HyperFluor™ 514 TSA Fluorescence System Kit

Introduction

Tyramide signal amplification (TSA) technology can be used to detect low-abundance targets in experiments such as tissue and cell immunofluorescence (IF), immunohistochemistry, and in situ hybridization (ISH), and can increase signal sensitivity by approximately 100-fold. Fluorescently labeled tyramine molecules are activated by HRP-labeled secondary antibodies in the presence of H_2O_2, triggering a large number of enzymatic reactions. This causes fluorescent molecules to bind to protein residues at the antigen-antibody binding site and in the surrounding tissue, forming extensive fluorescent deposits that achieve signal amplification. This labeling process is rapid (less than 10 minutes), and the deposited labels can be directly observed under standard or confocal microscopes. TSA fluorescence kits can also be combined with traditional immunofluorescence methods for multi-color imaging, and two or more tyramide reactions can be performed sequentially to label different targets on the same sample. Compared with conventional experiments, using TSA reagents can significantly improve signal sensitivity while maintaining stable specificity and resolution. Additionally, TSA reagents can substantially reduce the consumption of primary antibodies or probes.

The fluorescence-labeled signal in this kit is HyperFluor™ 514 (516 nm/548 nm), which can be easily detected using standard FITC filters.

Components and Storage

Components	200 Slides	400 Slides	Storage
1X Amplification Diluent	20 mL	40 mL	4°C
HyperFluor™ 514 Tyramide (200×, dry, dissolve in 100 µL DMSO)	1 tube	2 tubes	-20°C away from light
Blocking Reagent (BSA)	6 g	12 g	4°C
Shipping: Blue Ice	Shelf life: 2 years		

Protocol

1. Pre-experiment preparation (self-prepared or pre-made reagents):

   a) 1x PBS: e.g. PBS (Phosphate-Buffered Saline) (Cat. #: K2818)

   b) DMSO: Molecular Biology or HPLC level

   c) Fixative: e.g. 4% paraformaldehyde (PBS)

d) Permeable solution or osmotic reagent: e.g., 0.1% Triton X-100 in PBS

e) Antigen Retrieval Solution (Paraffin Section): e.g., 0.1 M Sodium Citrate Buffer pH 6.0

f) HRP catalytic reaction reagent: 30% H_2O_2

g) Primary antibody and HRP conjugated secondary antibody

h) Blocking Buffer: Dissolve 1 g of Blocking Reagent (BSA) in 100 mL of PBS to prepare the blocking buffer (prepare according to the required volume). BSA specifically recommended for TSA experiments, namely Bovine Serum Albumin (No fatty acids) (Cat. #: H1005), is advised.

i) The following reagents are used exclusively for Biotin-Tyramide series experiments (A8011 Biotin-tyramide and A8012 Biotin-XX Tyramide Reagent):

- 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 streptavidin
- Biotin solution: Biotin blocking wash buffer containing 0.5 mg/mL biotin.

2. Configure tyrosamide fluorescence storage solution (200×).

HyperFluor™ 514 Tyramide (200×, dry, dissolve in 100 µL DMSO) is supplied as a solid. Before initial use, add 100 µL of DMSO to each tube and mix thoroughly to prepare the tyramide fluorescence stock solution (200×). It is recommended to aliquot the prepared tyramide fluorescence stock solution into single-use portions based on the experimental needs. Store the aliquots protected from light at 4°C, where they are stable for up to 6 months.

3. Sample processing (for cell and tissue staining)

■ Cells as well as frozen tissue sectioned samples

1) Wash: 1× PBS wash cells/tissues twice

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

3) Wash: 1× PBS wash twice

4) Permeabilization: Cell samples were permeated with 0.1% Triton X-100 solution configured in PBS for 5 min at room temperature. Tissue samples can be increased with Triton X-100 concentration and transmission time as appropriate, such as 0.5% Triton X-100 (prepared with PBS) for 20 min at room temperature.

5) Wash: 1× PBS wash twice.

■ Paraffin tissue sections

1) Bake the pieces: Place the paraffin slices in an oven at 60°C for 30 min.

2) Dewaxing and hydration: at room temperature, soak the paraffin tissue with xylene and cut it twice for 5 minutes each time; At room temperature, the slice samples were immersed in anhydrous ethanol twice for 5 min each time. At room temperature, the sliced samples were submerged in ethanol (95%, 90%, 80%, 70%) with different concentration gradients in sequence, and rinsed once for 5 minutes each time. At room temperature, submerge the sections in pure water and rinse once for 3 minutes each time, then immerse the sections in 1× PBS once for 3 minutes each time, and carefully blot the excess liquid around the sectioned samples with filter paper.

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

3) Marking: Use a pencil to trace the sample outline around the sliced sample for subsequent transparency and labeling.

4) Antigen repair: 0.1 M citrate buffer (pH 6.0) was heated in the microwave until boiling, and the dewaxed and rehydrated tablets were placed in the buffer and boiled for 10 minutes. After antigen retrieval, it is taken out at room temperature to cool slowly.

***Note:** During this process, the tissue on the slide should be immersed in the buffer to ensure the antigen repair effect of the tissue. Different samples choose different antigen retrieval methods.

5) Wash: 1× PBS wash twice.

4. Endogenous peroxidase inactivation (optional)

The sample was covered with 3% H_2O_2 and incubated at room temperature for 60 min to quench the endogenous peroxidase activity.

