Tyramide SuperBoost™ Kits with Alexa Fluor™ Tyramides

Pub. No. MAN0015834 **Rev.** B.0

Table 1. Contents and storage

Material	Amount	Concentration	Storage[1]
Blocking buffer (10% Goat Serum) (Component A)	22.5 mL	1X	• 2–8°C • Desiccate • Protect from light • DO NOT FREEZE
Poly-HRP-conjugated secondary antibody or HRP-conjugated streptavidin (Component B)	22.5 mL (150 slides) or 7.5 mL (50 slides)	1X	
Alexa Fluor™ tyramide reagent (Component C1)	1 vial[2]	N/A	
Hydrogen Peroxide (Component C2)	28.5 mL	Stabilized 3% solution	
Reaction buffer (Component C3)[3]	6 mL	20X	
Reaction Stop Reagent (Component D)	2 × 8 mg (150 slides) or 1 × 8 mg (50 slides)	N/A	
Dimethylsulfoxide (DMSO) (Component E)	200 µL	N/A	

[1] When stored as directed, the product is stable for 6 months after receipt.

[2] Sufficient material is provided for up to 150 or 50 slides (depending on the catalog number) based on the protocol below. See Table 2 for more information of individual Tyramide SuperBoost™ Kits and standalone reagents.

[3] Reaction Buffer can be replaced with Tris Buffer, pH 7.4 for similar performance.

N/A: Not applicable.

Table 2. Labeled tyramide conjugates provided in Tyramide SuperBoost™ Kits or as standalone reagents.

Labeled tyramide	Ex/Em[1]	Tyramide SuperBoost™ Kits			Tyramide conjugate (standalone)[3]	Kit size[4]
		Goat Anti-Mouse IgG[2]	Goat Anti-Rabbit IgG[2]	Streptavidin		
Alexa Fluor™ 350	347/442	—	—	—	B40952	150 slides
Alexa Fluor™ 488	495/519	B40912	B40922	B40932	B40953	
Alexa Fluor™ 546	556/573	—	—	—	B40954	
Alexa Fluor™ 555	555/565	B40913	B40923	B40933	B40955	
Alexa Fluor™ 568	579/604	—	—	—	B40956	
Alexa Fluor™ 594	591/617	B40915	B40925	B40935	B40957	
Alexa Fluor™ 647	650/668	B40916	B40926	B40936	B40958	
Biotin-XX	N/A	B40911	B40921	B40931	B40951	
Alexa Fluor™ 488	495/519	B40941	B40943	—	—	50 slides
Alexa Fluor™ 594	591/617	B40942	B40944	—	—	

[1] Approximate fluorescence excitation and emission maxima, in nm.

[2] Poly-HRP-conjugated secondary antibody (Component B) provided in each kit can also be purchased separately as standalone reagents, if needed.

[3] Alexa Fluor™ tyramide reagents (Component C1) can be also be purchased separately as standalone reagents, if needed.

[4] Sufficient material is provided for up to 150 or 50 (depending on the catalog number) 18-mm × 18-mm coverslips using 100 µL per slide in most critical incubation steps. This volume can be adjusted for different size samples.

N/A: Not applicable.

Introduction

Tyramide SuperBoost™ Signal Amplification is a highly sensitive method for the detection of low-abundance targets in multiplexable fluorescent ICC/IHC/ISH experiments. Tyramide SuperBoost™ technology combines the brightness of Alexa Fluor™ dyes with poly-HRP-mediated tyramide signal amplification to discern signal from noise, yielding precision and sensitivity 10–200 times greater than standard ICC/IHC/ISH and 2–10 times that of other tyramide amplification techniques like TSA™ (Figures 2–3, page 4).

Tyramide signal amplification used in the Tyramide SuperBoost™ kits utilizes the catalytic activity of horseradish peroxidase (HRP) for high-density labeling of a target protein or nucleic acid sequence *in situ*. Typical ICC/IHC/ISH experiments using the Tyramide SuperBoost™ kits require 10–100 times less primary antibody than standard ICC/IHC/ISH experiments to achieve the same signal intensity. Since the Tyramide SuperBoost™ kits greatly enhance specific signal intensity over background, they can be easily optimized to detect specific signal in samples where high endogenous autofluorescence is observed.

