XFD546 tyramide reagent *Same Structure to Alexa Fluor™ 546 tyramide*

Catalog number: 11075
Unit size: 200 slides

Component	Storage	Amount
XFD546 tyramide reagent	Freeze (< -15 °C), Minimize light exposure	200 slides

OVERVIEW

XFD546 is manufactured by AAT Bioquest, and it has the same chemical structure of Alexa Fluor® 546 (Alexa Fluor® is the trademark of ThermoFisher). 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. XFD546 tyramide contains the bright XFD546 dye that can be readily detected with the standard TRITC 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

KEY PARAMETERS

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.

1. XFD 546 tyramide stock solution (100X)
Add 100 µL of DMSO into the vial of XFD 546 tyramide conjugate to make 100X tyramide stock solution. **Note:** Make single use aliquots, and store unused 100X stock solution at 2-8 ° C in dark place.

2. H_2O_2 stock solution
Add 10 µL of 3% hydrogen peroxide (Not provided) to 90 µL of ddH_2O. **Note:** Prepare the 100X H_2O_2 solution fresh on the day of use.

PREPARATION OF WORKING SOLUTION

1. XFD 546 tyramide working solution (1X)
Every 1 mL of Reaction Buffer requires 10 µL of tyramide stock solution and 10 µL of H_2O_2 stock solution. **Note:** The tyramide provided is enough for 100 tests based on 100 µL of tyramide working solution needed per coverslip or per well in a 96-well microplate. **Note:** The tyramide working solution must be used within 2 hours after preparation and avoid direct exposure to light. **Note:** Tris Buffer, pH=7.4 can be used. For optimal performance, use ReadiUse Tyramide (TSA)/Styramide (PSA) Optimized Reaction buffer (AAT Cat# 45090).

2. Secondary antibody-HRP working solution
Make appropriate concentration of secondary antibody-HRP working solution as 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 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

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

DISCLAIMER