

iFluor® 647 PSA™ Imaging Kit with Goat Anti-Rabbit IgG

 Catalog number: 45240
 Unit size: 100 Tests

Component	Storage	Amount (Cat No. 45240)
Component A: iFluor® 647 Styramide™ conjugate	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component B: Styramide™ Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (10 mL)
Component C: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)
Component D: Secondary Antibody-HRP (Goat Anti-Rabbit IgG-HRP)	Refrigerated (2-8 °C), Minimize light exposure	1 vial (100 µL) (100X)
Component E: Stabilized 3% Hydrogen Peroxide (H ₂ O ₂)	Refrigerated (2-8 °C)	1 bottle (200 µL)

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. iFluor® 647 PSA kit is a much superior replacement for Alexa Fluor 647 tyramide-based kit or other spectrally similar fluorescent tyramide or TSA kits.

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	Cy5 filter set
Excitation	Cy5 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)

1. Add 100 µL of DMSO into the vial of iFluor® 647-labeled Styramide™ conjugate (Component A) 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 solution (100X)

1. Add 10 µL of 3% hydrogen peroxide (Component E) to 90 µL of ddH₂O.

Note: Prepare the 100X H₂O₂ solution fresh on the day of use.

PREPARATION OF WORKING SOLUTION
Styramide working solution (1X)

1. Every 1 mL of Reaction Buffer requires 10 µL of Styramide™ stock solution and 10 µL of H₂O₂ 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

1. Dilute the 100X secondary antibody-HRP stock solution 1:100 in PBS with 1% BSA.

Note: The secondary antibody-HRP provided in this kit is sufficient for 100 tests based on 100 µL HRP working solution per coverslip or per well in a 96-well microplate.

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.

Multiplex with primary antibodies from different species

Important: When performing multiplexing experiments with PSA™ Imaging Kits, it is essential to maintain a specific imaging sequence. To ensure clear and accurate results, it is recommended to image the targets from least to most abundant, with the nucleus counterstained last. Following this optimized imaging sequence will enhance the precision and quality of your experiment.

1. Following Step 2 in the Styramide labeling section, you can multiplex cells or tissue samples either by employing another iFluor® PSA™ Imaging Kit or by utilizing conventional IHC/ICC protocols.
2. For multiplexing, employ a primary antibody from a host different from the one utilized in Step 4 of the Peroxidase labeling section, along with a fluorescent label that is spectrally compatible with the initial fluorescent label.

Multiplex with primary antibodies from the same species in IHC

1. Dilute 10X Citrate Buffer (pH 6.0) (Cat. No. 10000) 1:10 in distilled water.
2. Following Step 2 of the Styramide labeling process, immerse the tissue in a diluted citrate buffer (pH 6.0) and heat in a microwave

oven at 100% power until boiling (1–2.5 minutes).

3. Reduce the power to 20% and microwave for an additional 15 minutes.
4. Allow the tissue sample to cool to room temperature while it remains in the citrate buffer.
5. Wash the sample twice with 1X PBS. Then, repeat the Peroxidase labeling and Styramide labeling steps using a primary antibody of the same species, if desired.
6. Use an iFluor® PSA™ Imaging Kit that is spectrally compatible with the iFluor® 350 PSA™ Imaging Kit used in the first round.

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 instructions 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 ReadUse™ 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. Recommended nucleus counterstains.

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

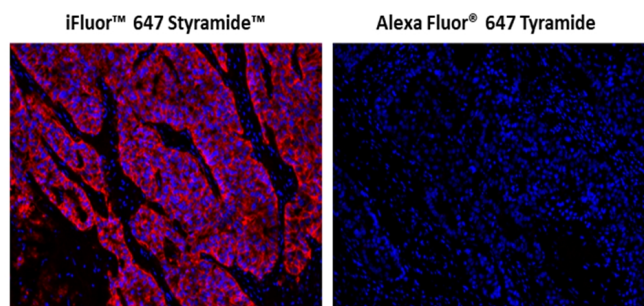


Figure 1. Fluorescence IHC in formaldehyde-fixed paraffin-embedded tissue. Human lung adenocarcinoma sections were incubated with Rabbit mAb EpCAM and then stained with an HRP-labeled Goat anti-Rabbit IgG secondary antibody followed by iFluor® 647 Styramide™ (Left) or Alexa Fluor® 647 tyramide (Right), respectively. Fluorescence images were taken using the Cy5 filter set and under the same

exposure time. The cell nuclei were stained with Nuclear Blue™ DCS1 (Cat#17510)

DISCLAIMER

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