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Tyramide SuperBoost™ Kits with Alexa Fluor™ Tyramides

USER GUIDE

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Revision A.0

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0029125 A.0 (English)

Revision	Date	Description
A.0	10 March 2023	New document for Tyramide SuperBoost™ Kits with Alexa Fluor™ Tyramides.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

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™ (Figure 1 and Figure 2).

Tyramide signal amplification used in the Tyramide SuperBoost™ kits uses 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 equal signal intensity. Because the 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.

The 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 (Figure 3, Figure 4, and Figure 5). In tissue samples, it is possible to use the primary antibodies from the same host species for easier multiplexing (Figure 6).

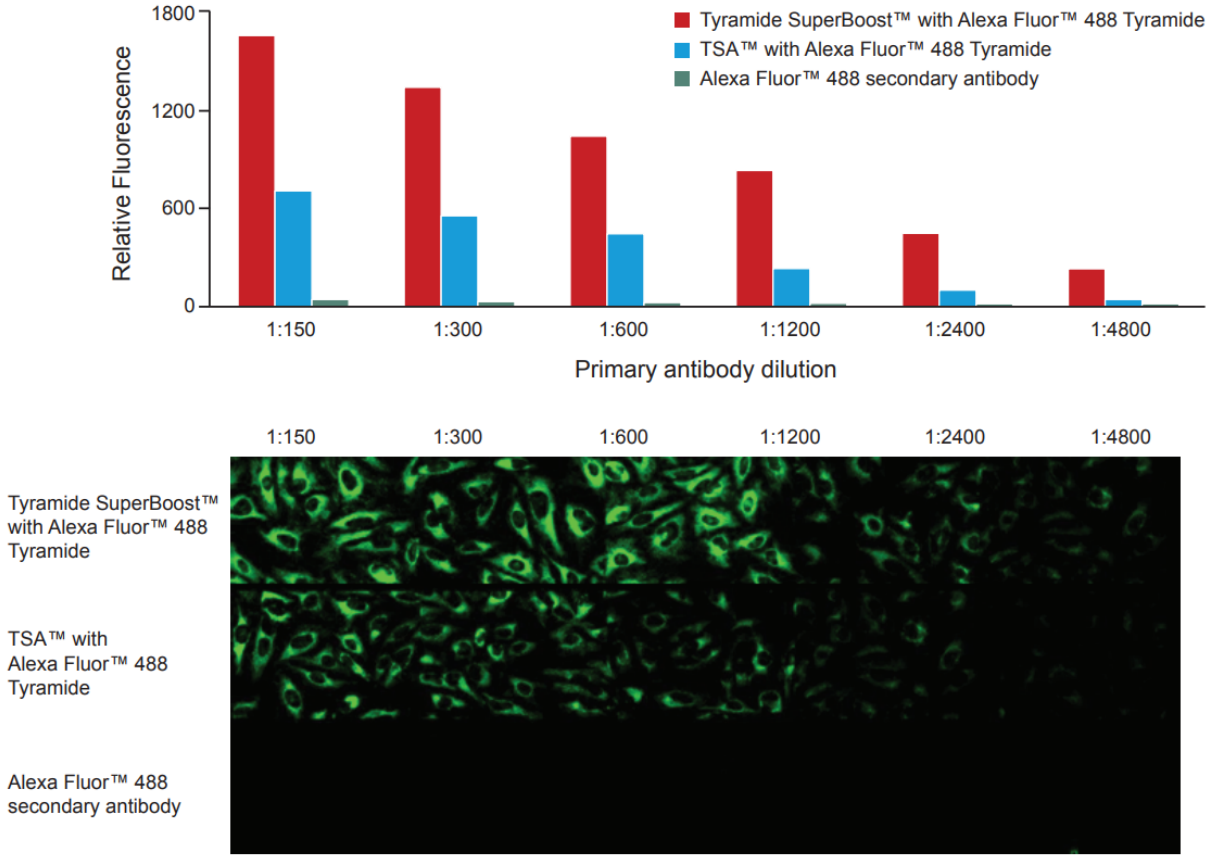


Figure 1 Sensitivity of Tyramide SuperBoost™ kits.

