ReadiCleave™ iFluor® 647 Styramide

Catalog number: 45520
Unit size: 100 Slides

Component	Storage	Amount (Cat No. 45520)
ReadiCleave™ iFluor® 647 Styramide	Freeze (< -15 °C), Minimize light exposure	100 Slides

OVERVIEW

Power Styramide™ Signal Amplification (PSA) system is one of the most sensitive fluorescence imaging methods that can detect extremely low-abundance targets in cells and tissues with improved fluorescence signal 10-50 times higher than the commonly used tyramide (TSA) reagents. ReadiCleave™ Styramides are the new iteration of our PSA products that add the reversible capability to chemically remove the PSA staining on tissue or in cells if needed. As our other PSA reagents, the ReadiCleave™ Styramides are an excellent collection of multicolor reagents to simultaneously detect multiple targets in the same tissue samples. They provide an additional benefit, i.e., the PSA staining can be removed (if desired) while preserving the integrity of tissue samples. A specific protein is first recognized by its selective primary antibody. Subsequently, the target is stained by the HRP-secondary antibody conjugate of its immunoglobulin class. The bright deep red, fluorescent ReadiCleave ™ iFluor® 647 Styramide is added subsequently. The HRP-antibody conjugate catalyzes the coupling reaction between ReadiCleave™ iFluor® 647 Styramide and the target protein in proximity. The deep red fluorescence imaging can be easily recorded with the Cy5 filter set. The deep red iFluor® 647 Styramide staining can be gently removed with nearly 100% efficiency using ReadiCleave™ AML Cleavage Buffer when needed.

AT A GLANCE

Protocol Summary

1. Fix/permeabilize/block cells or tissue.
2. Add the primary antibody in blocking buffer.
3. Add the HRP-conjugated secondary antibody.
4. Prepare the Styramide™ working solution and apply it to cells or tissue. Incubate at room temperature for 5-10 minutes.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC filter set
Excitation	FITC 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

ReadiCleave™ iFluor® 647 Styramide Stock Solution (100X)

1. To prepare a 100X stock solution of ReadiCleave™ iFluor® 647 Styramide, add 100 µL of DMSO to the vial containing the conjugate.

   Note: Prepare single-use aliquots of the 100X stock solution and store any unused portions at 2-8°C, protected from light. Avoid freeze-thaw cycles.

Hydrogen Peroxide Stock Solution (100X)

1. Add 10 µL of 3% hydrogen peroxide (not provided) to 90 µL of ddH2O.

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

PREPARATION OF WORKING SOLUTION

ReadiCleave™ iFluor® 647 Styramide Working Solution (1X)

1. For every 1 mL of Reaction Buffer, add 10 µL of ReadiCleave™ iFluor® 647 Styramide stock solution and 10 µL of H2O2 stock solution.

   Note: The provided ReadiCleave™ iFluor® Styramide is sufficient for 100 tests, with each test requiring 100 µL of ReadiCleave™ iFluor® Styramide working solution per coverslip or per well in a 96-well microplate.

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

Secondary Antibody-HRP Working Solution

1. Prepare the secondary antibody-HRP working solution according to the manufacturer's instructions.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol is applicable for both cells and tissues staining.

Cell Fixation and Permeabilization

1. Fix the cells or tissue using 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 following standard IHC protocols. Then, perform antigen retrieval using the preferred specific solution and protocol. Detailed instructions for the 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 the cell or tissue sample in a peroxidase quenching solution (e.g., 3% hydrogen peroxide) for 10 minutes. Rinse the sample twice with PBS at room temperature.

2. **Optional:** If using HRP-conjugated streptavidin, it is recommended to block endogenous biotin with a biotin blocking buffer.

3. Block the sample using your preferred blocking solution, such as PBS with 1% BSA, for 30 minutes at 4°C.

4. Remove the blocking solution. Add the primary antibody, diluted in the recommended antibody diluent, and incubate 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 at room temperature for 60 minutes.

   Note: Incubation time and concentration can be varied depending on the signal intensity.

7. Wash with PBS three times for 5 minutes each.

ReadiCleave™ iFluor® 647 Styramide Labeling

1. Prepare 100 µL of ReadiCleave™ iFluor® 647 Styramide working solution and apply it to each sample. Allow the samples to incubate at room temperature for 5-10 minutes.

   Note: If you observe a non-specific signal, you can shorten the incubation time with Styramide. It is important to optimize the incubation period using positive and negative control samples at various time points. Additionally, you can use a lower concentration of Styramide in the working solution.

2. Rinse with PBS three times.

Removal of ReadiCleave™ iFluor® 647 Styramide Staining

1. Prepare a 1X working solution, add 200 µL of ReadiCleave™ AML Cleavage Buffer (Cat. 7510, not provided) into 800 µL of ddH2O, and mix thoroughly.

   Note: For optimal results, use this solution within a few hours of preparation.

2. Add 100 µL of ReadiCleave™ AML Cleavage Buffer working solution to the tissue or cell samples.

   Note: Add a sufficient amount of ReadiCleave™ AML Cleavage Buffer working solution to ensure that the samples are fully submerged.

3. Heat the samples at 60°C for 60 minutes.

4. Remove the ReadiCleave™ AML Cleavage Buffer working solution and briefly rinse the samples with PBST.

5. Reprocess the tissue samples beginning with the Antigen Retrieval step in your IHC staining protocol.

Counterstain and Fluorescence Imaging

1. For optimal results, counterstain the cell or tissue samples as needed. AAT offers a range of nucleus counterstain reagents, which are detailed in Table 1. Please follow the instructions provided with each reagent.

2. Mount the coverslip using an anti-fade mounting medium to prevent fading.

   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. Vectashield® mounting media may not be compatible with some TSA/PSA conjugates.

3. Use the appropriate filter set to visualize the signal from the counterstain.

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