

## Biotin Styramide \*Superior Replacement for Biotin Tyramide\*

Catalog number: 45300  
Unit size: 100 Slides

Component	Storage	Amount (Cat No. 45300)
Biotin Styramide *Superior Replacement for Biotin Tyramide*	Freeze (< -15 °C), Minimize light exposure	100 Slides

### OVERVIEW

Power Styramide™ Signal Amplification (PSA™) system is one of the most sensitive methods that can detect extremely low-abundance targets in cells and tissues with improved fluorescence signal 10-50 times higher than the widely used tyramide (TSA) reagents. In combination with our superior iFluor® dyes that have higher fluorescence intensity, increased photostability and enhanced water solubility, the iFluor® dye-labeled Styramide™ conjugates can generate fluorescence signal with significantly higher precision and sensitivity (more than 100 times) than standard ICC/IF/IHC. PSA utilizes the catalytic activity of horseradish peroxidase (HRP) for covalent deposition of fluorophores *in situ*. PSA radicals have much higher reactivity than tyramide radicals, making the PSA system much faster, more robust and sensitive than the traditional TSA reagents. Compared to tyramide reagents, the Styramide™ conjugates have ability to label the target at higher efficiency and thus generate significantly higher fluorescence signal. Styramide™ conjugates also allow significantly less consumption of primary antibody compared to standard directly conjugate method or tyramide amplification with the same level of sensitivity. Biotin Styramide is a superior replacement for biotin tyramide that is widely used in the well-known tyramide signal amplifications (TSA).

### 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 Styramide™ working solution and apply in cells or tissue for 5-10 minutes at room temperature

### 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

#### Styramide™ stock solution (100X)

Add 100 µL of DMSO into the vial of Biotin-labeled Styramide™ conjugate to make 100X Styramide™ stock solution.

**Note:** Make single-use aliquots and store unused 100X stock solution at -20 °C in a dark place. Avoid repeat freeze-thaw cycles.

#### Hydrogen peroxide stock solution (100X)

Add 10 µL of 3% hydrogen peroxide (not provided) to 90 µL of ddH<sub>2</sub>O.

**Note:** Prepare the 100X H<sub>2</sub>O<sub>2</sub> solution fresh on the day of use.

### PREPARATION OF WORKING SOLUTION

#### Styramide™ working solution (1X)

Every 1 mL of Reaction Buffer requires 10 µL of Styramide™ stock solution and 10 µL of H<sub>2</sub>O<sub>2</sub> stock solution.

**Note:** The Styramide™ provided is enough for 100 tests based on 100 µL of Styramide™ working solution needed per coverslip or per well in

a 96-well microplate.

**Note:** The Styramide™ working solution must be used within 2 hours after preparation and avoid direct exposure to light.

#### 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 the preferred specific solution/protocol as needed. A 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 the 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.

#### Styramide™ labeling

1. Prepare and apply 100  $\mu$ L of Styramide™ 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 Styramide™. 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 Styramide™ 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.

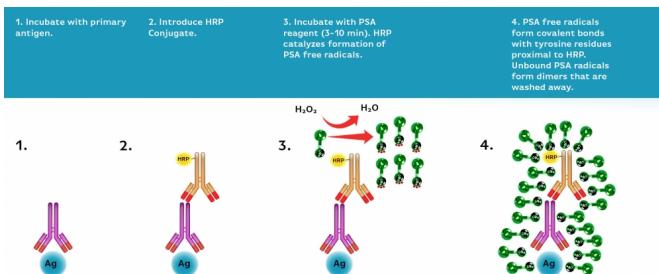
**Note:** To ensure optimal results, it is recommended to use either ReadiUse™ microscope mounting solution ([Cat. 20009](#)) or FluoroQuest™ TSA/PSA Antifade Mounting Medium \*Optimized for Tyramide and Styramide Imaging\* ([Cat. 44890](#)) instead of Vectashield® mounting media. There are instances 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 Styramide 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.** Power Styramide™ Signal Amplification (PSA™) system is one of the most sensitive methods that can detect extremely low-abundance targets in cells and tissues with improved fluorescence signal 10-50 times higher than the widely used tyramide (TSA) reagents. In combination with our superior iFluor® dyes that have higher fluorescence intensity, increased photostability and enhanced water solubility, the iFluor® dye-labeled Styramide™ conjugates can generate fluorescence signal with significantly higher precision and sensitivity (more than 100 times) than standard ICC/IF/IHC. PSA utilizes the catalytic activity of horseradish peroxidase (HRP) for covalent deposition of fluorophores *in situ*. PSA radicals have much higher reactivity than tyramide radicals, making the PSA system much faster, more robust and sensitive than the traditional TSA reagents.

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