5. Endogenous biotin blockade (optional)

When performing a Biotin-tyramide/Streptavidin assay, it is recommended to block the endogenous biotin in the sample to reduce the background. This step can be omitted for fluorescent molecule-Tyramide kits.

1) At room temperature, incubate the sample with the unlabeled streptavidin solution for 15 min. Samples were then washed 3 times at room temperature for 5 min each using biotin blocking wash buffer.

2) Incubate the sample with biotin solution for 30 min at room temperature to seal off excess biotin-binding sites on streptavidin. The samples were then washed 3 times with biotin blocking wash buffer for 5 min each time.

6. Immunolabeling

1) Blocking: Block with blocking buffer at room temperature for 30-60 minutes.

2) Primary antibody incubation: The primary antibody is diluted to the appropriate concentration using blocking buffer, and then the sample is incubated with the primary antibody at room temperature for 2 h or 4°C overnight.

3) Washing: 1× PBS wash 3 times at room temperature for 5 min each time.

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

5) Washing: 1× PBS wash 3 times at room temperature for 5 min each time.

6) Tyramide Working Solution Incubation: Each sample is typically incubated for 5-10 minutes at room temperature using 100 μL of HyperFluor™ 514 Tyramide Working Solution. The working fluid is recommended to be dispensed and used, and the excess unused is recommended to be discarded.

The HyperFluor™ 514 Tyramide working fluid configuration is shown in the table below:

Component	Volume	Final concentration
1X Dilute Amplification	100 μL	
HyperFluor™ 514 Tyramide storage solution (200×)	0.5 μL	1×
0.3% H_2O_2	1 μL	0.003%
***Note:** 1. 0.3% H_2O_2 is also recommended to be diluted before the experiment, as H_2O_2 is not stable. 2. Tyramide working solution was stored at 4°C in a dark place for up to 24 h. 3. The storage solution is the tyrosamide fluorescent storage solution (200×) configured in step 2.		

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

8) Antibody stripping (optional): If you need to perform immunofluorescence TSA reactions on multiple targets, you can perform antibody stripping (e.g., 0.1 M sodium citrate buffer pH 6.0 microwaved to boil for 10-15 minutes) followed by immunofluorescence labeling of the other target.

9) Fluorescently labeled Streptavidin labeling (optional): If labeled with Biotin-tyramide, fluorescently labeled Streptavidin can be used for fluorescent chromogenicism.

10) Nuclear staining (optional): Nuclear staining with DAPI or Hoechst, and after staining, wash 3 times with PBS.

11) Mounting: Mounting with anti-fluorescence quenching agent for mounting.

12) Microscopic imaging.

Note

1. Compared to fluorescent secondary antibodies, the TSA kit demonstrates higher sensitivity and signal intensity. Consequently, the primary antibody can be used at a lower concentration during experiments, which helps reduce background fluorescence caused by non-specific binding. We recommend testing a gradient of primary antibody concentrations to determine the optimal dilution.
2. To minimize autofluorescence, an autofluorescence quencher can be utilized.
3. Excessive signal intensity or high background may result from using primary or secondary antibodies at too

high concentrations. Titration within a range of 1:50 to 1:1000 is advised to identify 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 research use only.

6. Related product recommendations:

Type	Item number	Product Name
Fixation	K4640	Paraformaldehyde (4%)
Transparent	K4641	Animal Tissue Optical Clearing Kit
Antigen Retrieval	K4606	Universal Powerful Antigen Retrieval Solution(10X)
	K4601	Citrate Antigen Retrieval Solution (50X)
	K4603	Tris-EDTA Antigen Retrieval Solution (50X, pH9.0)
Permeabilization/Penetration Reagents	K4611	Immunostaining Permeabilization Buffer with Saponin
	K4612	Immunostaining Permeabilization Buffer with Triton X-100
	K4613	Enhanced Immunostaining Permeabilization Buffer with Triton X-100
Endogenous Blocking	K4615	Enhanced Endogenous Peroxidase Blocking Buffer
Immunostaining associated buffer	K4661	Immunol Staining Blocking Solution
	K4659	Immnol Staining Wash Buffer(10X)
	K4655	Immunol Staining Primary Antibody Dilution Solution
	K4657	Immnol Staining (Non-fluorescence) Secondary Antibody Dilution Solution
Mounting Medium	K2634	Enhanced Antifade Mounting Medium
	K2635	Antifade Mounting Medium with DAPI
Secondary antibody-HRP	K1221	HRP Goat Anti-Mouse IgG (H+L) Antibody
	K1223	HRP Goat Anti-Rabbit IgG (H+L) Antibody
TSA series	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
	K4302	HyperFluor™ 594 TSA Fluorescence System Kit
	K4304	HyperFluor™ 514 TSA Fluorescence System Kit
	K4305	HyperFluor™ 555 TSA Fluorescence System Kit
	K4306	HyperFluor™ 633 TSA Fluorescence System Kit
	K4307	HyperFluor™ 680 TSA Fluorescence System Kit
	K4308	HyperFluor™ 750 TSA Fluorescence System Kit
	K4310	TSA Multi-colour Immunofluorescence System Kit (4-Color)
	A8011	Biotin-tyramide
	A8012	Biotin-XX Tyramide Reagent
	H1001	1X Amplification Diluent

	H1005	Bovine Serum Albumin (No fatty acids)