Universal IF Toolkit (Anti-Mouse Dylight 488)

Cat #: KTD108-EN Size: 100 T

	Universal IF Toolkit (Anti-Mouse Dylight 488)
REF	**Cat #:** KTD108-EN	LOT	**Lot #:** Refer to product label
	Applications: Immunofluorescence detection of tissue and cell samples
	Storage: Store at -20℃ for 12 months, protected from light

Assay Principle

Immunofluorescence (IF) is based on the principle of antigen-antibody reaction. First, a known antigen or antibody is labeled with fluorescein to make a fluorescent antibody, and then the fluorescent antibody (or antigen) is used as a probe to detect tissue or cell's corresponding antigen (or antibody). Based on the tedious and time-consuming operation of traditional IF experiments, a wide variety of reagents and supplies, and poor stability and reliability of experimental results, Abbkine has developed a Universal IF Toolkit, carefully designed and researched, which includes: ①The operation is convenient, the components are complete, and there is no need to worry about complex reagents, and the experiment can be started immediately after opening the box; ② Provide optimized antibody diluent and SuperKine™ Enhanced Antifade Mounting Medium, which has strong anti-quenching effect and maintains excellent fluorescence intensity; ③Provide Dylight series secondary antibodies with stronger fluorescence intensity and higher specificity; ④DyLight 488, Goat Anti-Mouse IgG can be used to detect the corresponding primary antibody of rabbit origin, and the observed color is bright green.

Materials Supplied and Storage Conditions

Kit components	Size (100 T)	Storage conditions
Immunostaining Permeabilization Buffer	100 mL	4℃
EDTA Antigen Retrieval Solution pH 8.0 (20×)	100 mL	4℃
PBS (20×)	100 mL	4℃
Goat Serum Blocking Buffer	20 mL	-20℃
Antibody Wash Buffer (20×)	25 mL	4℃
Antibody Dilution Buffer	50 mL	4℃
DyLight 488, Goat Anti-Mouse IgG	100 µL	-20℃, protected from light
DAPI (500×)	100 µL	-20℃, protected from light
SuperKine™ Enhanced Antifade Mounting Medium	10 mL	-20℃, protected from light

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Materials Required but Not Supplied

- Fixation solution (4% Paraformaldehyde)
- Primary Antibody
- Fluorescence Microscope
- Pap Pen
- Deionized water
- Glass slide
- Humidified box

Reagent Preparation

Immunostaining Permeabilization Buffer: Ready to use as supplied. Store at 4℃.

EDTA Antigen Retrieval Solution pH8.0: Prepare before use, dilute EDTA Antigen Retrieval Solution pH 8.0 (20×) 20 times with deionized water to obtain EDTA Antigen Retrieval Solution pH8.0. Store at 4℃.

PBS: Prepare before use, dilute PBS (20×) 20 times with deionized water to obtain PBS. Store at 4℃.

Goat Serum Blocking Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Antibody Wash Buffer: Prepare before use, dilute Antibody Wash Buffer (20×) 20 times with deionized water to obtain Antibody Wash Buffer. Store at 4℃.

Antibody Dilution Buffer: Ready to use as supplied. Used to dilute Primary Antibodies and Secondary Antibodies. Store at 4℃.

DyLight 488, Goat Anti-Mouse IgG: Green fluorescent dye DyLight 488 labeled Goat Anti-Mouse IgG Secondary Antibody. The recommended dilution ratio is 1:200.

1×DAPI Staining Solution: Prepare before use, dilute DAPI (500×) 500 times with PBS to obtain 1×DAPI Staining Solution.

SuperKine™ Enhanced Antifade Mounting Medium: Ready to use as supplied. Store at -20℃, protected from light.

Assay Procedure

A. For Paraffin-Embedded Tissue

1．Deparaffinize to water: The slices were placed in Xylene Ⅰ for 15 min, Xylene Ⅱ for 15 min, Anhydrous Ethanol Ⅰ for 5 min, Anhydrous Ethanol Ⅱ for 5 min, 85% Ethanol for 5 min, 75% Ethanol for 5 min, and then washed with Deionized water.

2．Antigen Retrieval: The slices were placed in an Antigen Retrieval box filled with EDTA Antigen Retrieval Solution pH 8.0 (1×) and then the Antigen was repaired in microwave oven. Medium Power for 8 min, turn off for 8 min, Medium-Low Power for 7 min, during this process, the buffer should be prevented from excessive evaporation, slices cannot be dried. After natural cooling, the slices were placed in PBS and washed on the shaker for 3 times for 3 min each.

