Protocol

Cat. No. K4302

HyperFluor™ 594 TSA Fluorescence System Kit

Introduction

Tyramide signal amplification (TSA) technology can be used to detect low-abundance targets in tissue and cell immunofluorescence (IF), immunochemistry, in situ hybridization (ISH), and more, increasing signal sensitivity by about 100-fold. The fluorescently labeled tyramide molecule is catalyzed by the secondary antibody-labeled HRP in the presence of H2O2, resulting in a large number of enzymatic reactions, which make the fluorescent molecules bind to the protein residues around the tissue at the antigen-antibody binding site, forming a large number of fluorescent molecule deposits to achieve signal amplification. 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 conjunction with traditional immunofluorescence methods for multicolor imaging, or two or more tyrosamide reactions can be performed sequentially to label different targets on a sample. The use of TSA reagents can significantly improve signal sensitivity while maintaining stable specificity and resolution compared to normal experiments, and in addition, TSA reagents can significantly reduce the consumption of primary antibodies or probes.

The fluorescently labeled signal of this kit is HyperFluor™ 594 (591 nm/617 nm) and can be easily detected with a standard standard red/Texas Red filter.

Components and storage

Components \ Size	200 slides	400 slides	Storage
1X Amplification Diluent	20 mL	40 mL	4°C
HyperFluor™ 594 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. Preparation before the experiment

   Bring your own reagents or pre-made reagents:

a) 1x PBS

b) DMSO (Molecular Biology or HPLC Grade)

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 sections, not required for the rest of the experiments)

f) 30% H2O2

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 (A8011 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 streptavidin
- Biotin solution: Biotin blocking wash buffer containing 0.5 mg/mL biotin.

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

HyperFluorTM ^{594} Tyramide (200×, dry, dissolve in 100 μL DMSO) is in solid form, add 100 μL of DMSO per tube before first use, mix well, and make a caseamide fluorescent stock solution. It is recommended to divide the prepared caseamide fluorescent stock solution into several portions according to the amount used in each experiment and store it at 4°C in the dark.

3. Sample processing (for cell and tissue staining)

■ Samples of cells and frozen tissue sections

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

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

3) Washing: 1 × PBS twice for washing

4) Permeabilization: Cell samples were permeabilized with 0.1% Triton X-100 solution in PBS and stored at room temperature for 5 min. Tissue samples can be permeabilized by increasing the concentration and permeabilization time of Triton X-100 as appropriate, e.g., 0.5% Triton X-100 (prepared in PBS) for 20 minutes at room temperature.

5) Wash: 1 × PBS wash twice.

■ Paraffin tissue sections

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

2) Dewaxing and dehydration: At room temperature, soak the paraffin tissue with xylene and cut it twice for 5 min each time; At room temperature, the sliced samples were immersed in absolute ethanol and bleached twice for 5 minutes each time; At room temperature, the sliced samples were sequentially immersed in ethanol (95%, 90%, 80%, 70%) at different concentration gradients, and each concentration was rinsed once for 5 min each time. At room temperature, submerge the sections in pure water and rinse once for 3 minutes each, then submerge the sections in 1 × PBS and rinse once for 3 minutes each, carefully blotting excess liquid around the sliced samples with filter paper. Note: Xylene is toxic and volatile, so please operate it in a fume hood.

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

4) Antigen retrieval: 0.1 M citrate buffer (pH 6.0) was heated in a microwave oven until boiling, and the dewaxed and rehydrated tablets were placed in the buffer and boiled intermittently for 10 min. After antigen retrieval, it is removed and slowly cooled at room temperature. Note: During this process, the tissue on the slide should be immersed in buffer to ensure the antigen retrieval effect of the tissue. Different antigen retrieval methods are selected for different samples.

5) Wash: Wash twice × 1 PBS.

4. Endogenous peroxidase inactivation (optional).

The endogenous peroxidase activity of the sample was quenched by adding 3% hydrogen peroxide and incubating for 60 minutes at room temperature.

5. Endogenous biotin blockade (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//HyperFluor™ 594--Tyramide kits, this step can be omitted.

1) Incubate the samples with unlabeled streptavidin solution for 15 min at room temperature. Samples were then washed 3 times for 5 minutes at room temperature using Biotin Blocking Wash Buffer.

2) Incubate the samples with biotin solution for 30 minutes at room temperature to block the excess biotin binding sites on streptavidin. Samples were then washed 3 times with biotin blocking wash buffer for 5 minutes each.

6. Immunolabeling

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

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

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

6) Each sample is typically incubated for 5–10 minutes at room temperature using 100 μL of HyperFluorTM 594 Tyramide working solutionIt is recommended that the working solution be prepared and used now, and the excess unused is discarded.

The components of HyperFluor™ 594 Tyramide working solution is shown in the following table:

Components	volume	Final concentration
1X Amplification Diluent	100 μL	
HyperFluor™ 594 Tyramide (200 X) Stock Solution	0.5 μL	1×
0.3% H_2O_2	1 μL	0.003%
*Note: The staining solution can be stored at 4°C for up to 24 h in the dark. It is also recommended to dilute 0.3% H_2O_2 before the experiment, as H_2O_2 is not stable.		

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

8) Optional: If you need to perform an immunofluorescent TSA reaction on multiple targets, you can perform antibody stripping at this step, followed by immunofluorescence labeling of another target.

9) Optional: If labeling with Biotin-tyramide, fluorescently labeled Streptavidin can be used for fluorescence development.

10) Optional: Use the DAPI or Hoechst for nuclear staining, and after staining, wash 3 times in PBS.

11) Mounts are performed with antifluorescence quenching mountant.

12) Microscopic imaging.

Note

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 consider reducing autofluorescence, an autofluorescence quencher can be used.

3. Higher concentrations of primary or secondary antibodies may result in an excessively strong signal or high background, and can be fumbled 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 after each tyramide reaction by HRP quenching or antibody stripping.

5. This product is for scientific research use only.

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