SUNLONG

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# Six Color mIHC Fluorescence Kit

**Catalog Number: SL-IHC007**

Size: 20T / 100T

Multiplexing: Five labels / six colors

Sample type: paraffin sections, frozen sections, cell smears and cell climbing slices

Storage: 4℃; stable for 12 months

## KIT COMPONENTS

| No. | Component | 20T | 100T | Storage |
|---|---|---|---|---|
| 1 | TSA Fluorescence Dye 1 (TSA-480 Plus) | 10 μL (200×) | 50 μL (200×) | -20℃, protect from light |
| 2 | TSA Fluorescence Dye 2 (TSA-520 Plus) | 10 μL (200×) | 50 μL (200×) | -20℃, protect from light |
| 3 | TSA Fluorescence Dye 3 (TSA-570 Plus) | 10 μL (200×) | 50 μL (200×) | -20℃, protect from light |
| 4 | TSA Fluorescence Dye 4 (TSA-620 Plus) | 10 μL (200×) | 50 μL (200×) | -20℃, protect from light |
| 5 | TSA Fluorescence Dye 5 (TSA-690 Plus) | 10 μL (200×) | 50 μL (200×) | -20℃, protect from light |
| 6 | Nuclear Dye (DAPI, ready to use) | 2 mL | 10 mL | 2-8℃, protect from light |
| 7 | TSA Buffer | 3.2 mL | 16 mL | 2-8℃ |
| 8 | HRP Goat Anti-Rabbit/Mouse Universal Secondary Antibody | 3.2 mL | 16 mL | 2-8℃ |
| 9 | Antibody Diluent | 4 mL | 20 mL | 2-8℃ |
| 10 | 3% H2O2 | 4 mL | 20 mL | 2-8℃ |
| 11 | Anti-Fade Mounting Medium (dry-set) | 2 mL | 10 mL | 2-8℃ |
| 12 | Datasheet | 1 copy | 1 copy | - |

## PRINCIPLE

Tyramide signal amplification (TSA) is an enzymatic detection method that labels target proteins in situ using horseradish peroxidase (HRP). In the presence of HRP and H2O2, the tyramide-fluorophore substrate is activated and covalently binds to tyrosine residues near the antigen-antibody binding site, depositing large amounts of fluorophore and thereby amplifying the signal. Non-covalently bound antibodies from the previous round are removed by heat retrieval or antibody elution buffer, while the covalently bound fluorophore remains, allowing the next round of staining. Because only a single antibody is applied in each round, cross-reactivity and host-species matching are not a concern, and the dyes can be used alone or in combination for single, double, triple or higher multiplex fluorescence labelling.

## PROTOCOL

### 1. Sample preparation

Paraffin sections: xylene I 15 min - xylene II 15 min - absolute ethanol I 5 min - absolute ethanol II 5 min - 95% ethanol 5 min - 85% ethanol 5 min - 75% ethanol 5 min, then rinse with distilled water. Frozen sections and cell smears: fix for 10-30 min, wash with PBS 3 × 5 min, permeabilize with 0.3% Triton X-100 for 20 min, wash with PBS 3 × 5 min.

### 2. Antigen retrieval

Place tissue sections in EDTA-based antigen retrieval buffer (pH 9.0) or citrate buffer (pH 6.0) and heat in a microwave (medium heat 8 min, stop 8 min, medium-low heat 7 min); alternative methods such as pressure cooking 1-2 min, boiling at 100℃ for 15 min or water bath at 95℃ for 20 min can be used. Do not let the sections dry. Cool naturally, then wash in PBS (pH 7.4) 3 × 5 min. Frozen sections and cell samples may skip this step.

### 3. Block endogenous peroxidase

Immerse the slides in 3% $H_2O_2$ and incubate for 15 min at room temperature in the dark; wash in PBS (pH 7.4) 3 × 5 min.

### 4. Non-specific blocking

Draw a circle around the tissue with a hydrophobic IHC pen and apply Antibody Diluent (or 3% BSA / goat serum) to cover the tissue; block for 30 min at room temperature.

### 5. Primary antibody incubation

Remove the blocking solution and apply the diluted primary antibody; incubate in a humid chamber overnight at 4℃ or for 1-2 hours at 37℃.

### 6. Secondary antibody incubation

Wash in PBS 3 × 5 min, then apply the HRP goat anti-rabbit/mouse universal secondary antibody to cover the tissue; incubate for 50 min at room temperature in the dark; wash with PBS 3 × 5 min.

### 7. TSA fluorescence staining

Prepare the TSA working solution by diluting the concentrated TSA dye in TSA Buffer (optimal range 1:50-1:400; 1:200 is suitable in most cases). Apply the working solution to cover the tissue and incubate for 1-15 min at room temperature (optimal 5-10 min); wash with PBS 3 × 5 min. Signal too strong: reduce dye concentration, reaction time or primary antibody concentration; signal too weak: increase them.

### 8. Antibody elution

Paraffin sections: place slides in antigen retrieval buffer and heat in a 95℃ water bath for 25-40 min, or apply pre-warmed (37℃) mIHC Antibody Elution Buffer (C2208, recommended for frozen sections, cell smears and fragile tissue) for 5-20 min at 37℃, repeat once, then wash with PBS 3 × 5 min. Frozen sections and cell samples must use the elution buffer.

### 9. Repeat staining

Repeat steps 5-8 with the next primary antibody and the next TSA dye until all targets have been labelled. Only one antibody is applied in each round, so cross-reactivity and host-species matching are not a concern.

### 10. Counterstain, mount and image

After the final wash, apply DAPI nuclear dye for 5 min, wash with PBS 3 × 5 min, and mount with the anti-fade mounting medium; scan the slide on a fluorescence microscope or slide scanner.

## NOTES

1. Store the kit at 4℃; it is stable for 12 months.
2. The kit is suitable for paraffin sections, frozen sections, cell smears and cell climbing slices. For frozen sections and cell samples, mIHC Antibody Elution Buffer (C2208) is required and must be ordered separately.
3. Do not allow the sections to dry at any time during the procedure.
4. TSA dyes are light sensitive; keep them protected from light and avoid repeated freeze-thaw cycles.
5. This product is for research use only and is not intended for diagnostic procedures.

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**