HeLa cells were fixed and permeabilized with the Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Prohibitin (green) was labeled with various concentrations of antibody. The manufacturer recommendation was 1:150 dilution or 5 µg/mL. Anti-prohibitin antibody was then detected using the 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 an 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.

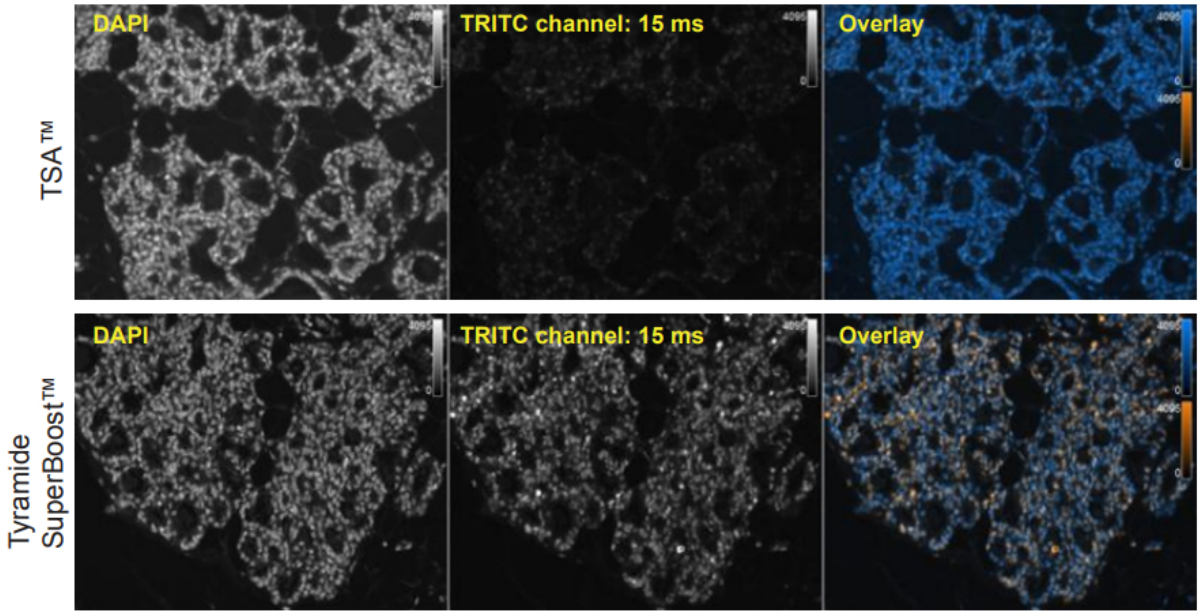


Figure 2 Tyramide SuperBoost™ kits are shown to be more sensitive than TSA™ kits.

FFPE-preserved mouse mammary tissue was processed for immunohistochemistry and labeled with anti-histone H3 antibody, which was detected with the 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 an 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.

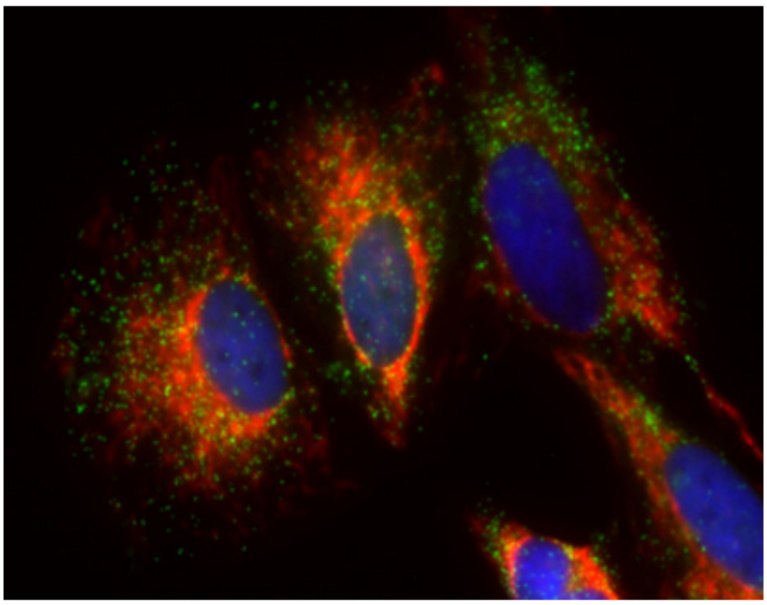


Figure 3 Multiplexing: Tyramide SuperBoost™ kits with GFP/RFP.

