Four-color Fluorescence kit

Cat#:4990411/4990412/4990413

[Product Name]

Four-color Fluorescence kit

[Model & Specification]

Cat No.	Specification	Component Name	Component specification	Quantity
4990411	20 Tests	Post Primary Reagent	6 mL/bottle	1 bottle
		Polymer Reagent	6 mL/bottle	1 bottle
		TSA 488 Reagent	20 μL/bottle	1 bottle
		TSA 546 Reagent	20 μL/bottle	1 bottle
		TSA 647 Reagent	20 μL/bottle	1 bottle
		DAPI Reagent	2 mL/bottle	1 bottle
		Optimized Reaction Buffer	6 mL/bottle	1 bottle
		Antifade Mounting Medium	1 mL/bottle	1 bottle
4990412	50 Tests	Post Primary Reagent	15 mL/bottle	1 bottle
		Polymer Reagent	15 mL/bottle	1 bottle
		TSA 488 Reagent	50 μL/bottle	1 bottle
		TSA 546 Reagent	50 μL/bottle	1 bottle
		TSA 647 Reagent	50 μL/bottle	1 bottle
		DAPI Reagent	5 mL/bottle	1 bottle
		Optimized Reaction Buffer	15 mL/bottle	1 bottle
		Antifade Mounting Medium	2 mL/bottle	1 bottle
4990413	100 Tests	Post Primary Reagent	30 mL/bottle	1 bottle
		Polymer Reagent	30 mL/bottle	1 bottle
		TSA 488 Reagent	100 μL/bottle	1 bottle
		TSA 546 Reagent	100 μL/bottle	1 bottle
		TSA 647 Reagent	100 μL/bottle	1 bottle
		DAPI Reagent	10 mL/bottle	1 bottle
		Optimized Reaction Buffer	30 mL/bottle	1 bottle
		Antifade Mounting Medium	3 mL/bottle	1 bottle

[Intended use]
Immunofluorescence staining in paraffin tissue sections.

[Test principle]
Primary antibody forms antigen-antibody complex with target antigen in the section, the reagent after primary antibody in this kit closes the non-specific protein and improves the binding efficiency, the ultrasensitive enzyme-labeled goat anti-mouse/rabbit IgG polymer binds to the primary antibody in the antigen-antibody complex, the fluorescent diluent diluted TSA fluorescent reagent can be catalyzed by the ultrasensitive enzyme-labeled goat anti-mouse/rabbit IgG polymer of the HRP to produce an activated fluorescent substrate, the activated substrate can covalently bind to residues such as tyrosine on the antigen to make stable covalent binding TSA fluorescent reagent on the samples. The substrate can covalently bind to tyrosine and other residues on the antigen, resulting in stable covalent binding of TSA fluorescein on the sample. Afterwards, the non-covalently bound primary antibody-secondary antibody-HRP complex is washed away by thermal repair, and the next primary antibody-secondary antibody-HRP is repeated for the second round of incubation, replaced by another TSA fluorescent reagent, and so on to achieve multiple labeling, fluorescent DAPI reagent re-staining to locate the nucleus, and anti-fluorescent quenching sealing reagent is used to seal the film.

[Main Ingredients]

Product Composition	Description
Post Primary Reagent	IgG Protein
Polymer Reagent	Goat anti-mouse/rabbit IgG-HRP polymer
TSA 488 Reagent	TSA 488 and Dimethyl Sulfoxide (DMSO)
TSA 546 Reagent	TSA 546 and Dimethyl Sulfoxide (DMSO)
TSA 647 Reagent	TSA 647 and Dimethyl Sulfoxide (DMSO)
DAPI Reagent	4,6-Diamidino-2-phenylindole (DAPI)
Optimized Reaction Buffer	Hydrogen peroxide and phosphate buffer (Na_2HPO_4, KH_2PO_4)
Antifade Mounting Medium	Glycerol and phosphate buffer (Na_2HPO_4, KH_2PO_4)

[Storage Conditions and Period of Validity]
Store at 2℃~8℃, validity period is 12 months.

[Sample Type]
Paraffin tissue sections;
The following reagents should be provided: xylene, anhydrous ethanol, 95% ethanol, 85% ethanol, antigen repair solution, 3% H_2O_2 solution, sealing solution, primary antibody, PBST solution.

