AcalephFluor488

IF Detection System Kit

(Goat Anti-Rabbit)

User Manual

Catalog # CRG1117

Used for immunofluorescence detection.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

AcalephFluor488 IF Detection System Kit is a non-biotin, one-step detection system that allows for the demonstration of antigens in paraffin-embedded tissue, cryostat sections, and cell preparations. This kit has been developed using a proprietary hyper labeling technology used to label IgG directly with more AcalephFluor488. One step system provides increased sensitivity, time savings and detection simplicity. All the components contain with PBS, proteins, stabilizers and preservatives. This kit is suitable for single, double and triple immunofluorescence detect. The color is emerald green with a correct result.

II. KIT COMPONENTS

Component	Volume	Storage
Permeabilization Buffer (20X)	5 ml x 1	2 to 8 °C
Antigen Retrieval Solution (10X)	30 ml x 3	2 to 8 °C
Blocking Buffer	10 ml x 1	2 to 8 °C
Antibody Solution Buffer	10 ml x 1	2 to 8 °C
Goat Anti-Rabbit IgG (H&L) - AcalephFluor488 (200X)	50 µl x 1	-20 °C
DAPI (1000X)	10 µl x 1	2 to 8 °C
Antifade Mounting Medium	500 µl x 1	2 to 8 °C
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III. STORAGE AND STABILITY

The kit is stable for up to 12 months.

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Xylene
2. Ethanol
3. Pipettor
4. Timer
5. Microwave
6. IHC pen
7. PBS
8. Fluorescence microscope

V. WORKING SOLUTION PREPARATION

All reagents need to be centrifuged before use.

1. Permeabilization Buffer

Add 1 ml Permeabilization Buffer (20X) into 19 ml PBS before use, mix.

2. Antigen Retrieval Solution

Add 10 ml Antigen Retrieval Solution (10X) into 90 ml ddH2O before use, mix.

3. Goat Anti-Rabbit IgG (H&L) - AcalephFluor488 Working Solution

Add 1 µl Goat Anti-Rabbit IgG (H&L) - AcalephFluor488 (200X) into 200 µl PBS before use, mix.

4. DAPI Working Solution

Add 1 µl DAPI (1000X) into 1 ml PBS before use, mix.

VI. PROCEDURE

For Paraffin Sections

1. De-paraffinizing (de-waxing) and rehydrating

1.1 Heat the slides in tissue-drying oven for 45 minutes at 60°C. Place the slides in a rack, and perform the following washes:

Xylene I: 15 mins

Xylene II: 15 mins

100% Ethanol: 5 mins

95% Ethanol: 5 mins

85% Ethanol: 5 mins

70% Ethanol: 5 mins

1.2 The slides are placed in a lab draught cupboard to rinse off ethanol.

1.3 Finally, wash the slides in the pure water.

2. Antigen or epitope retrieval

2.1 Add the appropriate antigen retrieval buffer to the microwaveable vessel.

2.2 Place the slides in the microwaveable vessel. Then place the vessel inside the microwave (1200W).

2.3 Boil for 8 mins in microwave (1200 W) under medium heat, then stop heating for 8 mins, followed by low and medium heat for 7 mins. Other heat-induced epitope retrieval methods can also be used, e.g., heated at 120 °C 1-2 min, 100 °C 20mins or 95 °C in a water bath.

Be sure to monitor for evaporation and watch out for boiling over during the procedure. Do not let the slides dry out.

2.4 After cooling down in room temperature, place the slides in PBS to wash 3 X 5 mins on a decolorizing shaker.

Notes: To get best results, antigen retrieval buffer and protocol should be determined according to the tissue types and antigen types.

3. Blocking

3.1 Drain slides and then use an IHC pen to draw a circle around each sample on your slide (to hold antibody solution within the target area).

3.2 Add Blocking Buffer inside the circle to cover the tissues, incubate 30 mins at room temperature.

4. Primary antibody incubation

Remove blocking buffer and add primary antibody diluted by recommended antibody diluent overnight at 4°C or 37°C for 1-2h, PBS wash for 3 X 5 mins.

5. Secondary antibody incubation

Add 100 µl Goat Anti-Rabbit IgG (H&L) - AcalephFluor488 to the sections and incubate for 30 - 60 minutes, PBS wash for 3 X 5 mins.

NOTE: Secondary antibody is light sensitive. Please avoid unnecessary light exposure.

6. DAPI incubation

Stain nuclear with 100 µl DAPI for 5-10 min, PBS wash for 3 X 5 mins.

7. Mounting the slides

Add antifade mounting medium (3-5 µl for each slice) to cover the section, and cover the microscope cover glass for observation.

For Cells

1. Fix cells

Fix cells by adding paraformaldehyde (PFA) 1- 4% in PBS 10 min at room temperature, PBS wash for 3 X 5 mins.

2. Permeation

Add 100 µl Permeabilization Buffer to the sections and incubate for 30 - 60 minutes, PBS wash for 3 X 5 mins.

Note: for membrane-associated protein staining, it can be insteaded by 100 µM digitonin or 0.5% saponin.

3. Blocking

Add 100 µl Blocking Buffer incubate for 30 mins at room temperature.

4. Primary antibody incubation

4.1 Remove blocking buffer and add primary antibody diluted by recommended antibody diluent overnight at 4°C or 37°C for 1-2h, PBS wash for 3 X 5 mins.

5. Secondary antibody incubation

5.1 Add 100 µl Goat Anti-Rabbit IgG (H&L) - AcalephFluor488 to the sections and incubate for 30 - 60 minutes, PBS wash for 3 X 5 mins.

NOTE: Secondary antibody is light sensitive. Please avoid unnecessary light exposure.

6. Mounting the slides

6.1 Add antifade mounting medium (3-5 µl for each slice) to cover the section, and cover the microscope cover glass for observation.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES