iFluor® 546 Tyramide

Catalog number: 45103
Unit size: 200 slides

Component	Storage	Amount (Cat No. 45103)
AF546 tyramide reagent	Freeze (< -15 °C), Minimize light exposure	200 Slides

OVERVIEW

For many immunohistochemical (IHC) applications, traditional enzymatic amplification procedures are sufficient for achieving adequate antigen detection. However, several factors limit their sensitivity and utility. 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 results in the ability to detect low-abundance targets with ultrasensitive precision and reduces the amount of antibodies and hybridization probes needed. In IHC applications, this method can also enhance sensitivity in cases where the primary antibody dilution needs to be increased to reduce nonspecific background signals or overcome weak immunolabeling due to suboptimal fixation procedures or low levels of target expression. The iFluor® 546 tyramide contains the bright iFluor® 546 that can be readily detected with the standard TRITC or Cy3 filter set. iFluor® dyes have higher fluorescence intensity, increased photostability, and enhanced water solubility, resulting in fluorescence signals with significantly higher precision and sensitivity. iFluor® 546 is an excellent replacement for Alexa Fluor® 546 tyramide (Alexa Fluor® is the trademark of ThermoFisher), TRITC tyramide, or other comparable fluorescent tyramide conjugates.

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

Emission	Cy3/TRITC filter set
Excitation	Cy3/TRITC 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 100 µL of DMSO to the vial of iFluor® tyramide and mix well.

Note: Make single-use aliquots and store unused 200X stock solution at 2-8 °C, protected from light. Avoid repeat freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Tyramide working solution (1X)

Add 100 µL of the tyramide stock solution into 20 mL of a buffer of your choice containing 0.003% H_2O_2.

Note: For optimal performance, use Tris Buffer, pH=7.4.

Note: A 20 mL solution is good for 200 tests. The tyramide working solution should be used immediately and made fresh on the day of use. Avoid direct exposure to light.

Secondary antibody-HRP working solution

Make an appropriate concentration of secondary antibody-HRP working solution per the manufacturer's recommendations.

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 the preferred specific solution/protocol as needed. A 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 a non-specific signal, you can shorten the incubation time with the tyramide reagent. You should optimize the incubation period using positive and negative control samples at various incubation time points. Or you can use a lower concentration of the tyramide reagent 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.

   Note: To ensure optimal results, it is recommended to use either ReadiUse™ microscope mounting solution (Cat. 20009) or FluoroQuest™ TSA/PSA Antifade Mounting Medium *Optimized for Tyramide and Styramide Imaging* (Cat. 44890) instead of Vectashield® mounting media. There are instances where Vectashield® mounting media may not be suitable for certain TSA/PSA conjugates.

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

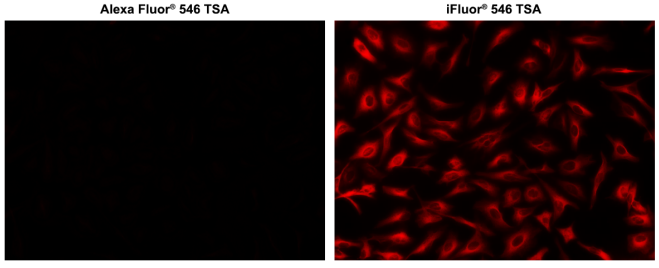

Figure 1. Microtubules of fixed HeLa cells were labeled with anti-α tubulin mouse mAb followed by HRP-labeled goat anti-mouse IgG (Cat No. 16728). The fluorescence signal was developed using Alexa Fluor® 546 tyramide or iFluor® 546 tyramide (Cat No. 45103) and detected with a TRITC/Cy3 filter set. iFluor® 546 tyramide shows significantly higher fluorescence intensity than Alexa Fluor® 546 tyramide under the same conditions.

DISCLAIMER

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