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Revised: August 2, 2021

Product Information

Tyramide Amplification Kits

Size: 50-150 slides per kit (actual number of tests may vary depending on sample size)

Kit Contents

Component	Amount	Catalog No.
Tyramide Stock Solution, 500X	100 uL	See Table 1
HRP Conjugate	100 ug	See Table 1
BSA	3 g	22013-3g
1X Tyramide Amplification Buffer Plus*	25 mL	99832-25mL
30% Hydrogen Peroxide*	100 uL	99823-100uL

* Kits now include our improved Tyramide Amplification Buffer Plus, which requires addition of the hydrogen peroxide before use (see protocol). If you have an older version of the kit with component 22027 (Ready-to-Use Tyramide Amplification Buffer), you do not need to add hydrogen peroxide.

Storage and Handling

Store kit at -20°C, protected from light. After first use, BSA, 1X Tyramide Amplification Buffer Plus, and 30% Hydrogen Peroxide may be stored at 4°C. Components are stable for at least 6 months from date of receipt when stored as recommended.

Before each use, warm 1X Tyramide Amplification Buffer to room temperature and mix well by vortexing or shaking to make sure all solids are completely dissolved. The buffer can be warmed in a 37°C water bath for convenience.

Danger: 30% hydrogen peroxide causes serious eye damage, is harmful if inhaled or swallowed, and may cause skin and respiratory irritation. Download the Safety Data Sheet (SDS) at www.biotium.com for more information.

Spectral Properties

See Table 1

Product Application

Tyramide Amplification Kits provide all critical reagents for secondary detection of mouse, rabbit, or biotinylated primary antibodies followed by tyramide signal amplification in cells or tissue sections. The HRP secondary reagent mediates covalent coupling of labeled tyramide to tyrosine-containing proteins in the vicinity of the reaction site. This allows the target proteins to be labeled with a large number of dyes or biotin molecules, creating stronger fluorescence signal compared to using dye-labeled secondary antibodies. Therefore the Tyramide Amplification Kits offer benefits of high sensitivity and signal-to-background ratio, enabling the visualization of low-abundance targets that cannot be efficiently labeled using traditional immunostaining methods.

Tyramide amplification can be used in combination with dye-labeled antibodies or other staining methods for multi-color imaging. Two or more tyramide reactions also can be performed sequentially to label different targets on one sample (see Technical Notes for more information).

These kits are available with Biotin-XX-Tyramide or Biotium's next-generation CF® Dye Tyramides in combination with our high-performance Goat Anti-Mouse, Goat Anti-Rabbit, or Streptavidin HRP conjugates. CF® Dyes provide superior brightness and photostability compared to other fluorescent dyes. Our Tyramide Amplification Buffer Plus provides enhanced sensitivity compared to our original tyramide amplification buffer.

Staining Protocol

The following protocol is provided for immunostaining and tyramide amplification of cells or tissue sections using 100 uL per sample (enough to cover one well of a 96-well plate or ~1 cm^2 tissue section). Volumes may be scaled for different specimen sizes.

A. Materials Required But Not Provided

- Buffer components: Phosphate buffered saline (1X PBS), Triton® X-100.
- Fixation and permeabilization reagents: Methanol (pre-chilled at -20°C) or formaldehyde solution.
- For endogenous peroxidase quenching: Sodium azide (NaN_3), additional 30% hydrogen peroxide.
- For endogenous biotin blocking (only for kits with biotin-XX tyramide): Unlabeled streptavidin, biotin, TWEEN® 20

B. Reagent Preparation

1. If using formaldehyde fixation, prepare 3.75% formaldehyde solution in PBS and pre-chill on ice. Use methanol-free formaldehyde if the target protein or structure can be disturbed by methanol (eg. actin, nucleolin).
2. If using formaldehyde fixation, prepare permeabilization buffer: 0.5% Triton® X-100 in PBS.
3. Prepare blocking buffer by dissolving 1 g of BSA in 100 mL of PBS with 0.5% Triton® X-100. We recommend making the blocking buffer for immediate use. Unused blocking buffer can be aliquoted and stored at -20°C.
4. Add 100 uL of PBS to the HRP Goat anti-Rabbit IgG, HRP Goat anti-Mouse IgG, or HRP streptavidin, and vortex to dissolve all the solids, making a 1 mg/mL stock solution. Unused stock solution can be stored at 4°C for up to 3 months. Do NOT add sodium azide (NaN_3) to the solution.
5. For streptavidin or biotin-XX-tyramide kits only: Prepare biotin blocking wash buffer: 1% BSA and 0.05% TWEEN® 20 in PBS.
6. For streptavidin or biotin-XX-tyramide kits only: Prepare unlabeled streptavidin solution: 0.1 mg/mL streptavidin in biotin blocking wash buffer (prepared in step 5).
7. For streptavidin or biotin-XX-tyramide kits only: Prepare biotin solution: 0.5 mg/mL biotin in biotin blocking wash buffer (prepared in step 5).