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 the Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602). Prohibitin was labeled with anti-prohibitin antibody and then detected with the Alexa Fluor™ 594 Tyramide SuperBoost™ Kit – Goat anti-Rabbit IgG (Cat. No. B40925) (red). Nuclei were labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

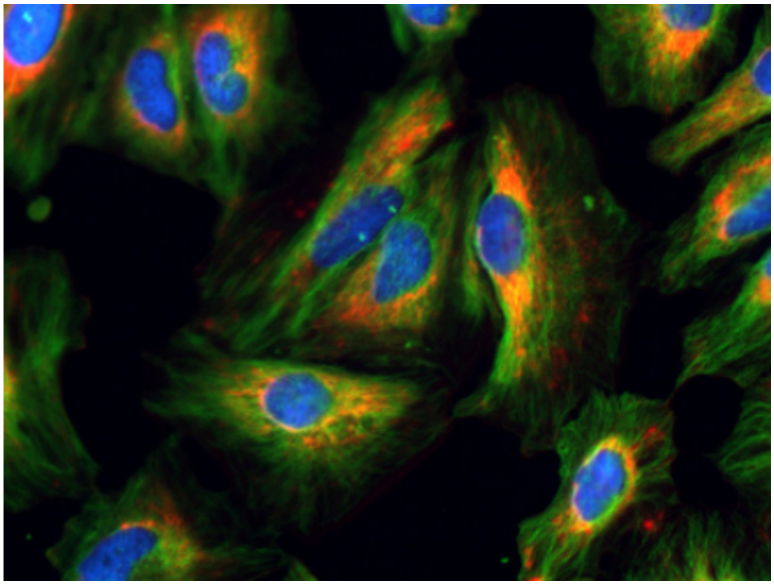


Figure 4 Multiplexing: Tyramide SuperBoost™ kits with secondary antibody.

HeLa cells were fixed and permeabilized with the 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 the 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.

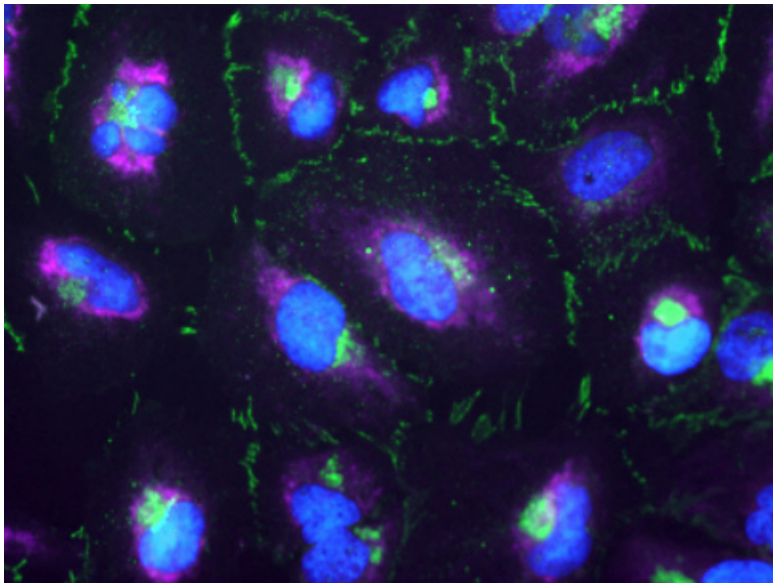


Figure 5 Multiplexing: Two proteins detected with two different colors of Tyramide SuperBoost™ kits.

