

## iFluor 555™ PSA™ Imaging Kit with Goat Anti-Mouse IgG

 Catalog number: 45270  
 Unit size: 100 Tests

Component	Storage	Amount
Component A: iFluor™ 555 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-Mouse IgG-HRP)	Refrigerated (2-8 °C), Minimize light exposure	1 vial (100 µL) (100X)
Component E: Stabilized 3% Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> )	Refrigerated (2-8 °C)	1 bottle (11 mL)

### 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™ 555 PSA kit is a much superior replacement for Alexa Fluor 555 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

Excitation	Cy3/TRITC filter set
Emission	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy3/TRITC filter set

### 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.

#### 1. Styramide™ stock solution (100X)

Add 100 µL of DMSO into the vial of iFluor™ 555-labeled Styramide™ conjugate (Component A) to make 100X Styramide™ stock solution.

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

#### 2. H<sub>2</sub>O<sub>2</sub> solution (100X)

Add 1 mL of 3% hydrogen peroxide (Component E) to 9 mL 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

#### 1. 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.

#### 2. Secondary antibody-HRP working solution

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 preferred specific solution/protocol as needed.

Protocol can be found at <https://www.aatbio.com/resources/guides/paraffin-embedded-tissueimmunohistochemistry-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.

- Optional: If using HRP-conjugated streptavidin, it is advisable to block endogenous biotins by biotin blocking buffer.
- Block with preferred blocking solution (such as PBS with 1% BSA) for 30 minutes at 4 °C.
- Remove blocking solution and add primary antibody diluted in recommended antibody diluent for 60 minutes at room temperature or overnight at 4 °C.
- Wash with PBS three times for 5 minutes each.
- 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.

- Wash with PBS three times for 5 minutes each.

### Styramide labeling

- 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 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 lower concentration of Styramide in the working solution.

- Rinse with PBS three times.

### Counterstain and fluorescence imaging

- 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.
- Mount the coverslip using a mounting medium with anti-fading properties.
- Use the appropriate filter set to visualize the signal from the Styramide labeling. **Table 1.** Products recommended for nucleus counterstain

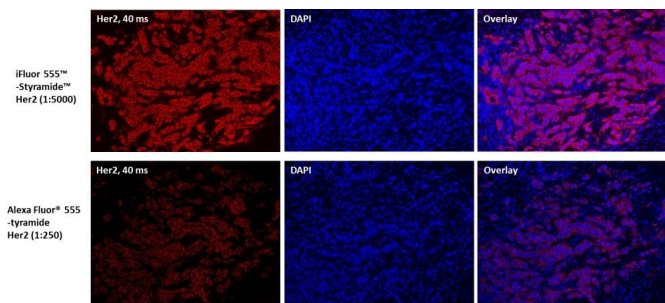
Cat#	Product Name	Ex/Em (nm)
17548	Nuclear Blue™ DCS1	350/461
17550	Nuclear Green™ DCS1	503/526
17551	Nuclear Orange™ DCS1	528/576
17552	Nuclear Red™ DCS1	642/660

HRP-labeled Goat anti-mouse IgG secondary antibody followed by iFluor™ 555 Styramide™ (Top) or Alexa Fluor® 555 tyramide (Bottom), respectively. Compared to tyramide amplification, the increase in sensitivity of Styramide™ super signal amplification allows use of less primary antibody. The same level of sensitivity can be achieved with a significant reduction in the amount of target antibody used (10-50 fold). Cell nucleus was stained with Nuclear Blue™ DCS1 (Cat#17548).

### DISCLAIMER

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### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Fluorescence IHC for HER2/ErbB2 detection in formaldehyde-fixed paraffin-embedded breast cancer tissue. Human Her2/Neu (c-erbB-2) positive tissue sections were incubated with mouse mAb HER2/ErbB2 diluted 1:5000 (Top) or 1:250 (Bottom), respectively. The tissue were then incubated with