Tyramide SuperBoost™ kits are simple to use and easily adapted to standard ICC, IHC, or FISH experimental protocols, using any cell or tissue type. Cells labeled using a Tyramide SuperBoost™ kit can be imaged using any type of microscope, producing high-resolution multiplex images (Figures 4–6, page 5). In tissue samples, it is possible to use the primary antibodies from same host species for easier multiplexing (Figure 7, page 6)

Benefits of SuperBoost™ kits

Enhancement of signal using Alexa Fluor™ tyramides: Tyramide SuperBoost™ kits utilize Alexa Fluor™ tyramides, which react with HRP to ultimately deposit bright and photostable Alexa Fluor™ dyes on surrounding proteins and other similar molecules. Tyramide SuperBoost™ kits are the only kits that combine the brightness of Alexa Fluor™ dyes with the enhancement of tyramide signal amplification to produce a superior signal.

Poly-HRP enhancement: Unlike TSA™, Tyramide SuperBoost™ kits employ poly-HRP-conjugated secondary antibodies (except streptavidin, which is conjugated with standard HRP). In poly-HRP systems, several HRP enzymes are conjugated with short polymers, enhancing the signal several fold over regular HRP systems. The poly-HRP is structured in such a way that the antibodies penetrate cells or tissue as efficiently as regular HRP-conjugated secondary antibodies. The molar enzyme/antibody protein ratio averages around 4.

Reaction Stop Solution: Like any enzyme system, it is possible to overdevelop the signal. Tyramide SuperBoost™ kits include an HRP stop solution to halt the HRP reaction. HRP stop solution can be used to obtain maximum signal without an increase in the background signal. Images produced with optimized HRP reaction times are as sharp as images produced with standard ICC/IHC/ISH methods, but with 10–200 times more sensitivity.

Highly cross-adsorbed secondaries: Tyramide SuperBoost™ kits utilize highly-cross-adsorbed secondary antibodies, which are then conjugated to form the poly-HRP. When performing multiple antibody labeling, this high cross-adsorption helps ensure specificity with minimal cross-labeling. Our goat anti-mouse poly-HRP shows no observed reactivity to mouse serum proteins or IgG from bovine, goat, human, rabbit, or rat. Likewise, our goat anti-rabbit poly-HRP shows no observed reactivity to rabbit serum proteins or IgG from bovine, goat, human, mouse, or rat.

Reduction of background: Tyramide SuperBoost™ kits include blockers for the elimination or reduction of endogenous peroxidase and fluorescent background signals. These blockers help ensure that only specific signals are enhanced while keeping non-specific or background signals in check.

Tyramide SuperBoost™ kits with Alexa Fluor™ tyramides contain all of the critical components needed to label and detect any type of cell or tissue sample that can be labeled with standard IHC/FISH techniques. The kits include sufficient reagents for labeling 150 or 50 18-mm × 18-mm coverslips using 100 µL of reaction volume per slide in most critical incubation steps, depending on the kit size (see Table 2, page 1). This volume can be adjusted for different size samples.

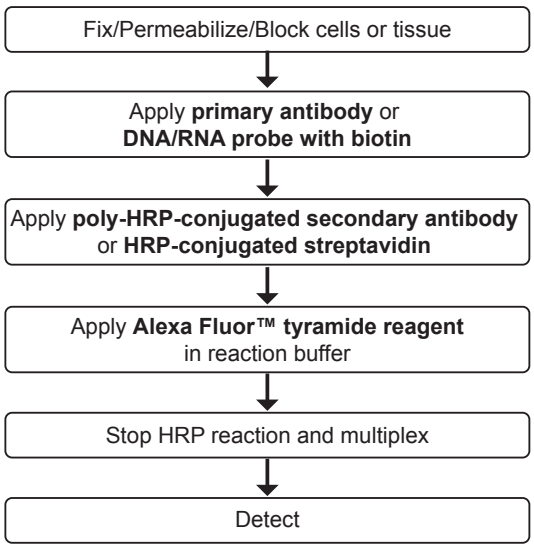

Figure 1. Typical labeling and detection workflow using Tyramide SuperBoost™ Kits with Alexa Fluor™ tyramides.

