TSA Multi-colour Immunofluorescence System Kit (4-Color)

Product Description:

Tyramide Signal Amplification (TSA) technology can be used to detect low-abundance targets in tissue and cell immunofluorescence (IF), immunochemistry, in situ hybridization (ISH), etc., increasing signal sensitivity by about 100-fold. The HRP-labeled secondary antibody can activate the fluorescently labeled tyramide molecules in the presence of H2O2, 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 labeling process is rapid (less than 10 minutes), and the deposition marks can be viewed directly under a standard or confocal microscope. TSA fluorescence kits can also be used in combination with traditional immunofluorescence methods for multicolor imaging, or two or more tyramide reactions can be performed sequentially to label different targets on one sample (for example, in this kit, three different fluorescent tyramide molecules were employed). The use of TSA reagents can significantly improve signal sensitivity while maintaining the specificity and resolution ratio compared to normal experiments, in addition, TSA reagents can significantly reduce the consumption of primary antibodies or probes.

This kit is a 4-color fluorescent labeling kit, including HF 488, HF 546, HF 633, and DAPI, which can realize multiplex labeling of samples. After one single-color fluorescent labelling, use an antibody stripping method (such as microwave heating treatment in an appropriate buffer) to remove the non-covalently bound antibody, then in the next labelling, employ another primary antibody, fluorescein tyramide, and so on.

Our Tyramide Signal Amplification (TSA) product family also includes several other single-staining kits: 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, K1052 with Cyanine 5 at excitation and emission wavelengths of 648 nm/667 nm, and K4301 with HyperFluor 488, which can detect the signal at the excitation and emission wavelengths of 495 nm/519 nm. You can purchase TSA kits with different wavelengths according to your own requirements.

Composition and storage conditions

Components	K4310-20 slides
HF 488 Tyramide (200×)	10 μL
HF 546 Tyramide (200×)	10 μL

HF 633 Tyramide (200×)	10 μL
DAPI Solution	2 X 1 mL
HRP Goat Anti-Mouse IgG (H+L) Antibody, 1 mg/mL	30 μL
HRP Goat Anti-Rabbit IgG (H+L) Antibody, 1 mg/mL	30 μL
1× Tyramide Amplification Buffer	6 mL
BSA	0.2 g
Fluorescence Mounting Medium	0.4 mL

Store DAPI Solution, HF 488 Tyramide, HF 546 Tyramide and HF 633 Tyramide at -20°C away from light and the others at -20°C for 6 months.

Staining protocol

1. Experiment preparation

Materials Not Supplied:

a) 1x PBS

b) Xylene

c) Ethanol

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

e) Permeabilization reagent (0.1% Triton X-100 in PBS）

f) 0.1 M sodium citrate buffer (pH 6.0)

g) Blocking buffer: 0.2 g of BSA can be dissolved in 20 mL of PBS to generate blocking buffer (configured in the desired amount and ratio).

2. General preparation Before IF

■ **Samples of cells and frozen tissue sections**

1) Wash: Wash the cells/tissues twice with 1× PBS.

2) Fixation: Fix with 3.7% formaldehyde or 4% paraformaldehyde at room temperature for 20 min.

3) Rinse the cells or tissues with PBS twice.

4) Permeabilization: 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 tissues with PBS twice.

■ **Paraffin tissue sections**

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

2) Deparaffinizing and rehydrating the section: Immerse the slides in xylen 2 times for 5 minutes each, Immerse the slides in 100% alcohol 2 times for 5 minutes each, Immerse the slides in 95% alcohol for 5 minutes, Immerse the slides in 90% alcohol for 5 minutes, Immerse the slides in 80% alcohol for 5 minutes, Immerse the slides in 70% alcohol for 5 minutes, Rinse the slides with deionized H2O for 3 min, 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.

3. Endogenous peroxidase inactivation (optional)

The endogenous peroxidase activity of the sample can be quenched by covering the sample with 3% hydrogen peroxide and incubating for 60 minutes at room temperature.