[Methods]
1. Baking: place the prepared tissue sections in a constant temperature drying blower oven at 60℃~65℃ and bake the sections for 1 h~1.5 h. The sections should be baked at 60℃~65℃ for 1 h~2.5 h.
2. Dewaxing: the sections were routinely dewaxed to water, xylene I for 10 min, xylene II for 10 min, anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, 95% ethanol for 5 min, 85% ethanol for 5 min, and rinsed with tap water for 3 times.
3. Antigen repair: the repair solution can be used as immunohistochemistry antigen repair buffer, the

repair solution should be heated to boiling in microwave oven, and then put into the tissue sections, and then continue to be heated in medium heat for 15 min, and then turn off the heat. After the repair solution cooled down naturally, remove the sections and rinse with tap water for 1 min.
4. Blocking: the tissue sections were immersed in 3% H_2O_2 for 12 min, then removed and rinsed with tap water for 1 min, and placed in tap water for spare.
5. Closure: remove the section and shake off excess water, dry the water around the tissue with filter paper, draw a circle around the tissue with an immunohistochemical pen, add a drop of sealing solution (commonly used 5% sheep serum) to the tissue, and then close it in the oven at 37℃ for 30 min.
6. Add primary antibody: shake off the sealing solution on the section, add the corresponding primary antibody directly to cover the tissue, cover the wet box lid, incubate at 37℃ for 1 h or 4℃ overnight.
7. Add secondary antibody: take out the wet box 25 ℃ recovery 20 min, PBST immersion wash 3 times, shake off the excess PBST, drop plus primary antibody reagent sealing non-specific proteins, 37 ℃ oven incubation for 15 min. take out the wet box 25 ℃ recovery 20 min, PBST immersion wash 3 times, shake off the excess PBST, drop plus secondary antibody to cover the tissues, 37 ℃ oven incubation for 30 min.
8. Color development: soak the sections in PBST for 3 times, mix the fluorescent reagent with the fluorescent diluent according to the ratio of 1:100, and cover the tissues with TSA fluorescent reagent reaction solution for 1 min~15 min at room temperature (the best time is 5 min~10 min), and terminate the reaction by washing with PBST for three times.
9. Antibody elution: Heat the antigen repair solution in microwave oven to boiling and then put it into the tissue sections, continue heating in medium heat for 10 min~25 min and then turn off the heat (adjust the time flexibly according to the affinity of different antibodies). When the repair solution cools down naturally, take out the slices and rinse them with PBST for three times.
10. Repeat steps 4~9 (change to another fluorescent reagent) - the second round of labeling.
11. Repeat steps 4~9 (using another fluorescent reagent) --- the third round of labeling.
12. Re-staining: rinse the sections with PBST for 2 min after the color development is completed, add DAPI fluorescent reagent dropwise in the circle after the sections are slightly shaken dry, incubate for 2 min~20 min at room temperature away from light, and rinse with PBST for 2 min.
13. After the slices were shaken dry slightly, add appropriate amount of anti-fluorescence quenching sealer (generally 30 μL, can be increased or decreased according to the size of the samples) to seal the slices, and observe the slices.

[Interpretation of results]
Interpretation of staining results should be left to the judgment of a professional, in conjunction with appropriate controls.

[Limitations of the experimental method]
The final staining results are affected by the quality of sample collection and sections, the suitability of the primary antibody, the proficiency of the operator and other factors, and the interpretation of the final results should be carried out by a professional.

[Product Performance Indicators]
Positive and blank control tests meet the requirements; there is no significant difference in the intensity and localization of staining of tissue slices from the same tissue source.

[Precautions]
1. Pay attention to wearing a lab coat and gloves during operation.
2. For professional users only, to ensure the accuracy of the results must be interpreted by a professional.
3. DAPI is a potential carcinogen, please use appropriate protective measures to avoid contact

between the reagent and skin and eyes, and handle with care.
4. Please read the instructions carefully before the experiment and use it within the validity period.
5. Waste generated during use should be handled in accordance with the “Regulations on the Management of Medical Waste”.
6. If you need a copy of the safety data (MSDS), please contact the supplier.

[Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
REF	Catalog Number	LOT	Product Batch Code
	Date of Manufacture		Expiration Date
	Manufacturer		Storage at 2℃~8℃
	Consult instructions for use		Store away from light

[Instruction Revision Date]
2024.03.15

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