Immuno Fluorescence Staining Kit (Anti-Mouse IgG-Cyanine3 & Anti-Rabbit IgG-FITC)

Cat. No: E-IR-R320 **Size: 50 Assays / 100 Assays / 200 Assays**

Cat.	Product	50 Assays	100 Assays	200 Assays	Storage
E-AB-1011	Goat Anti-Mouse IgG(H+L)(Cyanine3 conjugated)	120 μL	200 μL	200 μL×2	-20°C
E-AB-1014	Goat Anti-Rabbit IgG(H+L)(FITC conjugated)	120 μL	200 μL	200 μL×2	-20°C
E-IR-R110	Normal Goat Blocking Buffer (Ready-to-Use)	5 mL	10 mL	20 mL	2~8°C
E-IR-R103	DAPI Reagent (1 μg/mL)	5 mL	10 mL	20 mL	2~8°C
E-IR-R119	Anti-Fluorescence Quenching Agent	5 mL	10 mL	20 mL	2~8°C
Manual			**One copy**		

Introduction

Immuno Fluorescence Staining Kit are developed for immunofluorescence detection of cell or tissue sections. When there is an appropriate antibody to detect a specific target protein, fluorescence can be detected by Immuno Fluorescence staining kit.

Immuno Fluorescence Staining Kit (Anti-Mouse IgG-Cyanine3 & Anti-Rabbit IgG-FITC) contain a Cyanine3-labeled goat anti-mouse IgG (H+L) antibody and an FITC-labeled goat anti-rabbit IgG (H+L) antibody. This enables the simultaneous detection of mouse-derived primary antibodies (which show a vivid red color) and rabbit-derived primary antibodies (which show a bright green color).

The kit contains anti-fluorescence quenching sealing solution, which can make the fluorescence more lasting.

Experimental Procedure

1. Preparation of Immunofluorescence Staining

A. Preparation of Fixation Solution

It is recommended to use 4% paraformaldehyde as the fixation solution (E-IR-R113), or use ethanol, methanol or other types of fixative according to specific primary antibody or sample.

B. Preparation of permeate working solution

Use Triton X-100 as the permeable solution (E-IR-R122) and dilute with 1× PBS buffer to 0.5% Triton X-100 working solution.

C. Preparation of PBST Working Buffer

It is recommended to use PBST as washing Buffer. Use Elabscience® 10×PBST (E-BC-R335) and dilute to 1 ×PBST Working Buffer with deionized water at ratio of 1:9.

D. Preparation of Antibody Dilution Solution

It is recommended to use Elabscience® Antibody Dilution Buffer (E-IR-R106) or PBS as primary antibody dilution Buffer.

E. Dilute Primary Antibody

Dilute both primary antibodies according to the dilution ratio recommended for immunofluorescence staining in the primary antibody instruction manual to prepare an antibody mixture.

F. Dilute the Secondary Antibody

It is recommended to use Elabscience® antibody diluent (E-IR-R106) to dilute the two immunofluorescence secondary antibodies (Anti-Mouse IgG-Cyanine3 and Anti-Rabbit IgG-FITC) from this kit at a ratio of 1:100 into a mixed working solution. The dilution ratio can be increased or decreased appropriately according to the intensity of fluorescence.

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2. **Immuno Fluorescence Staining for Adherent Cells**

A. Immerse a clean cover glass in 70% ethanol for 5 min or a longer time, dry it on a sterile super clean table or wash it with cell culture-grade PBS or 0.9% NaCl solution for 3 times, then wash it with cell culture solution. Place the cover glass into the six-hole plate, seed the cells on the glass overnight to make it about 50% ~80% full.

B. Treat the cells according to the specific experimental purpose, then discard the culture solution, add 1 mL fixation solution, and room temperature incubate for 15~30 min or longer.

C. Discard the fixation solution, wash the glass with PBST working Buffer for 3~5 min, 3 times. Discard the liquid.

D. Permeate the cells at room temperature for 15 minutes with 0.5%Triton X- 100 (prepared with 1×PBS) (This step can be omitted for antigens that are expressed on cell membranes), and then wash the cover glass with 1×PBS for 3 minutes and repeat 3 times.)

E. Blocking. Add 100 µL Normal Goat Blocking Buffer (Ready-to-Use) (E-IR-R110) to each glass and incubate for 30 min.

F. Discard the blocking Buffer, add 100 µL diluted primary antibody mixture and incubate at 37°C in wet box for 60 min (Or incubate at 4°C overnight).

G. Discard the primary antibody, wash the glass with PBST Working Buffer for 3~5 min, 3~5 times.

H. Discard the liquid. Add 100 µL diluted secondary antibody mixture and incubate at 37°C in wet box for 60 min.

I. Wash the glass with PBST Working Buffer for 3~5 min, 3~5 times, avoiding light during the washing.

J. Nuclear staining. Add DAPI Reagent (1 µg/mL) (E-IR-R103) and incubate in wet box for 5 min, wash the glass with PBST Working Buffer for 5 min, wash for 4 times to remove the redundant DAPI Reagent.

K. Add one drop of Anti-Fluorescence Quenching Agent (E-IR-R119) on a slice glass, cover the glass with cells to avoid air bubbles. Make the cell contact with the Anti-Fluorescence Quenching Agent, do not reverse it.