Note: Retrieval solution and retrieval conditions are determined according to the tissue, the optimum heating time depends on the sample and target protein.

3．Circle: After the sections were shaken dry, circle the tissue with a Pap Pen (To prevent the outflow of antibodies).

4．Blocking: Add a drop of Goat Serum Blocking Buffer into the circle and incubated at room temperature for 30 min.

5．Primary Antibody Incubation: Remove Goat Serum Blocking Buffer, add a drop of Primary Antibody diluted with Antibody Dilution Buffer into the circle, and incubate in a humidified box for 1 h at room temperature or overnight at 4℃ (Add a little water in the humidified box to prevent evaporation of antibodies).

Note: For fluorescence co-location detection, A primary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another primary antibody.

6．Fluorescence Secondary Antibody Incubation: The slices were placed in Antibody Wash Buffer and washed on the shaker for 2 times for 5 min each, then place the slices in PBS and washed on the shaker for 5 min. After the sections were shaken dry, add a drop of Secondary Antibody diluted with Antibody Dilution Buffer into the circle, and incubate at room temperature for 1 h, protected from light.

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Note: After Fluorescence Secondary Antibody Incubation, all steps should be protected from light as much as possible. For fluorescence co-location detection, A secondary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another secondary antibody.

7．Redyeing with DAPI: The slices were placed in Antibody Wash Buffer and washed on the shaker for 2 times for 5 min each, then place the slices in PBS and washed on the shaker for 5 min. Add a drop of 1×DAPI Staining Solution into the circle, and incubate at room temperature for 5-10 min, remove 1×DAPI Staining Solution, wash with PBS for 3 times for 5 min each.

8．Mounting: Drain the liquid on the slice with blotting paper, add a drop of SuperKine™ Enhanced Antifade Mounting Medium on the tissue slice, coat it with a cover glass, let the slice touch the mounting fluid, and try to avoid air bubbles, then observe the samples under the fluorescence microscope.

B. For Blood Smear, Frozen Section and Cell Coverslips

1．Fixation: Slides were treated with poly-L-Lysine, anticoagulant blood was centrifuged and stratified for smear; Frozen Section are blow-dried by fan at room temperature; Grow cells on coverslips. The cell coverslips in the 24-well plate are taken as an example, remove medium, add 1 mL Fixation solution, Fixation at room temperature for 15-30 min, then remove Fixation solution, wash with PBS for 3 times for 3 min each.

Note: 4% Paraformaldehyde is recommended as Fixation solution, Ethanol, Methanol, and other Fixation solution may be used depending on the specific Primary Antibodies or Samples.

2．Permeabilizing: Add 1 mL Immunostaining Permeabilization Buffer incubate at room temperature for 20 min, then remove Immunostaining Permeabilization Buffer, wash with PBS for 3 times for 3 min each.

Note: Frozen sections can also be digested with 20 μg/mL protease K PBS for 15 min.

3．Blocking: Remove PBS, add 200 μL Goat Serum Blocking Buffer and incubated at room temperature for 30 min.

4．Primary Antibody Incubation: Remove Goat Serum Blocking Buffer, add 200 μL Primary Antibody diluted with Antibody Dilution Buffer, and incubate in a humidified box for 1 h at room temperature or overnight at 4℃ (Add a little water in the humidified box to prevent evaporation of antibodies).

Note: For fluorescence co-location detection, A primary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another primary antibody.

5．Fluorescence Secondary Antibody Incubation: Remove primary antibody, add 1 mL Antibody Wash Buffer and washed on the shaker for 2 times for 5 min each, then add 1 mL PBS and washed on the shaker for 5 min. Add 200 μL Secondary Antibody diluted with Antibody Dilution Buffer and incubate at room temperature for 1 h, protected from light.

Note: After Fluorescence Secondary Antibody Incubation, all steps should be protected from light as much as possible. For fluorescence co-location detection, A secondary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another secondary antibody.

6．Redyeing with DAPI: Remove secondary antibody, add 1 mL Antibody Wash Buffer and washed on the shaker for 2 times for 5 min each, then add 1 mL PBS and washed on the shaker for 5 min. Add 500 μL 1×DAPI Staining Solution and incubate at room temperature for 5-10 min, protected from light, then remove 1×DAPI Staining Solution, wash with PBS for 3 times for 5 min each.

