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Multiplex Fluorescent IHC Staining Kit

Cat. No.: IHCT003
Detection method: Fluorescent
Section type: FFPE
Size: 100T
Storage and Stability: Store at -20℃ stable for 12 months.

General Information

Component	Size	Storage
AbFluor® 520-tyramide	5 ml (Ready to use)	-20℃
AbFluor® 570-tyramide	5 ml (Ready to use)	-20℃
AbFluor® 620-tyramide	5 ml (Ready to use)	-20℃
AbFluor® 690-tyramide	5 ml (Ready to use)	-20℃
TSA+ enhancer	10 µL x 4	-20℃

Principle

Tyramide signal amplification (TSA) technology is an enzyme-based detection method that employs the catalytic activity of horseradish peroxidase (HRP) to help label a target protein *in situ*. The underlying principle of this approach is that in the presence of hydrogen peroxide, HRP converts a fluorescence-labeled tyramide substrate into a reactive form that covalently binds to tyrosine residues on proteins at or near the HRP. As a result, TSA enables the concentration of fluorescently labeled tyramide at or near the protein of interest, thereby significantly boosting the fluorescence signal and enhancing detection sensitivity.

The covalent bonds formed through this process enable multiple target proteins to be sequentially stained using TSA. Each staining round involves the application of specific antibodies to a protein of interest followed by thoroughly washing to remove the antibodies. However, the covalently bonded tyramide label remains intact. This process allows simultaneous detection of all biomarkers of interest upon the completion of the assay. Importantly, each staining cycle engages only one type of primary antibodies, efficiently eliminating concerns of cross-reaction between different antibodies. Moreover, the removal of antibodies before each staining cycle enables the use of primary antibodies from the same species in the same samples.

The fluorescence labeled tyramide provided in this kit can be used individually or in various combinations, making it suitable for single, double, or multiple labeling.

Applications

1. Sample preparation

Immerse FFPE sections in fresh xylene and incubate for 15 min. Repeat the incubation two more times using separate containers. Immerse slides in anhydrous ethanol and incubate for 5 min and then repeat once. Hydrate sections by sequentially placing slides in 95%, 85%, and 75% ethanol for 5 min each. Wash slides 3 times with distilled water.

2. Antigen Retrieval

Place the tissue slices in a container filled with appropriate antigen retrieval buffer and boil slides at 95°C-100°C for 25-40 min in a water bath. Transfer slides to room temperature (RT) for 20 min then wash slides 3 times with PBS (pH 7.4) for 5 min each. The sections should remain moisturized during the process. Other antigen retrieval methods, such as pressure cooker and microwave, could be used as well dependent on the tissue type, fixation method and primary antibodies.

3. Block Endogenous Peroxidase

Drain the liquid off the slides and add several drops of 3% hydrogen peroxide solution to cover and block tissue sections for 20 min at RT. Wash slides 3 times in PBS (pH 7.4) bath for 5 min each.

4. Nonspecific Target Blocking

Drain the liquid off the slides and then use a PAP pen to draw circles on the slides around tissue sections. Add goat serum (or other blocking buffer, such as BSA) directly on slides, covering the whole tissue and block slides for 30 min at RT.

5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with primary antibodies overnight at 4°C or 1-2 hours at RT. Wash slides 3 times in PBS (pH 7.4) bath for 5 min each.

6. Secondary Antibody Incubation

Drain the liquid off the slides and then incubate slides with secondary antibody for 50 minutes at RT. Wash slides 3 times in PBS (pH 7.4) bath for 5 min each.

7. Fluorescent Labeling of Tyrosine

Add 50 µL of ready-to-use AbFluor® fluorescent dye-labeled tyramide (AbFluor® fluorophore tyramide) to the slides and incubate for 3-15 min at RT (an optimal time frame is 5-10 min). Wash slides 3 times in PBS (pH 7.4) bath for 5 min each. To obtain your optimized staining, a revised staining procedure is recommended as following. Add 50 µL of ready-to-use AbFluor® fluorophore tyramide to the slides and incubate for 1 min. Following the incubation, wash off the staining solution and check the staining signal under a fluorescent microscope. Depending on the intensity of the staining signal, continue the staining process by adding additional 50 µL of the same AbFluor® fluorophore tyramide to the slides until the appropriate intensity is achieved. Wash slides 3 times in PBS (pH 7.4) bath for 5 min each, then proceed to the **step 8 (multiplex staining) or 12 (single staining)**.

8. Antibody Removal (optional)

Transfer the slices into the antigen retrieval buffer and **repeat step 2** if additional targets of interest need to be determined.

9. Multiplex (Double) Staining

Repeat **steps 3-7** to proceed with the second round of labeling using another AbFluor® fluorophore tyramide from the kit, then proceed to the **step 10 (triple staining) or 12 (double staining)**.

10. Multiplex (Triple) Staining

Repeat **step 8** and then **steps 3-7** to conduct the third round of labeling using a different AbFluor® fluorophore tyramide from the kit, then proceed to the **step 11 (quadruple staining) or 12 (triple staining)**.