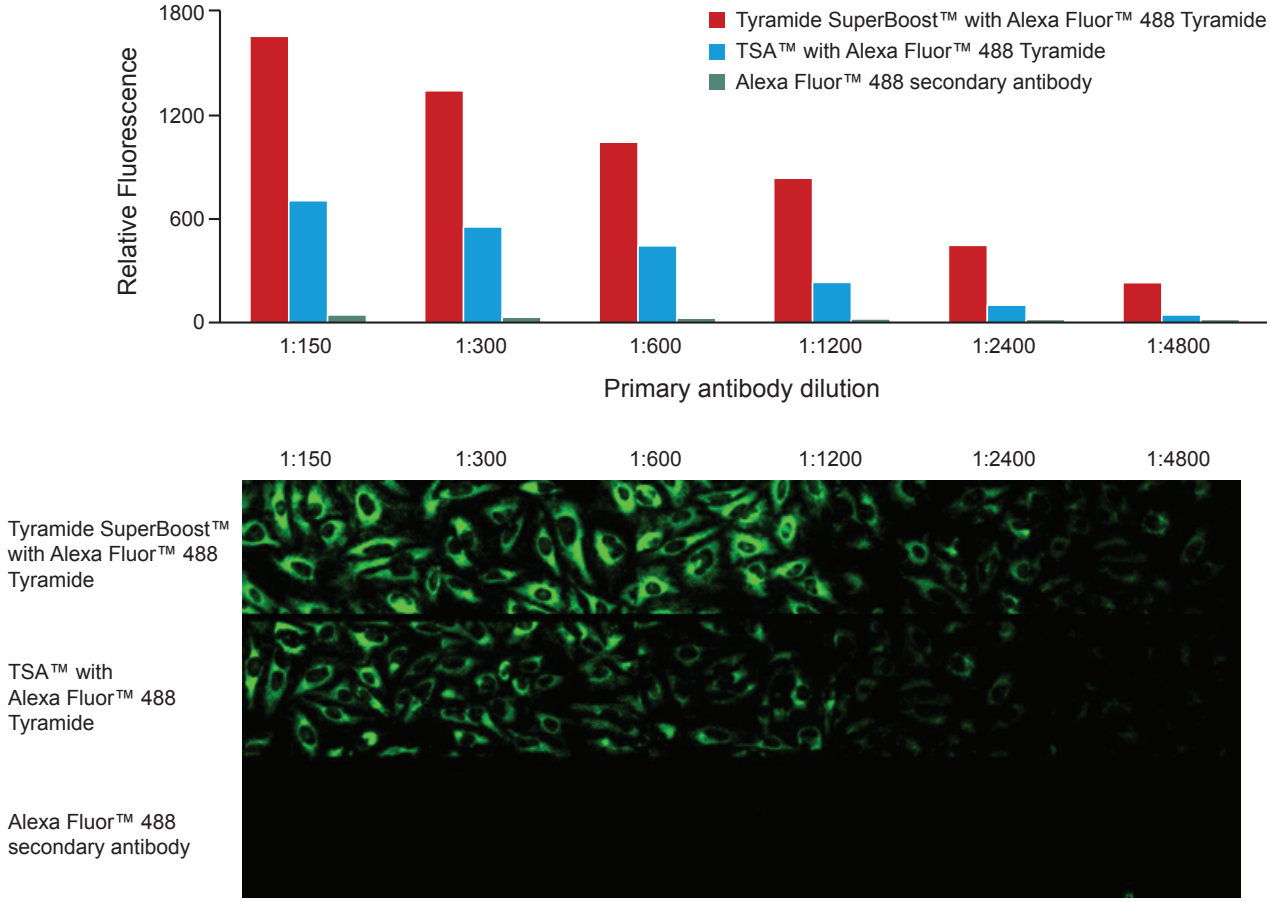

Figure 2. Sensitivity of Tyramide SuperBoost™ Kits. HeLa cells were fixed and permeabilized with Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Prohibitin (green) was labeled with various concentrations of anti-Prohibitin primary antibody. The manufacturer recommendation was 1:150 dilution or 5 µg/mL. Anti-Prohibitin antibody was then detected with Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG (Cat. No. B40922), with TSA™ Kit #12 with HRP—Goat Anti-Rabbit IgG and Alexa Fluor™ 488 Tyramide (Cat. No. T20922), or with Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor™ 488 conjugate (Cat. No. A11008). Images were taken and analyzed on EVOS™ FL Auto Imaging System (Cat. No. AMAFD1000) using the same exposure and gain. These images indicate that the Alexa Fluor™ 488 Tyramide SuperBoost™ Kit is more sensitive than both the TSA™ kits and directly labeled secondary antibodies. At this exposure and gain setting, prohibitin is not detectable with standard ICC methods.

