mFluor™ Green 620 Styramide

Catalog number: 45075
Unit size: 100 Slides

Component	Storage	Amount (Cat No. 45075)
mFluor™ Green 620 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-abundant targets in fluorescent immunocytochemistry (ICC), immunohistochemistry (IHC), and in situ hybridization (ISH). By utilizing bright and photostable mFluor™ dyes, Styramide™ conjugates deliver results of unparalleled sharpness and precision, surpassing the sensitivity of standard ICC/IHC/ISH methods by over 100 times, all the while reducing the consumption of primary antibodies. Like tyramide signal amplification (TSA), PSA™ leverages the catalytic activity of horseradish peroxidase (HRP) to generate high-density labeling of a target protein or nucleic acid sequence in situ. The enhanced reactivity of Styramide™ radicals over tyramide ensures faster, more robust labeling of the target, leading to fluorescence signals that are 10-50 times greater than those generated by tyramide (TSA) reagents. The mFluor™ Green 620 Styramide uses the bright red fluorescent dye mFluor™ Green 620 (Ex/Em = 525/623 nm) to label targets in situ. mFluor™ Green 620 is well-excited by the 532 nm laser line and exhibits minimal crosstalk in complex multicolor analysis with blue and green fluorescent probes or other spectrally compatible Styramide conjugates and PSA™ Imaging 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	623 nm
Excitation	525 nm
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 mFluor™ dye-labeled Styramide™ conjugate to make 100X Styramide™ stock solution.

   Note: Make single-use aliquots and store any unused 100X stock solution at < -15 °C, protect from light. Avoid repeated freeze-thaw cycles.

Hydrogen peroxide stock solution (100X)

1. 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)

1. 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

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

SAMPLE EXPERIMENTAL PROTOCOL

This protocol is applicable for both cell and tissue 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

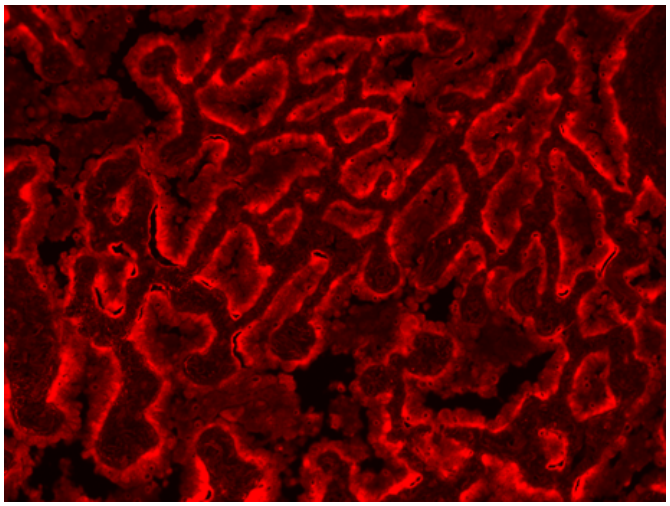

Figure 1. Formalin-fixed, paraffin-embedded (FFPE) human lung tissue was labeled with anti-EpCAM mouse mAb followed by HRP-labeled goat anti-mouse IgG (Cat No. 16728). The fluorescence signal was developed using mFluor™ Green 620 styramide and detected with a Cy3 filter set.

DISCLAIMER

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