11. Multiplex (Quadruple) Staining

Repeat **step 8** and then **steps 3-7** to proceed with the fourth round of labeling using the last AbFluor® fluorophore tyramide from the kit, then proceed to the **step 12**.

12. Counterstaining

Wash slides 3 times in PBS (pH 7.4) bath for 5 min each. Drain the liquid off the slides and then incubate slides with DAPI for 5-20 min at RT in a dark setting.

13. Slides Mounting

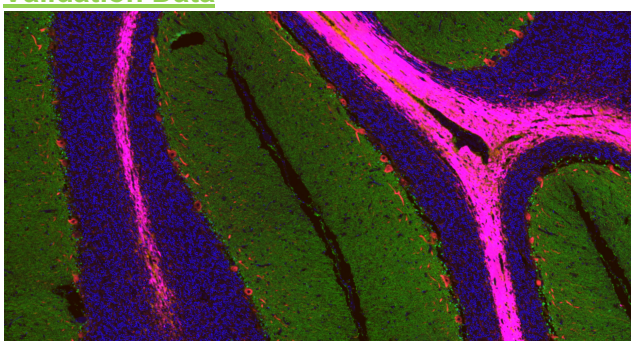
Wash slides 3 times in PBS (pH 7.4) bath for 5 min each. Drain the liquid off the slides and mount the sections with anti-fluorescence quenching mounting media and coverslip.

14. Visualization

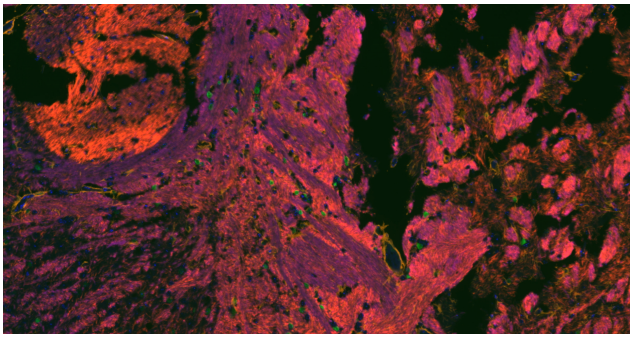
Staining results can be imaged on multiple systems, including fluorescent slide scanners, epi-fluorescent and confocal microscopes.

Dye	Excitation wavelength (nm)	Emission wavelength (nm)
DAPI	350	420
AbFluor® 520	490	520
AbFluor® 570	550	570
AbFluor® 620	590	620
AbFluor® 690	630	690

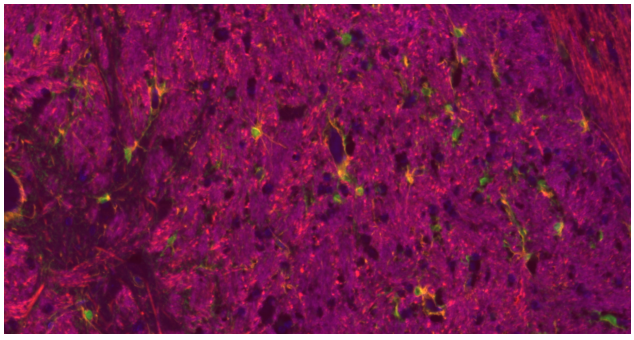
Validation Data



Immunohistochemical analysis of paraffin embedded human brain tissue slide using IHCT003 (Five Color mIHC Fluorescence Kit).



Immunohistochemical analysis of paraffin embedded mouse brain tissue slide using IHCT003 (Five Color mIHC Fluorescence Kit).



Immunohistochemical analysis of paraffin embedded rat brain tissue slide using IHCT003 (Five Color mIHC Fluorescence Kit).

Notes

1. TSA+ enhancer can boost the fluorescence signal by 5-10 times. Whether to add it can be determined by the specific fluorescence intensity. The recommended ratio of TSA+ enhancer to AbFluor® fluorophore tyramide is 1: 500.
2. AbFluor® fluorophore tyramide (Ready-to-use) can be stored at -20℃ for one year and at 4℃ for about two months.
3. AbFluor® fluorophores exhibit high quenching resistance, negating the need to avoid light from fluorescent lamps or work in dark environments. However, direct sunlight exposure must be prevented.
4. Prioritize the use of monoclonal antibodies, particularly those that have been validated by IHC.
5. It is suggested to use EDTA antigen retrieval buffer with pH 9.0 at 95℃ for 15-25 min for the initial round of antigen retrieval. For the second round or any subsequent rounds, opt for using the citric acid antigen retrieval buffer with pH 6.0 at 95℃ for 25-40 min.

Please cite this product as "IHCT003, Bioss Antibodies". For example: 'Co-staining of A and B was performed using the Multiplex Fluorescent IHC Staining Kit (IHCT003, Bioss Antibodies), which relies on the Tyramide Signal Amplification (TSA) technology, following the manufacturer's instructions.'