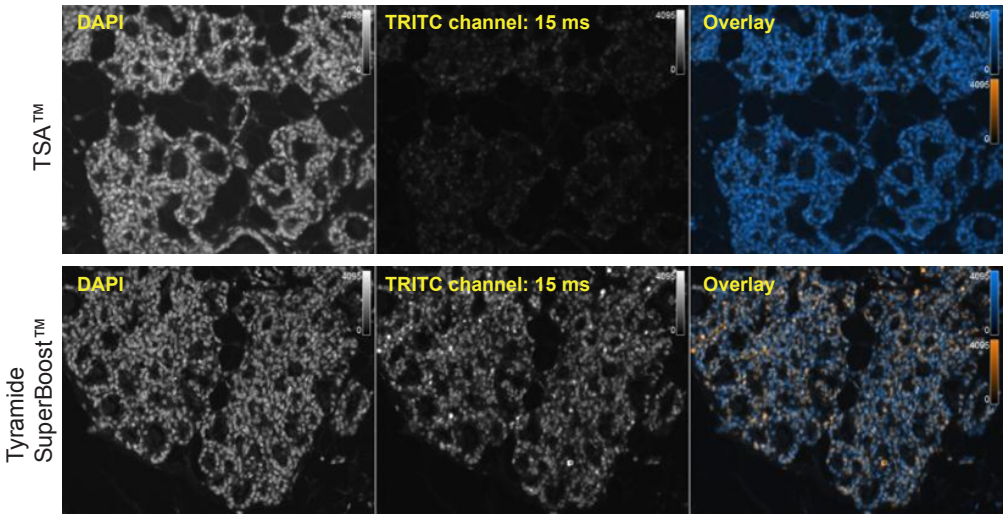

Figure 3. Tyramide SuperBoost™ Kits is more sensitive than TSA™ Kits in mouse mammary tissue. FFPE preserved mouse mammary tissue was processed for immunohistochemistry and labeled with anti-Histone H3 antibody, which was detected either with Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG (Cat. No. B40913) or with TSA™ Kit #40, with HRP—Goat Anti-Mouse IgG and Alexa Fluor™ 555 Tyramide (Cat. No. T30953). Images were taken and analyzed on EVOS™ FL Auto Imaging System (Cat. No. AMAFD1000) using the same exposure and gain. These images indicate that the SuperBoost™ Kit is more sensitive than TSA™ kits in mouse mammary tissue.

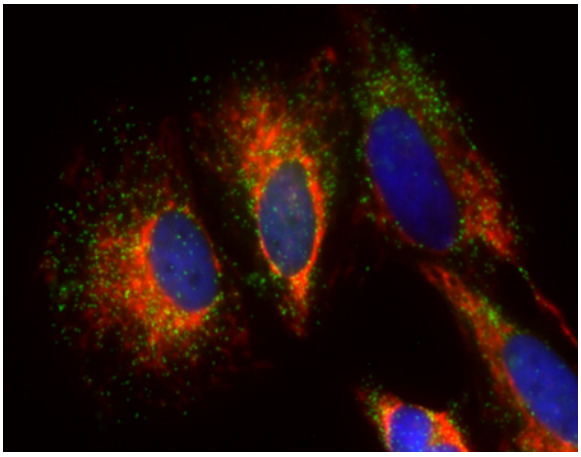

Figure 4. Multiplexing: Tyramide SuperBoost™ Kits with GFP/RFP. HeLa cells were treated with CellLight™ Peroxisome-GFP, BacMam 2.0 (Cat. No. C10604) to express GFP in peroxisomes (green). Cells were fixed and permeabilized with Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Prohibitin was labeled with anti-Prohibitin antibody and then detected with Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG (Cat. No. B40925) (red). Nucleus was labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

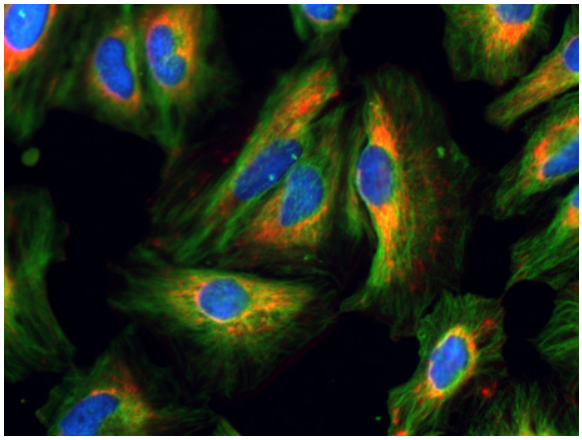

Figure 5. Multiplexing: Tyramide SuperBoost™ Kits with secondary antibody. HeLa cells were fixed and permeabilized with Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Tubulin (green) was labeled with anti-Tubulin primary antibody and then detected with Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor™ 488 conjugate (Cat. No. A11008). ATP Synthase (red) was labeled with anti-ATP Synthase Subunit IF1 Antibody (Cat. No. A21355) and then detected with Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG (Cat. No. B40915). Nucleus was labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606). Images were taken on a confocal microscope.