4. Immunofluorescence

1) Blocking: Block the sample with blocking buffer at room temperature for 30-60 min. (you can also choose goat or monkey serum according to your needs).

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.

3) Wash with 1× PBS 3 times, each 5 min at room temperature.

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

5) Wash with 1× PBS 3 times, each 5 min at room temperature.

6) Calculate the amount of Tyramide working solution required and dilute the Tyramide (200X) in a

200:1 ratio with 1× Tyramide Amplification Buffer. Typically, incubating for 5–10 minutes at room temperature using 100 μL of Tyramide working solution is preferable.

The working solution is recommended to be prepared right before the incubation, and the excess unused solution is recommended to be discarded.

You can also perform a concentration gradient to determine the most appropriate dilution ratio, the dilution ratio of Tyramide (200 X) can be adjusted according to your experiments from 1:50 to 1:1000.

Note: Tyramide working solution can be stored at 4°C in the dark for up to 24 h.

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

8) Antibody Stripping: Microwave the sample with a suitable buffer for 10-15 min intermittently, either 0.1 M sodium citrate buffer (pH 6.0) or other buffers such as Tris-EDTA Buffer (10 mM Tris Base, 1 mM EDTA Solution, 0.05% Tween 20, pH 9.0).

9) Wash 3 times with 1 × PBS for 5 min each at room temperature. *Note: After each fluorescent tyramide molecule labelling, the staining can be confirmed by fluorescence microscopy.*

10) Start from blocking step for the next target.

11) After immunofluorescence of all targets is finished. Incubate the sample with DAPI staining solution for 5 minutes at room temperature.

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

13) Mount the coverslip with Fluorescence Mounting Medium.

14) Visualize staining of the sample under a microscope.

Precautions

1. TSA kits show higher sensitivity and signal compared to fluorescent secondary antibodies. Therefore, the use of a lower concentration of primary antibody in the experiment can reduce the background fluorescence caused by non-specific binding, and we recommend setting the primary antibody concentration gradient 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 an excessively strong signal or high background, we recommend setting a dilution gradient of the fluorescently labeled tyramide from 1:50 to 1:1000 to find the optimal concentration.
4. Before use, centrifuge the product to the bottom of the tube before proceeding to subsequent experiments.

5. After the first use of 1× Tyramide Amplification Buffer, it is recommended to aliquot the buffer to several parts and store at -20°C to avoid repeating freeze-thaw cycles.
6. To prevent false negative or false positive results, a negative control and a positive control should be set up before the experiment. For tissue samples, it is recommended to set an unstained control (without the addition of antibodies or tyramide) to determine if the tissue is autofluorescent, excluding effects on background.
7. It is recommended to dilute Tyramide (200 ×) at ratio of 1:200. If you can't generate an ideal result, you can perform a gradient dilution from 1:50 to 1:1000 to find the most suitable concentration.
8. In multicolor labeling, different fluorescein is selected according to the antigen density (strong fluorescent molecules are selected for low-density antigens; weaker fluorescent molecules are selected for high-density antigens). The labelling sequence may have an impact on the final immunofluorescence effect, you need to explore it yourself.
9. This product is for scientific research purposes only.

Other products in the TSA series are summarized below

Catalog number	Product name
K1050	Fluorescein TSA Fluorescence System Kit
K1051	Cy3 TSA Fluorescence System Kit
K1052	Cy5 TSA Fluorescence System Kit
K4301	HyperFluor 488 TSA Fluorescence System Kit
B7773	Bovine Serum Albumin, BSA
C3362	DAPI (hydrochloride)
K2401	DAPI Solution (1 mg/mL)
K2402	DAPI Solution (10 μg/mL, ready to use)
K1222	HRP Goat Anti-Human IgG (H+L) Antibody
K1224	HRP Rabbit Anti-Goat IgG (H+L) Antibody
A3472	Hoechst 33342

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