iFluor® 570 Styramide

Catalog number: 45031
Unit size: 100 Slides

Component	Storage	Amount (Cat No. 45031)
iFluor® 570 Styramide	Freeze (< -15 °C), Minimize light exposure	100 Slides

OVERVIEW

The Power Styramide™ Signal Amplification (PSA™) system is a highly sensitive method for detecting low-abundance targets in cells and tissues, with fluorescence signals 10-50 times higher than tyramide (TSA) reagents. Paired with our iFluor® dyes, known for their fluorescence intensity, photostability, and water solubility, the iFluor® dye-labeled Styramide™ conjugates achieve precision and sensitivity surpassing standard ICC/IF/IHC methods by over 100 times. PSA™ relies on horseradish peroxidase (HRP) catalytic activity to covalently deposit fluorophores in situ, with radicals displaying higher reactivity than tyramide radicals, making the PSA™ system faster, more robust, and more sensitive than traditional TSA reagents. The Styramide™ conjugates label targets with higher efficiency, leading to significantly greater fluorescence signals while also enabling reduced primary antibody consumption compared to standard methods. iFluor® 570 Styramide is a replacement for Alexa Fluor™ 568 tyramide and similar fluorescent tyramide conjugates or TSA reagents, offering unmatched performance in sensitive imaging applications.

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

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

Styramide™ stock solution (100X)

Add 100 µL of DMSO into the vial of iFluor® dye-labeled Styramide™ conjugate to make a 100X Styramide™ stock solution.

Note: Make single-use aliquots, and store unused 100X stock solution at 2-8 °C in a dark place and avoid repeat freeze-thaw cycles.

Hydrogen peroxide stock solution (100X)

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

Styramide™ working solution (1X)

Every 1 mL of Reaction Buffer requires 10 µL of Styramide™ stock solution and 10 µL of H_2O_2 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 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 µL 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

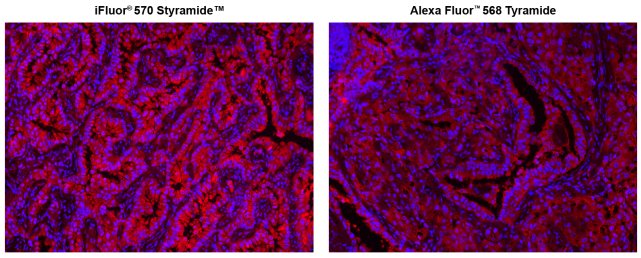

Figure 1. Fluorescence IHC was performed on formaldehyde-fixed, paraffin-embedded human lung adenocarcinoma-positive tissue using PSA™ and TSA amplified methods. First, the tissue sections were stained with rabbit anti-EpCam antibody and then incubated with polyHRP-labeled Goat anti-Rabbit IgG secondary antibody. The signal was developed using either iFluor® 570 Styramide™ (Cat No. 45031) or Alexa Fluor® 568 tyramide stain, respectively, and detected with a Cy3/TRITC filter set. Finally, the nuclei (blue) were counterstained with DAPI (Cat No. 17507).

DISCLAIMER

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