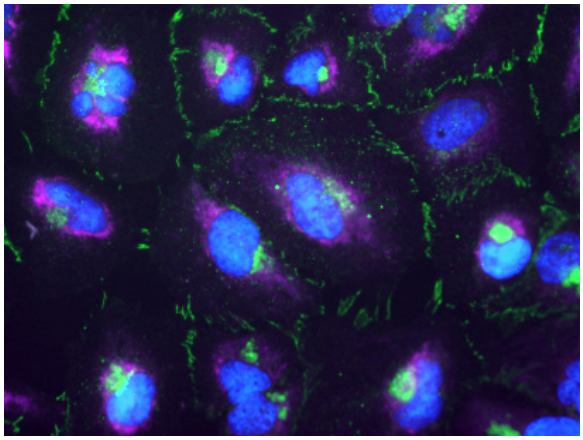

Figure 6. Multiplexing: Two proteins detected with two different colors of Tyramide SuperBoost™ Kits. HeLa cells were fixed and permeabilized with Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Prohibitin (purple) was labeled with anti-Prohibitin antibody and then detected with Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG (Cat. No. B40926) (far red). For ß-Catenin (green) detection, cells were incubated with anti-ß-Catenin antibody (Cat. No. 13-8400) and then detected with Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG (Cat. No. B40912). Nucleus was labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

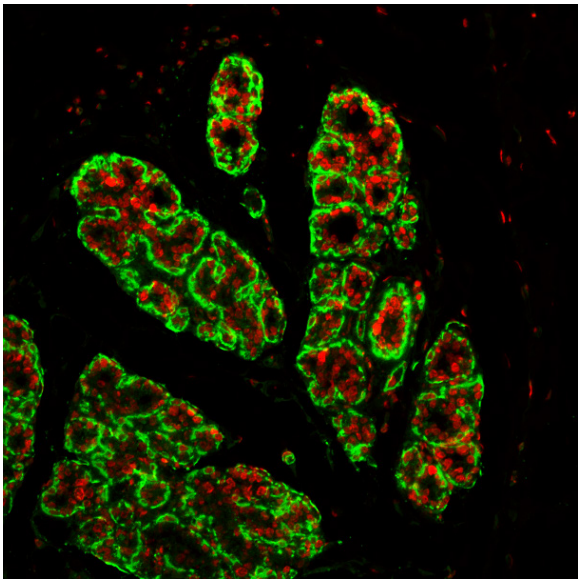

Figure 7. Multiplexing: Two proteins detected with primary antibodies from the same species. FFPE preserved mammary gland tissue was labeled with mouse anti-H3B and Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG (Cat. No. B40915) to detect the H3B protein (red). Primary and secondary antibodies were stripped using Citrate Buffer (pH 6.0) (Cat. No. 005000) in microwave. Actin (green) was then labeled with mouse anti-actin antibody and detected with Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG (Cat. No. B40912). Nucleus was labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

Before you begin

Required materials not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 3. Materials that might be needed, but not provided.

Item	Source
Cells or tissue	MLS; use positive and negative controls as needed
Slides, coverslips, containers	MLS
Conjugated probes for FISH	MLS
Primary or secondary antibodies, as needed[1]	MLS
PBS (phosphate buffered saline), pH 7.4 (without calcium, magnesium, or phenol red)	10010031
95% ethanol	MLS
Distilled water, highly purified	15230-147
Hydrophobic Barrier Pen	Fisher Scientific NC9545623
Image-iT™ Fixation/Permeabilization Kit	R37602
Endogenous Biotin-Blocking Kit	E21390
Citrate Buffer (pH 6.0), Concentrate	005000
ProLong™ Diamond Antifade Mountant or SlowFade™ Diamond Antifade Mountant	P36961 or S36963

[1] To search through the vast Thermo Fisher Scientific primary antibody collection, visit our Antibody seach tool at **www.thermofisher.com/us/en/home/life-science/antibodies**.

Cautions

DMSO is hazardous; avoid contact with skin and eyes and do not swallow. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.

Prepare reagents

Allow vials to warm to room temperature before opening.

1.1 100X Tyramide stock solution: Dissolve the Alexa Fluor™ tyramide reagent (Component C1) in 150 µL (for 150 slides) or 50 µL (for 50 slides) of DMSO (Component E). Invert the vial several times to dissolve any tyramide that might coat the sides of the vial.

You can store the 100X Tyramide stock solution at 2–8°C for up to 6 months in a sealed vial. Store the vial away from moisture, if possible.

