iFluor™ 430 Tyramide *Superior Replacement for Opal 480*

Catalog number: 45096
Unit size: 200 Slides

Component	Storage	Amount
iFluor™ 430 Tyramide *Superior Replacement for Opal 480*	Freeze (< -15 °C), Minimize light exposure	200 Slides

OVERVIEW

iFluor™ 430 tyramide is optimized to a superior replacement for Opal 480 or other spectrally similar fluorescent tyramide conjugates or TSA reagents. Tyramide reagetns can be used to detect extremely low-abundance targets in cells and tissues with significantly improved fluorescence signal than the direct fluorescence labeling reagents. In combination with our superior iFluor™ dyes that have higher florescence intensity, increased photostability and enhanced water solubility, the iFluor™ dye-labeled tyramide conjugates can generate fluorescence signal with significantly higher precision and sensitivity.

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 tyramide working solution and apply in cells or tissue for 5-10 minutes at room temperature

KEY PARAMETERS

Fluorescence microscope

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

iFluor™ 430 Tyramide stock solution (200X)
Add 100 µL DMSO to vial and mix well.

Note Unused tyramide stock solution can be stored at 2-8 °C.

PREPARATION OF WORKING SOLUTION

iFluor™ 430 Tyramide working solution (1X)
Add 100 µL of Tyramide stock solution into 20 mL of buffer of your choice containing 0.003% H_2O_2.

Note Tris Buffer, pH=7.4 can be used for optimal performance.

Note Tyramide working solution should be used immediately and made fresh on the day of use.

Note 20 mL solution is good for 200 tests.

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-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.

Tyramide labeling

1. Prepare and apply 100 µL of tyramide 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 tyramide. You should optimize the incubation period using positive and negative control samples at various incubation time points. Or you can use lower concentration of tyramide 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.

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

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.