7．Mounting: Add a drop of SuperKine™ Enhanced Antifade Mounting Medium on the glass slide, coat the cover glass with the cells, and let the cells contact the mounting fluid, and try to avoid air bubbles, then observe the samples under the fluorescence microscope.

C. For Non-Adherent Cells

1．Collection of cells: Cells were collected by centrifugation at 300 g for 5 min. Remove supernatant, then gently flick away the cells.

2．Fixation: Add 0.5 mL Fixation solution and gently suspend the cells. Fixation at room temperature for 15-30 min. Then centrifugate at 300 g for 5 min, remove Fixation solution, wash with PBS for 3 times for 3 min each.

3．Permeabilizing: Add 0.5 mL Immunostaining Permeabilization Buffer and gently suspend the cells, and incubate at room temperature for 20 min. Then centrifugate at 300 g for 5 min, remove Immunostaining Permeabilization Buffer, wash with PBS for 3 times for 3 min each.

4．Blocking: After centrifugation, remove PBS, add 100 μL Goat Serum Blocking Buffer and gently suspend the cells, incubate at

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room temperature for 30 min.

5．Primary Antibody Incubation: Centrifugate at 300 g for 5 min, remove Goat Serum Blocking Buffer, add 100 μL Primary Antibody diluted with Antibody Dilution Buffer to suspend the cells, and incubate for 1h at room temperature or overnight at 4℃ (It is recommended to use vertical rotating mixer with Low-speed rotation).

Note: For fluorescence co-location detection, A primary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another primary antibody.

6．Fluorescence Secondary Antibody Incubation: Centrifugate at 300 g for 5 min, remove primary antibody, add 0.5 mL Antibody Wash Buffer and washed for 2 times for 5 min each, then add 0.5 mL PBS and washed for 5 min. Centrifugate at 300 g for 5 min, remove PBS, add 100 μL Secondary Antibody diluted with Antibody Dilution Buffer to suspend the cells and incubate at room temperature for 1 h, protected from light.

Note: After Fluorescence Secondary Antibody Incubation, all steps should be protected from light as much as possible. For fluorescence co-location detection, A secondary antibody can be incubated first, then wash the non-specific binding antibody, and incubate with another secondary antibody.

7．Redyeing with DAPI: After centrifugation, remove secondary antibody, add 0.5 mL Antibody Wash Buffer and washed for 2 times for 5 min each, then add 0.5 mL PBS and washed for 5 min. Centrifugate at 300 g for 5 min, remove PBS, add 200 μL 1×DAPI Staining Solution to suspend the cells and incubate at room temperature for 5-10 min, protected from light. Then centrifugate at 300 g for 5 min, remove 1×DAPI Staining Solution, wash with PBS for 3 times for 5 min each.

8．Mounting: Centrifugate at 300 g for 5 min, remove most of PBS retained about 50 μL of liquid, gently suspend the cells and drop on the glass slide, distribute the cells evenly on the slide. Let dry slightly, add a drop of SuperKine™ Enhanced Antifade Mounting Medium on the glass slide, coat the cover glass with the cells, and let the cells contact the mounting fluid, and try to avoid air bubbles, then observe the samples under the fluorescence microscope.

Strawberry moment: Universal IF Toolkit is a multifunctional kit developed by Abbkine for immunofluorescence staining. It is very convenient and practical, and can quickly carry out IF experiments and obtain high-definition colorful imaging pictures. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

Recommended Products

Catalog No.	Product Name	Recommended reason
KTD107-EN	Universal IF Toolkit (Anti-Rabbit Dylight 488)	Bright green fluorescence, designed for "one-stop" Immunofluorescence
KTD109-EN	Universal IF Toolkit (Anti-Rabbit Dylight 594)	Vivid red fluorescence, designed for "one-stop" Immunofluorescence
KTD110-EN	Universal IF Toolkit (Anti-Mouse Dylight 594)	Vivid red fluorescence, designed for "one-stop" Immunofluorescence
A23210	DyLight 488, Goat Anti-Mouse IgG	Higher specificity, extremely low background, indispensable fluorescent secondary antibody
A23220	Dylight 488, Goat Anti-Rabbit IgG	Higher specificity, extremely low background, indispensable fluorescent secondary antibody
A23410	Dylight 594, Goat Anti-Mouse IgG	Higher specificity, extremely low background, indispensable fluorescent secondary antibody
A23420	Dylight 594, Goat Anti-Rabbit IgG	Higher specificity, extremely low background, indispensable fluorescent secondary antibody

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