1.2 100X H_2O_2 solution: Add 1 drop (approximately 50 µL) of Hydrogen Peroxide Solution (Component C2) to 1 mL of distilled water.

Note: Prepare the 100X H_2O_2 solution fresh on the day of use.

1.3 1X Reaction buffer: Add 1 drop (approximately 50 µL) of 20X Reaction buffer to 1 mL of distilled water.

Note: Prepare the 1X Reaction buffer fresh on the day of use. Tris buffer at pH 7.4 can be substituted for Reaction buffer for similar performance. Other HRP enzyme compatible buffers are possible replacements for reaction buffer, but are not tested.

1.4 Reaction Stop Reagent stock solution: Add 1.45 mL of 95% ethanol to one vial of Reaction Stop Reagent (Component D). Vortex the vial to dissolve any stop reagent coating the sides of the bottle.

Reaction Stop Reagent stock solution will be diluted 1:11 in PBS before use to prepare a working solution. Unused portion of the stock solution can be stored at –20°C for 6 months.

1.5 Reaction Stop Reagent working solution: Dilute the Reaction Stop Reagent stock solution (prepared in Step 1.4) 1:11 in PBS.

Note: Prepare the Reaction Stop Reagent working solution fresh on the day of use.

Methods

Procedural guidelines

- When using the Tyramide SuperBoost™ kits for the first time, we highly recommend that you optimize the protocol following the guidelines in "Appendix: Signal optimization and troubleshooting" on page 11.
- You can use a hydrophobic barrier pen (wax pen) to hold liquid reagents on the sample slide or coverslip.
- Do not let the cells or tissue samples dry out.
- For longer incubations, we recommend using a humidified chamber (for example, a covered box with damp paper towel).

Prepare cells (fixation and permeabilization)

Fix and permeabilize cells according to standard fixation and permeabilization protocols. If fluorescent proteins (GFP/RFP) are present, we recommend using the Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602) to prepare your cells.

Prepare tissues

Tyramide SuperBoost™ system is compatible with all types of tissues that can be labeled with standard IHC/FISH techniques. Deparaffinize and dehydrate the tissue according to standard IHC protocols before treating it for endogenous peroxidase activity in Step 2.1.

Peroxidase labeling

2.1 *Optional:* If needed, quench the endogenous peroxidase activity of the sample by adding enough drops of 3% Hydrogen Peroxide Solution (Component C2) to cover the sample and incubate for 60 minutes at room temperature.

2.2 Rinse the cells or tissue three times with 1X PBS at room temperature.

2.3 *Optional*: If using HRP-conjugated streptavidin, block endogenous biotin in the sample with Endogenous Biotin-Blocking Kit (Cat. No. E21390) as recommended by the manufacturer. Rinse the cells or tissue three times with 1X PBS at room temperature before proceeding to the next step.

2.4 Add 2–3 drops (approximately 100–150 µL) of Blocking buffer (Component A) to the sample and incubate for 60 minutes at room temperature.

2.5 Label the cells or tissue with primary antibody with mouse or rabbit as the host.

If using a SuperBoost™ kit with Streptavidin, use a biotin-conjugated primary antibody or other ligand. Dilute the antibody or biotin-conjugated ligand in Blocking buffer (10% goat serum) or another compatible blocking solution such as 2% BSA or BlockAid™ Blocking Solution (Cat. No. B10710), and incubate with the cells or tissue for 60 minutes at room temperature or overnight at 2–8°C.

For FISH, incubate the DNA/RNA probes according to manufacturer's protocol.

2.6 Rinse the cells or tissue for 10 minutes with PBS at room temperature. Repeat this step three times.

2.7 Add 2–3 drops (approximately 100–150 µL) of poly-HRP-conjugated secondary antibody or HRP-conjugated streptavidin (Component B) to the cells or tissue and incubate for 60 minutes at room temperature or overnight at 2–8°C.

Note: If you observe non-specific signal, you can shorten this incubation period.

2.8 Rinse the cells or tissue for 10 minutes with PBS at room temperature. Repeat this step three times.

Tyramide labeling

3.1 Prepare a tyramide working solution according to Table 4.

IMPORTANT! Do not use any unused solution after 2 hours of preparation.

Table 4. Tyramide working solution

Component	Number of coverslips (18-mm × 18-mm)				
	5	10	20	50	100
100X Tyramide stock solution (Step 1.1)	5 µL	10 µL	20 µL	50 µL	100 µL
100X H_2O_2 solution (Step 1.2)	5 µL	10 µL	20 µL	50 µL	100 µL
1X Reaction buffer (Step 1.3)	500 µL	1 mL	2 mL	5 mL	10 mL

Note: The volumes in this table are based on 100 µL of tyramide working solution needed per 18-mm × 18-mm coverslip. This volume can be adjusted based on the size of the coverslip or the volume needed per well in a microplate.