C. Fixation and Blocking

Fix, permeabilize, and block cell or tissue samples following general immunohistochemistry protocols. The following steps are provided as examples. Fixation and other procedures may need to be optimized for specific applications.

1. For paraffin sections: Perform deparaffinization and rehydration according to standard protocols. If necessary, perform antigen retrieval as recommended for your primary antibody.
2. For unfixed cells or cryosections: Perform either methanol fixation or formaldehyde fixation depending on the requirements for specific applications. For cultured cells, wash with PBS or HBSS prior to fixation. For cryosections, allow slides to warm to room temperature and proceed directly to fixation.

   Methanol fixation: Add appropriate amount of pre-chilled methanol to cover the sample. Incubate at -20°C for 10 minutes. Remove methanol and rinse the sample twice with PBS at room temperature.

   Formaldehyde fixation and permeabilization: Fix samples on ice with 3.75% formaldehyde solution (see step 1 in Reagent Preparation) for 15 minutes. Wash 3 times with PBS. Then incubate samples with permeabilization buffer (see Reagent Preparation) at room temperature for 10 minutes. Wash 3 times with PBS.
3. Optional: For tissue sections or other samples where endogenous peroxidases are a source of background, we recommend treating samples with peroxidase quenching buffer (0.3% hydrogen peroxide/0.1% NaN_3 in PBS, prepared just before use). Incubate samples with quenching buffer for 15 minutes at room temperature, then rinse 3 times with PBS.
4. Incubate samples with blocking buffer (see Reagent Preparation) for 1 hour at room temperature.

D. Endogenous Biotin Blocking

When performing streptavidin/biotin detection, we highly recommend blocking endogenous biotin in samples to reduce background. This is not necessary for kits with CF® Dye Tyramides and HRP secondary antibodies.

1. Incubate samples with unlabeled streptavidin solution (see Reagent Preparation) for 15 minutes at room temperature. Wash samples 3 times with biotin blocking wash buffer (see Reagent Preparation) for 5 minutes at room temperature for each wash.
2. Incubate samples with biotin solution (see Reagent Preparation) for 30 minutes at room temperature to block the extra biotin binding sites on streptavidin. Wash samples 3 times with biotin blocking wash buffer for 5 minutes at room temperature for each wash.

E. Antibody Binding and Tyramide Signal Amplification

1. Dilute the primary antibodies using the blocking buffer (see Reagent Preparation) to the appropriate concentration according to manufacturer's guidelines. Incubate samples with primary antibodies at room temperature for 1 hour or 4°C overnight.
2. Wash samples 3 times with PBS for 5 minutes at room temperature for each wash.
3. Optional: If you are using a biotinylated secondary antibody, incubate samples with secondary antibody at room temperature for 1 hour as described in step 1, then wash samples as described in step 2.
4. Dilute the HRP conjugate to 5 ug/mL (1:200 dilution) in blocking buffer. Incubate samples with this solution at room temperature for 1 hour.
5. Wash samples 3 times with PBS for 5 minutes at room temperature for each wash.
6. Prepare working amplification buffer with hydrogen peroxide at a final concentration of 0.0015% by performing a serial dilution of hydrogen peroxide as described below. Prepare 100 uL working amplification buffer for each sample.

   a. Add 1 uL of 30% hydrogen peroxide to 200 uL of 1X Tyramide Plus Buffer and mix well to make a 0.15% hydrogen peroxide solution.

   b. Add 1 uL of the 0.15% hydrogen peroxide solution to 100 uL of 1X Tyramide Plus Amplification Buffer, for a final concentration of 0.0015% hydrogen peroxide.

