HyperFluor™ 488 TSA Fluorescence System Kit

Product description

Tyramide signal amplification (TSA) technology can be used to detect low-abundance targets in tissue and cellular immunofluorescence (IF), immunochemistry, and in situ hybridization (ISH) experiments. This technology can increase the signal sensitivity by about 100 times. The HRP-labeled secondary antibody can activate the fluorescently labeled tyramide molecules in the presence of H_2O_2, and convert the labeled tyramide into an oxidized, highly reactive free radical that can covalently bind to tyrosine residues in the vicinity of the reaction site. The reaction is rapid (less than 10 minutes) and the deposited labeling can be observed directly under a standard or confocal microscope. TSA fluorescence kits can also be used in combination with traditional immunofluorescence methods for multicolor imaging, and two or more tyramide reactions can be performed sequentially to label different targets on one sample. The use of TSA reagents significantly improves signal sensitivity while maintaining the specificity and resolution ratio compared to common experiments, in addition, TSA reagents can significantly reduce the consumption of primary antibodies or probes.

The fluorescent molecule of this kit is HyperFluor™ 488 (495 nm/519 nm), which can be detected by standard FITC filters. Compared to fluorescein in the Fluorescein TSA Fluorescence System Kit (K1050), HyperFluor™ 488 is more photostable, allowing for longer observation and image capture, it also obtains low self-quenching and high fluorescence quantum yield.

Our products Tyramide Signal Amplification (TSA) kits include several kits with different wavelength: K1050 with fluorescein for detection at excitation and emission wavelengths of 494 nm/517 nm, K1051 with Cyanine 3 for detection at excitation and emission wavelengths of 550 nm/570 nm, and K1052 with Cyanine 5 at excitation and emission wavelengths of 648 nm/667 nm. You can purchase TSA kits with different wavelengths according to your requirements.

Components and storage conditions

Components	K4301-200 slides	K4301-400 slides
1X Amplification Diluent	20 mL	40 mL
HyperFluor™ 488 Tyramide (200×，dry, dissolve in 100 µL	1 tube	2 tubes

DMSO)		
Blocking Reagent (BSA)	6 g	12 g
Store the HyperFluor™ 488 Tyramide in the dark at -20°C. Keep 1X Amplification Diluent and Blocking Reagent in the dark at 4°C. Shelf life: 2 years upon receipt.		

Staining protocol

1. Experiment preparation

■ Materials Not Supplied

a) 1x PBS

b) DMSO (Molecular Biology level or HPLC level)

c) fixation solution (3.7% formaldehyde or 4% paraformaldehyde in PBS, for cell samples, you can also use methanol or acetone under -20℃)

d) Permeabilization Buffer (0.1% Triton X-100 in PBS）

e) 0.1 M sodium citrate buffer (pH 6.0) (for antigen retrieval of paraffin section, not required for others)

f) 30% H_2O_2

g) Primary and HRP-conjugated secondary antibodies

h) Blocking buffer: Dissolve 1 g of Blocking Reagent (BSA) in 100 mL of PBS as blocking buffer (prepare the volume according to your experiment).

i) The following reagents are only used for Biotin-Tyramide series (A 8011 Biotin-tyramide and A8012 Biotin-XX Tyramide Reagent) experiments

- Biotin blocking wash buffer: PBS with 1% BSA and 0.05% Tween 20
- Unlabeled Streptavidin Solution: Biotin blocking wash buffer containing 0.1 mg/mL of streptavidin
- Biotin solution: Biotin blocking wash buffer containing 0.5 mg/mL biotin.

2. Prepare the tyramide fluorescent stock solution (200 X).

Add 100 μL DMSO per tube to HyperFluor™ 488 Tyramide (200 X, dry, dissolve in 100 μL DMSO) before first use. The stock solution is recommended to be divided into several parts according to the usage amount of each experiment and stored at 4°C in the dark.

3. General preparation Before IF

■ Cell and frozen tissue section samples

1) Rinse the cells or tissue with PBS twice.

2) Fix the cells or sections with 3.7% formaldehyde or 4% paraformaldehyde at room temperature for 20 min. For methanol or acetone fixation: add appropriate amount of pre-chilled methanol or acetone to cover the sample. Incubate at -20°C for 10 minutes.

3) Rinse the cells or tissue with PBS twice.

4) Permeabilize the cells with 0.1% Triton X-100 in PBS for 5 minutes at room temperature. For tissue frozen sections, you can increase the concentration of Triton X-100 and the time, such as 0.5% Triton X-100 in PBS for 20 min at room temperature.

5) Rinse the cells or tissue with PBS twice.

■ **Paraffin tissue sections**

1) Bake the slides at 60°C for 30 minutes.

