immediately before staining. CF®750 is not stable in the oxidizing amplification buffer, particularly if stored premixed with buffer after one hour.

7. Dilute the tyramide conjugate in working amplification buffer at a final concentration of 2 uM.

8. Incubate samples with amplification buffer containing tyramide conjugate for 10 minutes at room temperature, then rinse three times with PBS.

Note: Amplification time may require optimization for your specific target.

9. Mount samples with mounting medium and image fluorescence using the appropriate excitation and emission settings.

Related Products

Cat. No.	Product
22029	Tyramide Amplification Buffer Plus
22027	Ready-to-Use Tyramide Amplification Buffer
33000... 33018	Tyramide Amplification Kit with HRP Goat Anti-Mouse and CF® Dye or Biotin Tyramide
33001... 33019	Tyramide Amplification Kit with HRP Goat Anti-Rabbit and CF® Dye or Biotin Tyramide
33002... 33020	Tyramide Amplification Kit with HRP Streptavidin and CF® Dye or Biotin Tyramide
92170... 96090	CF® Dye Tyramides
92176	Biotin-XX Tyramide
96019	DNP Tyramide
40083... 41040	NucSpot® Nuclear Stains
29129... 29086	Streptavidin Conjugates
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
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23008- 23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23001- 23002	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23003- 23004	EverBrite™ Hardset Mounting Medium (with or without DAPI)
23017- 23018	EverBrite™ TrueBlack® Hardset Mounting Medium (with or without DAPI)

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.

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