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Product Data Sheet

Tyramide - AcalephFluor680 Reagent (200X)

Catalog #	Source	Reactivity	Applications
CRG1080		N/A	mIHC

Description	AcalephFluor680 labled Tyramide for Multiplex IHC staining or enhanced fluorescent IHC staining
Form	Liquid in PBS
Directions for Use	Add 10 µl of Tyramide reagent into 2 ml of PBS buffer containing 0.003% H2O2. 2 ml solution is good for 20 assays. Tyramide working solution should be used immediately and made fresh on the day of use.
Platform	Ex/Em = 680/701 nm
Application	For multiplex immunohistochemical (mIHC) applications, the traditional enzymatic amplification procedures are sufficient for achieving adequate antigen detection. However, several factors limit the sensitivity and utility of these procedures. Tyramide signal amplification (TSA) has proven to be a particularly versatile and powerful enzyme amplification technique with improved assay sensitivity. TSA is based on the ability of HRP, in the presence of low concentrations of hydrogen peroxide, to convert labeled tyramine-containing substrate into an oxidized, highly reactive free radical that can covalently bind to tyrosine residues at or near the HRP. To achieve maximal IHC detection, tyramine is prelabeled with a fluorophore. The signal amplification conferred by the turnover of multiple tyramide substrates per peroxidase label translates ultrasensitive detection of low-abundance targets and the use of smaller amounts of antibodies and hybridization probes. In immunohistochemical applications, sensitivity enhancements derived from TSA method allow primary antibody dilutions to be increased to reduce nonspecific background signals, and can overcome weak immunolabeling caused by suboptimal fixation procedures or low levels of target expression.
Storage/Stability	Store at 4 °C in dark for 1 year, do not freeze.

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SAMPLE EXPERIMENTAL PROTOCOL

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.

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.
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.