User Manual

Tyramide dye kit

Size

6-color kit (500 stainings, 100 µl/staining)
4-color kit (250 stainings, 100 µl/staining)

Storage and Handling

Store Tyramide Amplification Buffer Plus at +4°C. Protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended. 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.

Store the tyramide dyes at -20°C, protected from light. Product is stable for at least 12 months from date of receipt. Before use, dissolve each dye with 200 µl DMSO. Aliquot in vials and store at ≤ -20°C. The aliquots can be stored in the freezer for at least 12 months. Avoid freeze-thaw cycles. However, a vial can tolerate one freeze-thaw cycle.

Product Description

Fluorescence tyramide dyes are used for tyramide signal amplification (TSA) to generate strong fluorescence signals in immunohistochemistry (IHC), immunocytochemistry (ICC) and *in situ* hybridization (FISH). TSA is based on the ability of horseradish peroxidase (HRP), in the presence of low concentrations of hydrogen peroxide, to convert tyramide-containing dyes into an oxidized, highly reactive free radical that can covalently bind to tyrosine residues at or near the HRP. This leads to significant amplification of the signal at the target.

Multiple TSA procedures can be performed sequentially to label different targets in the same sample, by performing HRP quenching or antibody stripping after each tyramide reaction. Since the tyramide dye is covalently attached to the sample it will remain.

Protocol for signal development

1. Prepare 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 1 uL of 30% hydrogen peroxide to 200 uL of 1X Tyramide Plus Buffer or water and mix well to make a 0.15% hydrogen peroxide solution.

b) Add 1 uL of the 0.15% hydrogen peroxide solution to 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 1. 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 10 minutes.

4. Wash samples 2 times with water or PBS.

The samples are now ready for fluorescence imaging or for another round of multistaining.

Fluorochrome spectra

Name	Absorption max	Emission max
AZDye™ 405	402 nm	424 nm
CF®430	426 nm	498 nm
AZDye™ 488	494 nm	517 nm
AZDye™ 555	555 nm	572 nm
AZDye™ 594	590 nm	617 nm
AZDye™ 647	649 nm	671 nm
Cyanine 3	555 nm	572 nm
Cyanine 5	648 nm	671 nm

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