3.2 Apply 100 µL of the tyramide working solution to the cells or tissue and incubate for 2–10 minutes at room temperature.

3.3 Apply 100 µL of Reaction Stop Reagent prepared in Step 1.5.

IMPORTANT! Incubation period in Step 3.2 and the timing of Stop reagent addition in Step 3.3 are crucial in getting high resolution images with specific signal. We highly recommend that you optimize the incubation period using positive and negative control slides at various incubation time points when conducting this experiment for the first time. For details, see page 11.

3.4 Rinse the cells or tissue three times with PBS.

3.5 *SuperBoost™ kits containing Biotin-XX tyramide only:* If using a kit containing Biotin-XX tyramide, then use the conjugated streptavidin as recommended by the manufacturer. Some of the recommended streptavidin conjugates are listed in Table 5.

Table 5. Streptavidin conjugates recommended for the detection of Biotin-XX

Streptavidin conjugate	Ex/Em (nm)	Cat. No.
Alexa Fluor™ 350 Streptavidin	346/442	S11249
Alexa Fluor™ 405 Streptavidin	402/421	S32351
Alexa Fluor™ 488 Streptavidin	495/519	S11223
Alexa Fluor™ 555 Streptavidin	555/565	S21381
Alexa Fluor™ 594 Streptavidin	590/617	S11227
Alexa Fluor™ 647 Streptavidin	650/668	S21374

Multiplex with primary antibodies from different species

After Step 3.4 or 3.5, cells or tissue samples can be multiplexed with another Tyramide SuperBoost™ Kit or using standard IHC/ICC protocols.

When muliplexing, use a primary antibody from a host that is different than the one used in Step 2.5, and a fluorescent label that is spectrally compatible with the first fluorescent label.

Multiplex with primary antibodies from the same species in IHC

For tissue samples (IHC), Tyramide SuperBoost™ kits are compatible with the method described by Tóth and Mezey (J Histochem Cytochem, 2007).

In summary, dilute Citrate Buffer (pH 6.0), Concentrate (Cat. No. 005000) 1:20 in distilled water. After Step 3.4 or 3.5, place the tissue in the diluted citrate buffer (pH 6.0) and heat in a microwave oven on 100% power until boiling (1–2.5 minutes). Reduce the power to 20% and keep microwaving for an additional 15 minutes. Let the tissue sample cool to room temperature while keeping it in the citrate buffer. Wash the sample twice with 1X PBS, and repeat Steps 2.1 to 3.5 with a primary antibody of the same species, if desired. Use a tyramide that is spectrally compatible with the tyramide used in the first round.

Counterstain and detect

4.1 Counterstain the cells or tissue as needed using standard protocols. Few of the reagents recommended for counterstaining are listed in Table 6.

Table 6. Products recommended for counterstain

Target for counterstain	Product	Cat. No.
Nucleus	NucBlue™ Fixed Cell ReadyProbes™ Reagent	R37606
	NucGreen™ Dead 488 ReadyProbes™ Reagent	R37109
	NucRed™ Dead 647 ReadyProbes™ Reagent	R37113
Actin cytoskeleton	ActinGreen™ 488 ReadyProbes™ Reagent	R37110
	ActinRed™ 555 ReadyProbes™ Reagent	R37112
Cell membrane	Wheat Germ Agglutinin, Alexa Fluor™ 488 Conjugate	W11261
	Wheat Germ Agglutinin, Alexa Fluor™ 594 Conjugate	W11262
	Wheat Germ Agglutinin, Alexa Fluor™ 647 Conjugate	W32466

4.2 Mount the coverslips using a mountant with antifade properties such as the ProLong™ Diamond Antifade Mountant (Cat. No. P36961) or the SlowFade™ Diamond Antifade Mountant (Cat. No. S36963). For optimal results, follow the instructions provided with the mountant.

4.3 Analyze the cells or tissue using a compatible imaging instrument. The Tyramide SuperBoost™ system is compatible with all types of fluorescent microscopes equipped with compatible fluorescent filters. High content analyzers also have been successfully used to analyze the cells and tissues on slides and plates.

Appendix A: Signal optimization and troubleshooting

We highly recommend optimizing the experimental conditions to acquire the most specific signal and minimal background.