2) Deparaffinizing and rehydrating the section:

2.1 Immerse the slides in xylen 2 times for 5 minutes each.

2.2 Immerse the slides in 100% alcohol 2 times for 5 minutes each.

2.3 Immerse the slides in 95% alcohol for 5 minutes.

2.4 Immerse the slides in 90% alcohol for 5 minutes.

2.5 Immerse the slides in 80% alcohol for 5 minutes.

2.6 Immerse the slides in 70% alcohol for 5 minutes.

2.7 Rinse the slides with deionized H2O for 3 min.

2.8 Rinse the slides with 1×PBS for 3 minutes. Drain the excess 1×PBS.

Note: Xylene is toxic and volatile, please operate in a fume hood.

3) Marking: Trace the outline of the sample around the slice with a pencil for subsequent permeabilization and labeling.

4) Antigen retrieval: Microwave 0.1 M citrate buffer (pH 6.0) buffer to boil. Immerse slides into preheated retrieval solution, boil intermittently for 10 min. After antigen retrieval, remove the coplin jar with retrieval solution and slides from microwave oven, and let it cool to room temperature slowly.

Note: During this process, the tissue on the slide should be immersed in the buffer to ensure the antigen retrieval effect. You may choose different antigen retrieval buffer for different samples.

5) Wash with 1×PBS twice.

4. Endogenous peroxidase inactivation (optional).

Cover the sample with 3% hydrogen peroxide and incubate at room temperature for 60 min to quench the endogenous peroxidase activity.

5. Endogenous biotin block (optional)

When performing the Biotin-tyramide/Streptavidin assay, it is recommended to block endogenous biotin in the sample to reduce the background. For Fluorescein/Cyanine 3/Cyanine 5/HyperFluor™ 488-Tyramide kits, this step can be omitted.

5.1 Incubate the samples with unlabeled streptavidin solution for 15 min at room temperature. Then wash samples 3 times at room temperature for 5 min each using biotin blocking wash buffer.

5.2 Incubate the samples with biotin solution for 30 min at room temperature to block excess biotin binding sites on streptavidin. Then wash samples 3 times for 5 min each with biotin blocking wash buffer.

6. Immunofluorescence

6.1 Blocking: Block the sample with blocking buffer at room temperature for 30-60 min.

6.2 Dilute the primary antibody to an appropriate concentration with blocking buffer. Incubate the samples with the primary antibody at room temperature for 2 h or 4°C overnight.

6.3 Wash 3 times with 1×PBS for 5 min each at room temperature.

6.4 Dilute the HRP-conjugated secondary antibody using blocking buffer (typically a final concentration of 5 μg/mL is appropriate for most TSA experiments, you can use a concentration gradient to determine the optimal concentration) and incubate the samples with the solution for 1 h at room temperature.

6.5 Wash 3 times with 1×PBS for 5 min each at room temperature.

6.6 Typically, each sample needs 100 μL of HyperFluor™ 488 Tyramide working solution, incubate for 5-10 min at room temperature. The working solution is recommended to be prepared right before the incubation, and the excess unused solution is recommended to be discarded.

The components of HyperFluor™ 488 Tyramide working solution is shown in the following table:

components	volume	Final concentration
1X Amplification Diluent	100 μL	
HyperFluor™ 488 Tyramide (200 X) Stock Solution	0.5 μL	1×
0.3% H_2O_2	1 μL	0.003%

Note: The working solution can be stored at 4°C in the dark up to 24 h. Dilution of 30% H_2O_2 to 0.3% is also recommended before the experiment, as H_2O_2 is not stable.

6.7 Wash with 1× PBS 3 times for 5 min each at room temperature.

6.8 Optional: If you need to perform TSA reactions for multiple targets, you can perform antibody stripping at this step, followed by immunofluorescent labeling of another target.

6.9 Optional: If Biotin-tyramide is used for labeling, fluorescently labeled streptavidin can be used.

6.10 Optional: Nuclear staining using DAPI or Hoechst, then wash with PBS 3 times after staining.

6.11 Mount the coverslip using a mounting medium with anti-fading properties.

6.12 Visualize staining of the sample under a microscope.

Notes

1. TSA kits show higher sensitivity and signal compared to fluorescent secondary antibodies. Therefore, using a low concentration of primary antibodies is possible, which can reduce background fluorescence due to non-specific binding, and we recommend setting a concentration gradient of the primary antibody to find the optimal concentration.
2. To reducing autofluorescence, autofluorescence quenching kits can be used.
3. Higher concentrations of primary or secondary antibodies may result in a too much strong signal or high background, we recommend setting a concentration gradient of the fluorescently labeled tyramide from 1:50 to 1:1000 to find the optimal concentration.
4. 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.
5. This product is for scientific purposes only.

Other products that you may need as listed below

Catalog No.	Product name
K1221	HRP Goat Anti-Mouse IgG (H+L) Antibody
K1222	HRP Goat Anti-Human IgG (H+L) Antibody
K1223	HRP Goat Anti-Rabbit IgG (H+L) Antibody
K1224	HRP Rabbit Anti-Goat IgG (H+L) Antibody
K1079	Streptavidin-Cy3

K1080	Streptavidin-Cy5
K1081	Streptavidin-FITC
C3362	DAPI (hydrochloride)
A3472	Hoechst 33342

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com