Cy3 tyramide

Catalog number: 11065
Unit size: 1 mg

Component	Storage	Amount
Cy3 tyramide	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

For many immunohistochemical (IHC) applications, the traditional enzymatic amplification procedures are sufficient for achieving adequate antigen detection. However, several factors limit the sensitivity and utility of these procedures. 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. To achieve maximal IHC detection, tyramine is prelabeled with a fluorophore. 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. Cy3 tyramide contains the bright Cy3 that can be readily detected with the standard Cy3 filter set.

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

Cat. #	Product Name	Unit	Ex (nm)	Em (nm)
11070	AF 488 Tyramide reagent	200 slides	491	518
11075	AF 546 Tyramide reagent	200 slides	554	570
11082	AF 594 Tyramide reagent	200 slides	590	617
11061	Azido-Cy5 Tyramide	1 mg	644	665
11065	Cy3 Tyramide	1 mg	555	565
11066	Cy5 Tyramide	1 mg	644	665
45100	iFluor™ 488 Tyramide	200 slides	491	514
45105	iFluor™ 555 Tyramide	200 slides	552	567
45110	iFluor™ 647 Tyramide	200 slides	649	665

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	Cy3/TRITC filter set
Emission:	Cy3/TRITC filter set
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Cy3/TRITC filter set

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 (1000X):
Add appropriate amount of DMSO to make 1-5 mM of Tyramide stock solution.

Note Unused Tyramide stock solution can be stored at 2-8° C.

PREPARATION OF WORKING SOLUTION

Tyramide working solution (1X):
Add 1 µL of Tyramide stock solution into 1 mL of buffer of your choice containing 0.003% H_2O_2.

Note Tris Buffer, pH=7.4 can be used for optimal performance.

Note Tyramide working solution should be used immediately and made fresh on the day of use.

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-tissue-immunohistochemistry-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

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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

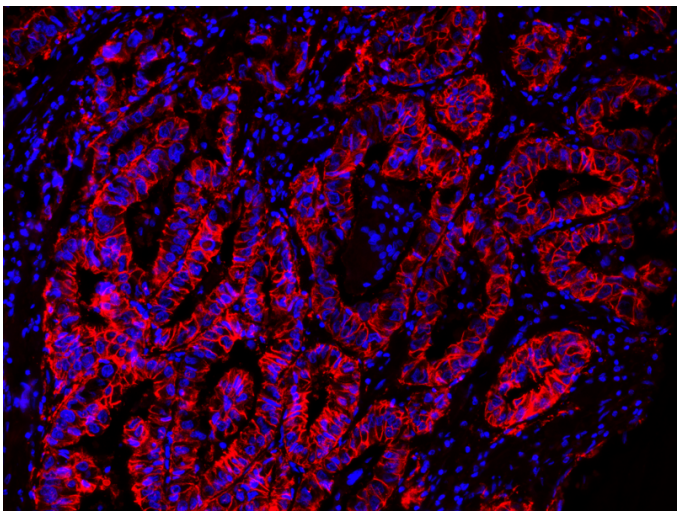

Figure 1.

Immunofluorescent image of paraffin-embedded human lung carcinoma labeled with EpCAM Rabbit mAb followed with HRP-labeled goat anti-rabbit IgG (H+L) (Cat#16793). The signal was developed with AAT's Cy3 tyramide (Cat#11065, Red). Cells were also counterstained with DAPI (Blue).

DISCLAIMER