TSA Multiplex Fluorecence Staining Kit (mIHC, 4 Plex)

Cat. No: E-IR-R333 **Size: 50T / 100T**

Cat	product	50T	100T	Storage
E-IR-R333A	TSA-520 Fluorophore (200×)	25 μL	50 μL	Store at 2-8°C, protected from light
E-IR-R333B	TSA-570 Fluorophore (200×)	25 μL	50 μL	Store at 2-8°C, protected from light
E-IR-R333C	TSA-690 Fluorophore (200×)	25 μL	50 μL	Store at 2-8℃, protected from light
E-IR-R333D	TSA Reaction Buffer	9 mL	18 mL	Store at 2-8°C, protected from light
E-IR-R333E	Polymerized HRP-Conjugated Goat Anti-Mouse/Rabbit Secondary Antibody	8 mL	15 mL	Store at 2-8°C, protected from light
E-IR-R333F	Antifade Mounting Medium with DAPI	5 mL	10 mL	Store at 2-8°C, protected from light
E-IR-R333G	Antibody Diluent	15 mL	30 mL	Store at 2-8°C, protected from light
E-IR-R333H	Endogenous Peroxidase Blocking Solution	15 mL	30 mL	Store at 2-8°C, protected from light
E-IR-R333I	mIHC Antibody Elution Buffer	20 mL	40 mL	Store at 2-8°C, protected from light

Product Introduction

Tyramide signal amplification (TSA) is a highly sensitive detection technology used for protein visualization in tissue samples. The ElabPlex™ TSA Multiplex IHC Kit is designed for sensitive and reproducible detection of multiple protein targets within a single sample using TSA-based multiplex immunofluorescence (mIHC).
The system supports sequential staining with spectrally distinct fluorophores, enabling comprehensive spatial analysis of biomarkers. The kit includes polymer HRP-conjugated secondary antibodies and a panel of tyramide fluorophores (TSA 480, 520, 570, 620, 690, and 780), allowing flexible assay design. This approach supports the use of primary antibodies from the same host species and minimizes cross-reactivity during multiplex staining.

Assay Principle

Tyramide signal amplification (TSA) is based on HRP-catalyzed activation of tyramide substrates in the presence of hydrogen peroxide (H_2O_2). The activated intermediates generate highly reactive radicals that covalently bind to nearby protein residues, primarily tyrosine, resulting in localized and stable signal deposition at antigen sites.
This enzymatic reaction produces significant signal amplification, typically **10–100-fold higher** than conventional immunofluorescence methods.
In multiplex applications, the covalent nature of fluorophore deposition allows signals to remain stable during subsequent antibody removal steps, enabling sequential detection of multiple targets within the same sample.

Toll-free: 1-888-852-8623
Web: www.elabscience.com
Tel: 1-832-243-6086
Email: techsupport@elabscience.com
Fax: 1-832-243-6017

Instructions for Use

Staining Procedure

1. **Sample Preparation:**
   Paraffin sections: deparaffinization and rehydration.
   Frozen sections or cell smears: fix for 10–30 min, wash, and permeabilize with 0.3% Triton X-100 for 20 min (optional for membrane proteins). Wash and rehydrate.
2. **Antigen Retrieval:**
   Perform heat-induced antigen retrieval using pH 9.0 EDTA buffer (E-IR-R104) or pH 6.0 citrate buffer (E-IR-R105). This step may be omitted for frozen sections or cell slides. For fragile tissues (e.g., bone), use enzymatic or mild retrieval methods.
3. **Endogenous Peroxidase Blocking:**
   Incubate with peroxidase blocking solution at room temperature for 15 min in the dark. Wash with PBS/TBS (pH 7.4), 3 × 3 min.
4. **Blocking:**
   Incubate with goat serum (E-IR-R110) or antibody diluent for 30 min at room temperature.
5. **Primary Antibody Incubation:**
   Apply diluted primary antibody and incubate at 4°C overnight or 37°C for 1–2 h.
6. **Polymer-HRP Incubation:**
   Wash slides and incubate with Polymer-HRP for 30 min at room temperature. Wash 3 × 3 min.
7. **TSA Fluorescent Staining:**
   Dilute TSA dye (1:50–1:400; recommended 1:200). Incubate for 5–10 min at room temperature. Wash 3 × 3 min.
8. **Antibody Elution:**
   Perform heat-mediated elution (100°C, 20–40 min) or use elution buffer (37°C, 2 × 10 min). Wash 3 × 3 min.
9. **Multiplex Labeling:**
   Repeat Steps 3–8 for the **2nd and 3rd targets**.
   For the **4th target**, repeat Steps 3–7 (omit elution).
10. **Counterstaining and Mounting:**
    Apply DAPI-containing mounting medium and coverslip. Incubate 5 min.
11. **Imaging:**
    Acquire images using fluorescence or multispectral imaging systems.

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Fluorescence imaging systems or digital slide scanners must be equipped with the following filter channels.

	Excitation Wavelength	Emission Wavelength
DAPI	350	420
TSA-480 Fluorophore (200×)	440	480
TSA-520 Fluorophore (200×)	488	519
TSA-570 Fluorophore (200×)	555	570
TSA-620 Fluorophore (200×)	594	615
TSA-690 Fluorophore (200×)	682	702
TSA-780 Fluorophore (200×)	750	780

Instrument Procedure:
Configure the instrument program according to the staining protocol. Load each kit component into the designated reagent reservoirs, then place the samples in the instrument and initiate the run.

Storage Conditions

1. Store at 2–8°C, protected from light. Do not freeze. The kit is stable for 12 months under recommended storage conditions. Reagents remain valid for up to 6 months after opening; avoid prolonged exposure to strong light.
2. The production date and expiration date are indicated on the outer packaging label.
3. The kit may be transported at room temperature for up to one week in a foam container with ice packs and properly sealed.

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