L. Observe the result by fluorescence microscope.

3. **Immuno Fluorescence Staining for Suspension Cells**

A. Collect the cells by centrifugation into a 1.5 mL centrifuge tube. Scatter the cells gently after discarding the supernatant.

B. Add 0.5 mL fixation solution to re-suspend the cells gently, and room temperature incubate for 15~30 min or a longer time.

C. Centrifuge the cells and discard the fixation solution, wash the cells with PBST Working Buffer for 3~5 min, 3 times.

D. Permeate the cells at room temperature for 15 minutes with 0.5%Triton X- 100 (prepared with 1×PBS) (This step can be omitted for antigens that are expressed on cell membranes), and then wash the cover glass with 1×PBS for 3 minutes and repeat 3 times.)

E. Discard the PBST working Buffer and left about 50 µL liquid for the last time, re-suspend the cells, add the cells to a clean slide glass, make sure that the cells are uniform distributed.

F. Slightly dry the cells to make the cells are attached to the slide and not easy to flow with the liquid. If the conditions permit, the cells can be attached to the slide by centrifugation using a suitable centrifuge.

G. Blocking. Add 100 µL Normal Goat Blocking Buffer (Ready-to-Use) (E-IR-R110) to each glass and incubate for 30 min.

H. Discard the blocking Buffer, add 100 μL diluted primary antibody mixture and incubate at 37°C in wet box for 60 min (Or incubate at 4°C overnight).

I. Discard the primary antibody, wash the glass with PBST working Buffer for 3~5 min, 3~5 times.

J. Discard the liquid. Add 100 μL diluted secondary antibody mixture and incubate at 37°C in wet box for 60 min.

K. Wash the glass with PBST Working Buffer for 3~5 min, 3~5 times, avoiding light during the washing.

L. Nuclear staining. Add DAPI Reagent (E-IR-R103) and incubate in wet box for 5 min, wash the glass with PBST Working Buffer for 5 min, wash for 4 times to remove the redundant DAPI Reagent.

M. Add one drop of Anti-Fluorescence Quenching Agent (E-IR-R119) to a slice glass, cover the glass with cells to avoid air bubbles. Make the cell contact with the Anti-Fluorescence Quenching Agent, do not reverse it.

N. Observe the result by fluorescence microscope.

4. **Immuno Fluorescence Staining for Tissue Slice**

① For paraffin section, dewaxing, hydration and antigen repair should be completed first.

② For frozen sections, follow the steps below:

a: Room temperature incubate with fixation solution for 15~30 min longer time.

b: Discard the fixation solution, wash the slice with PBST Working Buffer for 3~5 min, 3 times. Discard the liquid.

c: Use 0.5% Triton X-100 to permeate cells at room temperature for 15 min (this step can be omitted for antigens expressed on cell membranes), and soak slides with 1×PBST for 3 min each time, 3 times.

A. Blocking. Add 100 μL Normal Goat Blocking Buffer (Ready-to-Use)(E-IR-R110) to each glass and incubate for 30 min.

B. Discard the blocking Buffer, add 100 μL diluted primary antibody mixture and incubate at 37°C in wet box for 60 min (Or incubate at 4°C overnight).

C. Discard the primary antibody, wash the glass with PBST Working Buffer for 3~5 min, 3~5 times.

D. Discard the liquid. Add 100 μL diluted secondary antibody mixture and incubate at 37°C in wet box for 60 min.

E. Wash the glass with PBST Working Buffer for 3~5 min, 3~5 times, avoiding light during the washing.

F. Nuclear staining. Add DAPI Reagent (E-IR-R103) and incubate in wet box for 5 min, wash the glass with PBST Working Buffer for 5 min, wash for 4 times to remove the redundant DAPI Reagent.

G. Add one drop of Anti-Fluorescence Quenching Agent (E-IR-R119) on a slide glass, cover the glass with cells to avoid air bubbles. Make the cell contact with the Anti-Fluorescence Quenching Agent, do not reverse it.

H. Observe the result by fluorescence microscope.

Storage

Store at 2~8/-20°C, Refer to the label. Valid for 12 months. Secondary antibody (E-AB-1011 and E-AB-1014) and DAPI Reagent (1 μg/mL) (E-IR-R103) should be stored away from light.

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Notice

1. A fluorescence microscope or laser confocal microscope is required.

2. The anti - fluorescence quenching mounting solution can mitigate fluorescence quenching. However, light exposure should still be minimized, and the observation time under a fluorescence microscope should be specifically shortened.

3. Fluorescent substances are prone to quenching. Fluorescence microscopy observation is recommended as soon as possible after staining. If immediate observation is not possible, the samples should be stored at 4°C protected from light. However, the storage duration should generally not exceed one week, as prolonged storage may affect the observation quality.

4. The efficacy of immunofluorescence staining is closely related to the primary antibody. It is recommended to select a primary antibody with extensive literature coverage and clearly labeled as suitable for immunofluorescence staining for this experiment.

5. If the fluorescence is too weak during observation, the concentration of the primary antibody may be appropriately increased. If the fluorescence remains insufficient, the concentration of the fluorescently labeled antibody can be further increased.

6. The relevant reagents for immunofluorescence staining must be prepared independently, and cover slips and slides must be provided by the user.

7. This product is intended solely for scientific research by qualified professionals and shall not be used for clinical diagnosis, treatment, food or pharmaceutical applications, nor stored in ordinary residential premises.

8. To ensure your safety and health, please wear laboratory clothing and disposable gloves during operations.

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