   Note: If you are using an older version of this kit with component 22027 (Ready-to-Use Amplification Buffer), hydrogen peroxide is not required.
7. Prepare staining solution by diluting the CF® Dye or biotin-XX tyramide stock solution 1:500 in the working amplification buffer prepared in step 6. Make 100 uL of staining solution for each sample. The staining solution can be stored at room temperature, protected from light, for up to 24 hours.
8. Incubate samples with the staining solution for 10 minutes at room temperature.
9. Wash samples 3 times with PBS for 5 minutes at room temperature for each wash.
10. Mount samples with mounting medium. For tissue samples on slides, cover with coverslip and seal. The samples are now ready for fluorescence imaging. The excitation and emission maxima for the CF® Dyes are listed in Table 1.

F. Technical Notes

1. Immunofluorescence imaging using the Tyramide Amplification Kits generally shows higher sensitivity and stronger signal compared to using CF® Dye secondary antibody conjugates. As a result, the primary antibody can be applied at a lower concentration to minimize background fluorescence from non-specific binding. We recommend doing a primary antibody titration to find the optimal concentration.
2. If background fluorescence is a concern, we recommend running a negative control side-by-side which is not incubated with primary antibody. Make sure this negative control is not cross-contaminated by reagents from the positive samples during incubation and washing. For tissue samples, we also advise imaging an unstained control (with no antibody or tyramide added) to determine the contribution of tissue autofluorescence to background.
3. We recommend using the HRP conjugate at 5 ug/mL. Lowering the concentration may compromise the signal intensity and sensitivity.
4. We recommend using CF® Dye or biotin-XX tyramide at 1:500 dilution from the stock solution. Higher concentration may result stronger signal in addition to potentially higher background, and *vice versa*. You may perform a titration (from 1:100 to 1:1000) to find the optimal concentration for your specific application.
5. Multiple Tyramide Amplification Kits can be used sequentially to label different targets on the same sample by performing HRP quenching or antibody stripping after each tyramide reaction. The CF® Dye or biotin that is covalently attached to the sample will remain. For more details please visit www.biotium.com to see our *Tech Tip: Multi-Color Fluorescence Imaging Using Biotium's Tyramide Amplification Kits*.

Table 1. Catalog and Component Numbers

Catalog No.	Antibody Conjugate	Tyramide	Ex/Em
33000	HRP Goat anti-Mouse 20401-100ug	CF®488A Tyramide 99824-100uL	490/515 nm
33001	HRP Goat anti-Rabbit 20403-100ug		
33002	HRP Streptavidin 29049-100ug		
33003	HRP Goat anti-Mouse 20401-100ug	CF®543 Tyramide 99825-100uL	541/560 nm
33004	HRP Goat anti-Rabbit 20403-100ug		
33005	HRP Streptavidin 29049-100ug		
33006	HRP Goat anti-Mouse 20401-100ug	CF®568 Tyramide 99826-100uL	562/583 nm
33007	HRP Goat anti-Rabbit 20403-100ug		
33008	HRP Streptavidin 29049-100ug		
33009	HRP Goat anti-Mouse 20401-100ug	CF®594 Tyramide 99827-100uL	593/614 nm
33010	HRP Goat anti-Rabbit 20403-100ug		
33011	HRP Streptavidin 29049-100ug		
33012	HRP Goat anti-Mouse 20401-100ug	CF®640R Tyramide 99828-100uL	642/662 nm
33013	HRP Goat anti-Rabbit 20403-100ug		
33014	HRP Streptavidin 29049-100ug		
33015	HRP Goat anti-Mouse 20401-100ug	CF®680R Tyramide 99829-100uL	680/701 nm
33016	HRP Goat anti-Rabbit 20403-100ug		
33017	HRP Streptavidin 29049-100ug		
33018	HRP Goat anti-Mouse 20401-100ug	Biotin-XX Tyramide 99830-100uL	N/A
33019	HRP Goat anti-Rabbit 20403-100ug		
33020	HRP Streptavidin 29049-100ug		

Related Products

Catalog No.	Product
23007	TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22002	TWEEN® 20
22015	Fixation Buffer
22016	Permeabilization Buffer
22020	10X Phosphate-Buffered Saline (PBS)
20406	HRP Goat anti-Rat IgG (H+L), Highly Cross-Adsorbed
20474	HRP Goat anti-Chicken IgY (H+L), Highly Cross-Adsorbed
20470	HRP Goat anti-Human IgG (H+L), Highly Cross-Adsorbed
40061	RedDot™2 Far Red Nuclear Counterstain

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable CF® Dyes, including our full selection of dye tyramides, primary antibodies, secondary antibodies, streptavidin, phalloidins, and much more.

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