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 β-Catenin (green) detection, cells were incubated with anti-β-Catenin antibody (Cat. No. 13-8400) and then detected with Alexa Fluor™ 488 Tyramide SuperBoost™ Kit – Goat anti-Mouse IgG (Cat. No. B40912). Nuclei were labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

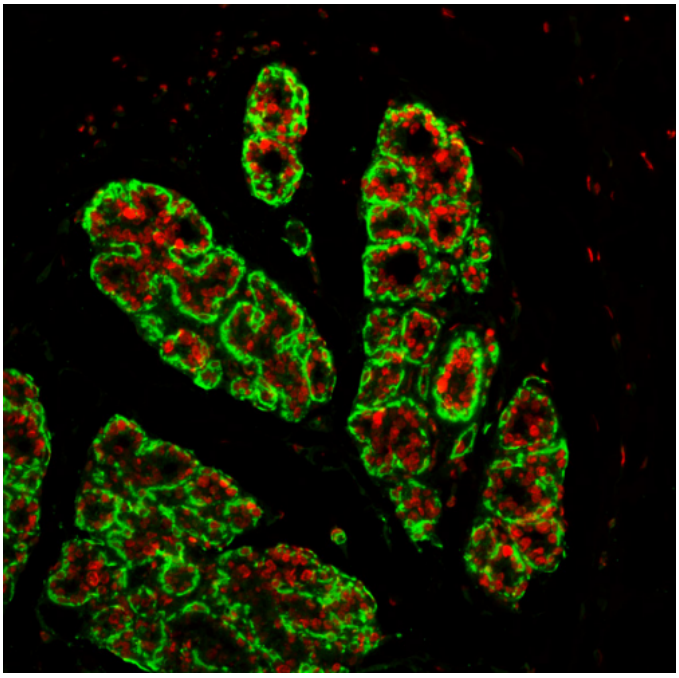


Figure 6 Multiplexing: Two proteins detected with primary antibodies from the same species.
FFPE-preserved mammary gland tissue was labeled with mouse anti-H3B and the Alexa Fluor™ 594 Tyramide SuperBoost™ Kit – Goat anti-Mouse IgG (Cat. No. B40915) to detect the H3B protein (red). Primary and secondary antibodies were stripped using Citrate Buffer (pH 6.0) (Cat. No. 005000) in a microwave. Actin (green) was then labeled with mouse anti-actin antibody and detected with the Alexa Fluor™ 488 Tyramide SuperBoost™ Kit – Goat anti-Mouse IgG (Cat. No. B40912). Nuclei were labeled with NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) (blue). Images were taken on a confocal microscope.

Contents and storage

Table 1 Standard kit components.

Item	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) 7.5 mL (50 slides)	1X	
Alexa Fluor™ Tyramide Reagent (Component C1)	1 vial[2]	—	
Hydrogen peroxide (Component C2)	28.5 mL	Stabilized 3% solution	

Table 1 Standard kit components. *(continued)*

Item	Amount	Concentration	Storage[1]
Reaction Buffer[3] (Component C3)	6 mL	20X	• 2–8°C • Desiccate • Protect from light • Do not freeze
Reaction Stop Reagent (Component D)	2 x 8 mg (150 slides) 1 x 8 mg (50 slides)	—	
Dimethylsulfoxide (DMSO) (Component E)	200 µL	—	

[1] When stored as directed, the product is stable for 6 months after receipt.
[2] Sufficient material is provided for 50 or 150 slides based on the protocols. See Table 2 for more information about individual Tyramide SuperBoost™ kits and stand-alone reagents.
[3] Reaction Buffer can be replaced with Tris Buffer, pH 7.4 for similar performance.

Table 2 Labeled tyramide conjugates provided in Tyramide SuperBoost™ kits or as stand-alone reagents.

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

Table 2 Labeled tyramide conjugates provided in Tyramide SuperBoost kits or as stand-alone reagents. *(continued)*

Labeled tyramide	Ex/Em[1]	Tyramide SuperBoost™ kits			Tyramide conjugate (stand-alone)[2]	Kit size[3]
		Goat Anti-Mouse IgG[4]	Goat Anti-Rabbit IgG[4]	Streptavidin		
Alexa Fluor™ 594 Tyramide	591/617 nm	B40942	B40944	—	—	50 slides
Alexa Fluor™ Plus 750 Tyramide	750/790 nm	—	—	—	B56131	900 slides

[1] Approximate fluorescence excitation and emission maxima

[2] Alexa Fluor™ tyramide reagents (Component C1) provided in each kit can be purchased as stand-alone reagent.

