Immuno Fluorescence Staining Kit (Anti-Mouse IgG-Elab Fluor® 488)

Cat. No: E-IR-R326 **Size: 50 Assays/ 100 Assays / 200 Assays**

Cat.	Product	50 Assays	100 Assays	200 Assays	Storage
E-AB-1056	Goat Anti-Mouse IgG(H+L)(Elab Fluor® 488 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 Fluorence Staining Kits are developed for immunofluorescence detection of cell or tissue sections. When there is an appropriate antibody to detect specific target protein, fluorescence can be detected by immunefluorescence staining kit.

Immuno Fluorescence Staining Kit (Anti-Mouse IgG- Elab Fluor® 488) contains Goat Anti-Mouse IgG (H+L) (Elab Fluor® 488 conjugated), this secondary antibody can detect primary antibody from mouse with green fluorescence.

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 1x 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 the primary antibody according to the manual of primary antibody.

F. Dilute the Secondary Antibody

It is recommended to use Elabscience ® Antibody Dilution Buffer (E-IR-R106) or PBS as secondary antibody dilution Buffer. Dilute the secondary antibody with antibody dilution Buffer at the dilution of 1:50. 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 into 70% ethanol for 5 min or a longer time, dry it in the sterile super clean table or washit with cell culture grade PBS or 0.9% NaCl solution for 3 times, then washit with cell culture solution. Place the cover glass into 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 ora longer time.

   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 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 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, avoid 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 tore-suspend the cells gently, and room temperature incubate for 15~30 min ora longer time (or overnight at 4°C).

   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 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 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, avoid 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) 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.

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 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 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, avoid 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-1056) and DAPI Reagent (1 µg/mL) (E-IR-R103) should be stored at -20°C and avoid light.

Notice

1. This result should be observed by fluorescence microscope or laser confocal microscope.
2. In the case of the use of anti-fluorescence quenching sealing solution, quenching can be slowed down, but it is still advisable to avoid light as much as possible, especially the need to minimize the observation time under the fluorescence microscope.
3. All fluorescent substances are easily quenched, so it is advisable to observe them under fluorescence microscope as soon as possible after staining. If it can not be observed in time, it can be stored away from

light at 4°C, but the storage time should not exceed one week, and the observation effect may become worse and worse with the extension of the storage time.

4. The effect of immunofluorescence staining is closely related to the primary antibody. It is recommended to select the primary antibody that has been reported in many literatures and indicated that it can be used for immunofluorescence staining for this experiment.

5. If the fluorescence is found to be too weak during observation, the concentration of primary antibody can be appropriately increased; If the fluorescence is still weak, the concentration of fluorescently labeled antibodies can be appropriately increased.

6. It is necessary to prepare relevant reagents for immunofluorescence staining by oneself, and cover glass and slide should be prepared by oneself

7. This product is only used for scientific research by professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary homes.

8. For your safety and health, please wear the lab coat and disposable gloves before the experiments.

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