Cy7 tyramide

Catalog number: 11064
Unit size: 1 mg

Component	Storage	Amount
Cy7 tyramide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Tyramide signal amplification (TSA) has proven to be a particularly versatile and powerful enzyme amplification technique with improved assay sensitivity. TSA is based on the ability of HRP, in the presence of low concentrations of hydrogen peroxide, to convert labeled tyramine-containing substrate into an oxidized, highly reactive free radical that can covalently bind to tyrosine residues at or near the HRP. The signal amplification conferred by the turnover of multiple tyramide substrates per peroxidase label translates ultrasensitive detection of low-abundance targets and the use of smaller amounts of antibodies and hybridization probes. In immunohistochemical applications, sensitivity enhancements derived from TSA method allow primary antibody dilutions to be increased to reduce nonspecific background signals, and can overcome weak immunolabeling caused by suboptimal fixation procedures or low levels of target expression. Cy7 tyramide contains the popular Cy7 fluorophore that can be readily detected with the standard Cy7 filter set. Its NIR excitation and emission make the probe an ideal choice for the applications where the common tyramides may have an interference resulted from the inherent fluorescence of tissues or other samples.

AT A GLANCE

Protocol Summary

1. Fix/permeabilize/block cells or tissue
2. Add primary antibody in blocking buffer
3. Add HRP-conjugated secondary antibody
4. Prepare tyramide working solution and apply in cells or tissue for 5-10 minutes at room temperature

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy7 filter set
Emission	Cy7 filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Tyramide stock solution (200X)
Add appropriate amount of DMSO to make 1 or 2 mg/mL stock solution and mix well. **Note:** Unused Tyramide stock solution can be stored at 2-8 °C.

PREPARATION OF WORKING SOLUTION

Tyramide working solution (1X)
Add 100 µL of Tyramide stock solution into 20 mL of buffer of your choice containing 0.003% H_2O_2. **Note:** Tris Buffer, pH=7.4 can be used for similar performance. **Note:** Tyramide working solution should be used immediately and made fresh on the day of use. **Note:** Concentration should be optimized initially for final appropriate concentration for the tests.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol is applicable for both cells and tissues staining.

Cell fixation and permeabilization

1. Fix the cells or tissue with 3.7% formaldehyde or paraformaldehyde, in PBS at room temperature for 20 minutes.
2. Rinse the cells or tissue with PBS twice.
3. Permeabilize the cells with 0.1% Triton X-100 solution for 1-5 minutes at room temperature.
4. Rinse the cells or tissue with PBS twice.

Tissue fixation, deparaffinization and rehydration
Deparaffinize and dehydrate the tissue according to the standard IHC protocols. Perform antigen retrieval with preferred specific solution/protocol as needed. Protocol can be found at: https://www.aatbio.com/resources/guides/paraffin-embedded-tissueimmunohistochemistry-protocol.html

Peroxidase labeling

1. Optional: Quench endogenous peroxidase activity by incubating cell or tissue sample in peroxidase quenching solution (such as 3% hydrogen peroxide) for 10 minutes. Rinse with PBS twice at room temperature.
2. Optional: If using HRP-conjugated streptavidin, it is advisable to block endogenous biotins by biotin blocking buffer.
3. Block with preferred blocking solution (such as PBS with 1% BSA) for 30 minutes at 4 °C.
4. Remove blocking solution and add primary antibody diluted in recommended antibody diluent for 60 minutes at room temperature or overnight at 4 °C.
5. Wash with PBS three times for 5 minutes each.
6. Apply 100 µL of secondary antibody-HRP working solution to each sample and incubate for 60 minutes at room temperature. **Note:** Incubation time and concentration can be varied depending on the signal intensity.
7. Wash with PBS three times for 5 minutes each.

Tyramide labeling

1. Prepare and apply 100 µL of Tyramide working solution to each sample and incubate for 5-10 minutes at room temperature. **Note:** If you observe non-specific signal, you can shorten the incubation time with Tyramide. You should optimize the incubation period using positive and negative control samples at various incubation time points. Or you can use lower concentration of Tyramide in the working solution.
2. Rinse with PBS three times.

Counterstain and fluorescence imaging

1. Counterstain the cell or tissue samples as needed. AAT provides a series of nucleus counterstain reagents as listed in Table 1. Follow the instruction provided with the reagents.

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

3. Use the appropriate filter set to visualize the signal from the Tyramide labeling. **Table 1.** Products recommended for nucleus counterstain

Cat#	Product Name	Ex/Em (nm)
17548	Nuclear Blue™ DCS1	350/461
17550	Nuclear Green™ DCS1	503/526
17551	Nuclear Orange™ DCS1	528/576
17552	Nuclear Red™ DCS1	642/660

EXAMPLE DATA ANALYSIS AND FIGURES

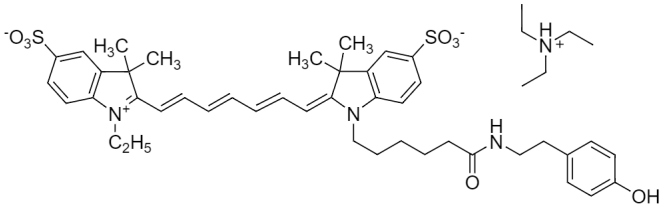

Figure 1. Chemical structure for Cy7 tyramide.

DISCLAIMER

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