[3] Sufficient material is provided for up to 50, 150, or 900 (based on catalog number) 18-mm x 18-mm coverslips using 100 µL per slide in most critical incubation steps. This volume can be adjusted for different size samples.

[4] Poly-HRP-conjugated secondary antibody (Component B) provided in each kit can be purchased as stand-alone reagent.

Required materials not supplied

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

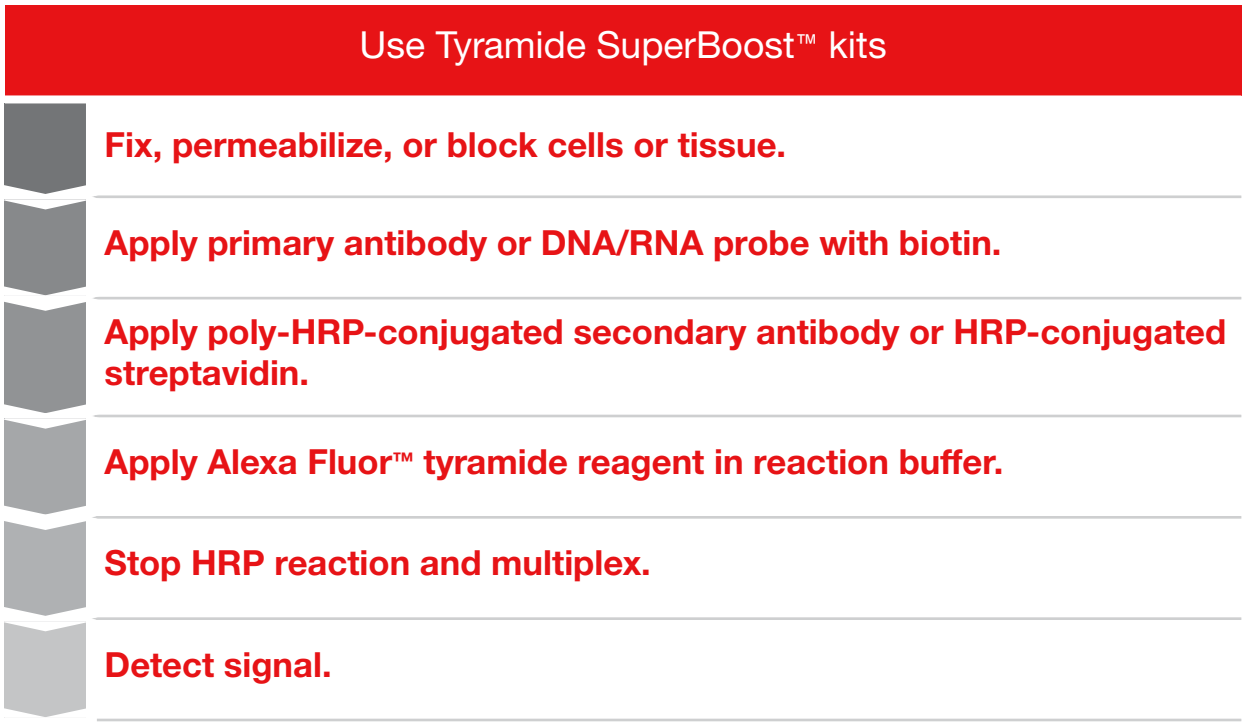
Item	Source
Cells or tissue	MLS (Use positive and negative controls if needed)
Slides, coverslips, or containers	MLS
Conjugated probes for FISH	MLS
Primary or secondary antibodies[1]	MLS
PBS (phosphate buffered saline), pH 7.4 (No calcium, magnesium, or phenol red in solution)	10010031
95% ethanol	MLS
Distilled water, ultrapure	15230-147
Hydrophobic barrier pen	R3777
Image-iT™ Fixation/Permeabilization Kit	R37602
Image-iT™ 4% Formaldehyde Fixative Solution in PBS (methanol-free)	FB002
Image-iT™ Glyoxal 3% Fixative Solution	I28700
Image-iT™ Paraformaldehyde 4% Fixative Solution in 0.1 M Phosphate Buffer (methanol-free)	I28800

