Manual

Tyramide Dye kits

KROMNIGON
Multiplex IHC made easy

Applications

The 3-color and 5-color Tyramide Dye kits are optimized for tyramide signal amplification (TSA) detection with the StreptaClick-HRP Multiplex IHC kits. The kits can also be used for other TSA protocols.

Kit contents

Components	3-color kit (250 stainings, 100 µl/staining)	5-color kit (250 stainings, 100 µl/staining)
1xTyramide Amplification Buffer Plus*	25 ml	25 ml
30% Hydrogen peroxide*	100 µl	100 µl
Tyramide Dyes	488, 555, 647	CF430, 488, 555, 594, 647

***** Tyramide Amplification Buffer Plus kit from Biotium (https://biotium.com/product/tyramide-amplification-buffer-plus/)

Storage and Handling

Tyramide Dyes
Upon arrival, store the tyramide dyes at -20°C, protected from light. Before use, dissolve each vial in 100 µl DMSO + 100 µl PBS. Aliquot in vials and store at ≤ -20°C. The aliquots can be stored in the freezer for at least 12 months. Once thawed, store at +4°C and use within a week.

Tyramide Amplification Buffer Plus
Store 1xTyramide Amplification Buffer Plus and 30% Hydrogen peroxide at +4°C. Protect from light. Warm 1X Tyramide Amplification Buffer to room temperature and mix well by vortexing or shaking to make sure all solids are completely dissolved before each use. The buffer can be warmed in a 37°C water bath for convenience.

Protocol for development of tyramide signal amplification (one reaction a 100 ul)

1. Prepare a working amplification buffer with hydrogen peroxide at a final concentration of 0.0015% by performing a serial dilution of hydrogen peroxide as described below.

a) Add 2 uL of 30% hydrogen peroxide to 400 uL of water and mix well to make a 0.15% hydrogen peroxide solution.

b) Add 1 uL of the 0.15% hydrogen peroxide solution to each 100 uL of 1X Tyramide Plus Amplification Buffer, for a final concentration of 0.0015% hydrogen peroxide.

2. Prepare working staining solution by mixing 2 ul tyramide dye (from stock aliquot) with 100 ul working amplification buffer prepared in step 1b. The working staining solution can be stored at room temperature, protected from light, for up to 24 hours.

3. Apply 100 uL of working staining solution to each sample. Incubate at room temperature, protected from light, for 12 minutes.

4. Wash samples twice with distilled water. The samples are now ready for fluorescence imaging or for another round of multistaining using the StreptaClick-HRP Multiplex IHC kit.

Fluorochrome absorption and emission

Name	Absorption max	Emission max
CF430 tyramide	428 nm	498 nm
488 tyramide	494 nm	517 nm
555 tyramide	555 nm	572 nm
594 tyramide	590 nm	617 nm
647 tyramide	648 nm	671 nm