Amount of primary antibody or probe

To optimize the amount of primary antibody or probe used in Step 2.5, we recommend testing the following conditions:

- **Slide 1:** Same primary antibody or probe dilution as the standard method
- **Slide 2:** 5-fold dilution of the amount used for Slide 1
- **Slide 3:** 10-fold dilution of the amount used for Slide 2 (further dilution may be necessary)
- **Slide 4:** Negative control (antibody or probe omitted)

You can dilute the antibody or probe in Component A (10% goat serum) or another compatible blocking solution such as 2% BSA or BlockAid™ Blocking Solution (Cat. No. B10710).

Incubation time for tyramide labeling

Incubation step for the tyramide labeling reaction (Step 3.2) is crucial for getting high resolution images with specific signal. To optimize the incubation time for this step, perform 0, 2.5, 5, 7.5 and 10 minute incubations using positive and negative control slides.

- If non-specific signal is present in negative controls or if the signal is blurry in positive controls, decrease the incubation time.
- If dim or no signal is present in positive controls, increase the incubation time

Troubleshooting

Observation	Recommended action
Excess signal	Optimize the primary antibody dilution
	Shorten the incubation time with the tyramide reagent working solution
	Decrease the tyramide reagent concentration
Low signal	Optimize the primary antibody dilution and incubation time
	Lengthen the incubation time with the tyramide reagent working solution
	Use antigen retrieval techniques to unmask the signal
Low resolution or blurry signal	Shorten the incubation time with the tyramide reagent working solution
	Check the dilution of the Stop reagent
High background	Lengthen the incubation time with the H_2O_2 solution (Step 2.1) to decrease endogenous peroxidase activity
	Decrease the primary antibody concentration
	Lengthen the incubation time for the blocking step (Step 2.4)
	Increase the number and/or the length of the wash steps
	Shorten the incubation time with the tyramide reagent working solution
	Use a lower concentration of secondary antibody than recommended
	Check for endogenous biotin (if using streptavidin conjugates) and use Endogenous Biotin-Blocking Kit (Cat. No. E21390) to minimize interference from endogenous biotin

Appendix B: Ordering information

Unless otherwise indicated, all products are available through **thermofisher.com**.

Tyramide SuperBoost™ Kits

Product	Amount[1]	Cat. No.
Biotin XX Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	150 slides	B40911
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	150 slides	B40912
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	150 slides	B40913
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	150 slides	B40915
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	150 slides	B40916
Biotin XX Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	150 slides	B40921
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	150 slides	B40922
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	150 slides	B40923
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	150 slides	B40925
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	150 slides	B40926
Biotin XX Tyramide SuperBoost™ Kit - Streptavidin	150 slides	B40931
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Streptavidin	150 slides	B40932
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Streptavidin	150 slides	B40933
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Streptavidin	150 slides	B40935
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Streptavidin	150 slides	B40936
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	50 slides	B40941
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	50 slides	B40942
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	50 slides	B40943
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG	50 slides	B40944

[1] Amount is based on 18-mm × 18-mm coverslips.

SuperBoost™ standalone reagents

We recommend first time users to use the complete Tyramide SuperBoost™ kits listed above. These reagents need to be optimized for best results.

Product	Amount[1]	Cat. No.
Alexa Fluor™ 350 Tyramide reagent	150 slides	B40952
Alexa Fluor™ 488 Tyramide reagent	150 slides	B40953
Alexa Fluor™ 546 Tyramide reagent	150 slides	B40954
Alexa Fluor™ 555 Tyramide reagent	150 slides	B40955
Alexa Fluor™ 568 Tyramide reagent	150 slides	B40956
Alexa Fluor™ 594 Tyramide reagent	150 slides	B40957
Alexa Fluor™ 647 Tyramide reagent	150 slides	B40958
Biotin-XX Tyramide reagent	150 slides	B40951
SuperBoost™ Goat anti-Mouse Poly HRP	22.5 mL[2]	B40961
SuperBoost™ Goat anti-Rabbit Poly HRP	22.5 mL[2]	B40962
Streptavidin - HRP	2.5 mg	43-4323
DMSO, Anhydrous	10 x 3 mL	D12345
Reaction stop reagent (same as Amplex™ Red/UltraRed Stop Reagent)	100 reactions	A33855

[1] 150 slide amount is based on 18-mm × 18-mm coverslips.
[2] Sufficient for 150 slides, based on 18-mm × 18-mm coverslips.

Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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Revision history: Pub. No. MAN0015834

Revision	Date	Description
B.0	September 2016	Add Multiplex protocol with primary antibodies from same species, provide directions for making solutions for the 50-slide kit
A.0	April 2016	New document

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