(continued)

Item	Source
Image-iT™ Paraformaldehyde 3%, Glutaraldehyde 0.35% Fixative Solution in 0.1 M Sodium Cacodylate Buffer (methanol-free)	I28900
Endogenous Biotin-Blocking Kit	E21390
Citrate buffer, pH 6.0, concentrate	005000
ProLong™ Glass Antifade Mountant or SlowFade™ Glass Antifade Mountant	P36982 or S36917

[1] To search the Thermo Fisher Scientific primary antibody collection, visit the antibody search tool at **Thermo Fisher Scientific antibody search tool**.

Workflow



2 Methods

Procedural guidelines

- When using the Tyramide SuperBoost™ kits for the first time, optimize the protocols following the guidelines in “Signal optimization” on page 19.
- A hydrophobic barrier pen (wax pen) can be used to hold liquid reagents on the sample slide or coverslip.
- Do not let the cells or tissue samples dry out.
- For longer incubations, a humidified chamber (e.g., a covered box with a damp paper towel) can be used.

Before you begin

Prepare reagents

Warm vials to room temperature before opening.

- **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 can coat the sides of the vial.
  - For Alexa Fluor™ Plus 750 Tyramide (Cat. No. B56131) – Dissolve Alexa Fluor™ Plus 750 Tyramide in 900 µL of DMSO (Component E) to make 100X Tyramide stock solution. Invert the vial several times to dissolve any tyramide that can coat the sides of the vial.

  The 100X Tyramide stock solution can be stored in a sealed vial at 2–8°C for up to 6 months away from moisture.

- **100X H_2O_2 solution** – Add 1 drop (approx. 50 µL) of Hydrogen Peroxide Solution (Component C2) to 1 mL of distilled water.

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

- **1X Reaction Buffer** – Add 1 drop (approx. 50 µL) of 20X Reaction Buffer to 1 mL of distilled water.

  Note: Prepare new 1X Reaction Buffer 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.

- **Reaction Stop Reagent stock solution** – Add 1.45 mL of 95% ethanol to one vial of Reaction Stop Reagent (Component D).

  Reaction Stop Reagent stock solution is 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.

- **Reaction Stop Reagent working solution** – Dilute the Reaction Stop Reagent stock solution 1:11 in PBS.

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

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 the cells.

Prepare tissues

The 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 1.

Perform peroxidase labeling

1. *(Optional)* If needed, quench the endogenous peroxidase activity of the sample by adding sufficient drops of 3% hydrogen peroxide solution (Component C2) to cover the sample and incubate for 60 minutes at room temperature.

2. Rinse the cells or tissue 3 times with 1X PBS at room temperature.

3. *(Optional)* If using HRP-conjugated streptavidin, block endogenous biotin in the sample with the Endogenous Biotin-Blocking Kit (Cat. No. E21390) as recommended by the manufacturer. Rinse the cells or tissue 3 times with 1X PBS at room temperature before proceeding to the next step.

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.

5. Label the cells or tissue with primary antibody (mouse or rabbit 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 (e.g., 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.

6. Rinse the cells or tissue for 10 minutes with PBS at room temperature. Repeat the rinse 3 times.

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 nonspecific signal, shorten the incubation period.

8. Rinse the cells or tissue in PBS for 10 minutes at room temperature. Repeat the rinse 3 times.

Perform tyramide labeling

1. Prepare a tyramide working solution according to Table 3.

   IMPORTANT! Do not use extra solution 2 hours after preparation.

   Table 3 Tyramide working solution component volumes.

Component	Number of coverslips (18 mm x 18 mm)				
	5	10	20	50	100
100X Tyramide stock solution	5 µL	10 µL	20 µL	50 µL	100µL
100X H_2O_2 solution	5 µL	10 µL	20 µL	50 µL	100µL
1X Reaction Buffer	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 x 18-mm coverslip. This volume can be adjusted based on the size of the coverslip or the volume needed per well in a microplate.

