MACS molecular

Anti-His antibodies

Anti-His-FITC	130-092-675
Anti-His-PE	130-092-691
Anti-His-Biotin	130-092-692

Index

1. Description

Clone	GG11-8F3.5.1 (isotype: IgG1).
Product format	1 mL Anti-His antibody, monoclonal mouse Anti-His antibody conjugated to fluoresceinisothiocyanate (FITC), R-phycoerythrin (PE)- or Biotin. The antibody is supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	For up to 100 stainings.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The Anti-His antibody conjugate detects proteins, which are tagged with the His epitope (HHHHHH) and are expressed in prokaryotic or eukaryotic cells.

▲ Note: The Anti-His antibody conjugates show best sensitivity and specificity against C-terminal His-tagged proteins.

Product applications

- Flow cytometry of cells expressing His-tagged proteins.
- Immunofluorescence of cells expressing His-tagged proteins by fluorescence microscopy.

1.2 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Inside Stain Kit (# 130-090-477) for fixation and permeabilization of cells.
- (Optional) Mouse IgG1-FITC isotype control antibody (# 130-092-213) and Mouse IgG1-PE isotype control antibody (#130-092-212).
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation.
- (Optional) Anti-Biotin-APC (#130-090-856).

2. General protocol for immunofluorescent staining

2.1 Protocol for intracellular staining in suspension

▲ Volumes for fluorescent labeling given below are for **up to 10^6** cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^6 cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash 10^6 cells by adding 1–2 mL of buffer. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
2. (Optional) Stain for cell surface antigens, which are sensitive to fixation, according to the manufacturer's recommendations. Subsequently, wash cells by adding 1–2 mL of buffer. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 cells per 500 µL of buffer.
4. Add 500 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes at room temperature.
5. Centrifuge for 5 minutes at 300×g. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer. Centrifuge for 5 minutes at 300×g and aspirate supernatant carefully.

   ▲ Note: Fixed cells may be stored at 2–8 °C for up to 1 week.
7. (Optional) Stain for cell surface antigens, which are not sensitive to fixation, according to the manufacturer's recommendations. Subsequently, wash cells by adding 1–2 mL of buffer. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit). Centrifuge for 5 minutes at 300×g and aspirate supernatant carefully.
9. Resuspend cells in 100 µL of Inside Perm. Add 10 µL of Anti-His-Antibody.

   ▲ Note: We strongly recommend staining an aliquot of the cells with the mouse IgG1 fluorochrome-conjugated or biotin-conjugated control antibody.
10. (Optional) Add additional staining antibodies for cytosolic antigens to the solution.

    ▲ Note: For efficient permeabilisation upon intracellular staining the volume of Inside Perm should be at least 5× the total volume of staining antibodies.
11. Mix well and incubate for 10 minutes in the dark at room temperature.

140-001-862.01

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

12. Wash cells by adding 1 mL of Inside Perm. Centrifuge at 300×g for 5 minutes and aspirate supernatant carefully.

    ▲ **For Biotin:**

    i) Resuspend the cell pellet in 100 µL Inside Perm and add 10 µL Anti-Biotin-APC.

    ii) Mix well and incubate for 10 minutes in the dark at room temperature.

    iii) Wash cells by adding 1 mL of Inside Perm, centrifuge and carefully aspirate the supernatant completely.

13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analyzed. Mix well before flow cytometric acquisition.

    ▲ Note: Samples may be stored at 2–8 °C in the dark for up to 24 hours.

    ▲ Note: Do not use propidium iodide (PI) or 7-AAD staining.

2.2 Protocol for extracellular staining

Volumes for fluorescent labeling given below are for **up to 10^7** cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^7 cells, use twice the volume of all indicated reagent volumes and total volumes).

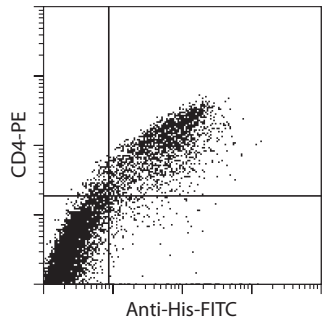
1. Resuspend up to 10^7 cells in 100 µL of buffer.
2. Add 10 µL of conjugated Anti-His antibody.
3. Mix well and refrigerate for 10 minutes in the dark (2–8 °C).

   ▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

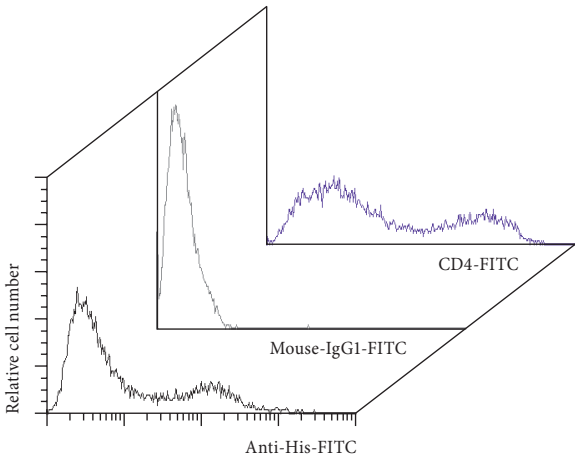
3. Examples of immunofluorescent staining with Anti-His antibodies

293HEK cells transiently transfected with His-tagged CD4 (vector pMACS K^K.His(C) CD4) were stained intracellularly with Anti-His-FITC and CD4-PE (# 130-091-231) (a) or CD4-FITC (# 130-080-501) (b) and analyzed by flow cytometry.

(a)



(b)



Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

140-001-862.01