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

3. Apply 100 µL of Reaction Stop Reagent prepared in step 4.

   IMPORTANT! The incubation period in step 2 and the timing of Stop reagent addition in step 3 are crucial in getting high resolution images with specific signal. We 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 “Optimize the incubation time for tyramide labeling” on page 19.

4. Rinse the cells or tissue 3 times with PBS.

5. For SuperBoost™ kits containing only Biotin XX tyramide, use the conjugated streptavidin as recommended by the manufacturer. Some recommended streptavidin conjugates are listed below in Table 4.

Table 4 Streptavidin conjugates recommended for the detection of Biotin XX tyramide.

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

Multiplex with primary antibodies from different species

After step 4 on page 15 or step 5 on page 16, cells or tissue samples can be multiplexed with another Tyramide SuperBoost™ kit or using standard IHC/ICC protocols.

When multiplexing, use a primary antibody from a host different from the one used in step 5 on page 14 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 Toth 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 4 on page 15 or step 5 on page 16, 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 added 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 step 1 on page 14 to step 5 on page 16 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

1. Counterstain the cells or tissue if needed using standard protocols. Few of the reagents recommended for counterstaining are listed in Table 5.

Table 5 Products recommended for counterstain.

Counterstain target	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

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.

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.

Troubleshooting

Observation	Possible cause	Recommended action
Excess signal	Kit signal output was greater than expected.	Optimize the primary antibody dilution.
		Shorten the incubation time with the tyramide reagent working solution.
		Decrease the tyramide reagent concentration.
Low signal	Kit signal output was low or weak.	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	Kit produced a poor signal resulting in a low resolution image.	Shorten the incubation time with the tyramide reagent working solution.
		Check the dilution of the Stop reagent.
High background	Reaction steps were not optimized.	Lengthen the incubation time with the H_2O_2 solution (step 1 on page 14) to decrease endogenous peroxidase activity.
		Decrease the primary antibody concentration.
		Lengthen the incubation time for the blocking step (step 4 on page 14).
		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.

Signal optimization

Optimize the amount of primary antibody or probe

To optimize the amount of primary antibody or probe used in step 5 on page 14, we recommend testing the following conditions:

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

Optimize the incubation time for tyramide labeling

The incubation step for the tyramide labeling reaction (step 2 on page 15) 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 nonspecific 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.

Ordering information

Tyramide SuperBoost Kits

Item	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		B40912
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG		B40913
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG		B40915
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG		B40916
Biotin XX Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40921
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40922
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40923
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40925
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40926
Biotin XX Tyramide SuperBoost™ Kit - Streptavidin		B40931
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Streptavidin		B40932
Alexa Fluor™ 555 Tyramide SuperBoost™ Kit - Streptavidin		B40933
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Streptavidin		B40935
Alexa Fluor™ 647 Tyramide SuperBoost™ Kit - Streptavidin		B40936
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG	50 slides	B40941
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Mouse IgG		B40942
Alexa Fluor™ 488 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40943
Alexa Fluor™ 594 Tyramide SuperBoost™ Kit - Goat anti-Rabbit IgG		B40944

[1] Amount of 150 slides is based on use of 18-mm x 18-mm coverslips.

SuperBoost™ stand-alone reagents

Item	Amount[1]	Cat. No.
Alexa Fluor™ 350 Tyramide Reagent	150 slides	B40952
Alexa Fluor™ 488 Tyramide Reagent		B40953
Alexa Fluor™ 546 Tyramide Reagent		B40954
Alexa Fluor™ 555 Tyramide Reagent		B40955
Alexa Fluor™ 568 Tyramide Reagent		B40956
Alexa Fluor™ 594 Tyramide Reagent		B40957
Alexa Fluor™ 647 Tyramide Reagent		B40958
Biotin XX Tyramide Reagent		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 regent (same as Amplex™ Red/UltraRed Stop Reagent)	100 reactions	A33855

[1] Amount of 150 slides is based on use of 18-mm x 18-mm coverslips.
[2] Sufficient for 150 slides based on use of 18-mm x 18-mm coverslips.

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

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  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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