MACS molecular

Anti-HA antibodies

Contents

1. Description

Components 1 mL monoclonal Anti-HA antibodies conjugated to various dyes.

FITC	130-092-256
PE	130-092-257
APC	130-098-404
Biotin	130-092-258

Clone GG8-1F3.3.1 (isotype: mouse IgG1).

Capacity 100 tests or up to 10^8 total cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The monoclonal Anti-HA antibody specifically recognizes proteins that are tagged with the HA epitope (YPYDVPDYA). The Anti-HA-HRP antibody enables direct protein detection in Western blot and ELISA analyses without the need for a secondary antibody. Anti-HA antibodies conjugated to FITC, PE, and APC enable direct flow cytometry and fluorescence microscopy analyses, while indirect fluorescent labeling of HA fusion protein expressing cells is possible with the biotin-conjugated antibody.

1.2 Applications

- Identification and enumeration of cells expressing HA-tagged proteins by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

- Anti-HA antibodies should be used at a dilution of 1:11.

1.4 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 bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-HA-Biotin.
- (Optional) Inside Stain Kit (# 130-090-477) for fixation and permeabilization of cells.
- (Optional) Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

2.1 Protocol for intracellular staining of cells in suspension

▲ Volumes given below are for **up to 10^6** nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation according to the manufacturer´s recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 cells in 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 at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.

   ▲ **Note:** Fixed cells may be stored at 2–8 °C for up to 1 week.

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7. (Optional) Stain cell surface antigens that are not sensitive to fixation according to manufacturer´s recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 100 µL of Inside Perm. Add 10 µL of the Anti-HA antibody.

   ▲ **Note:** We strongly recommend staining an aliquot of the cells with an isotype control antibody for flow cytometric analysis.
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 (19–25 °C).
12. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
13. (Optional) If Anti-HA-Biotin was used:

    i) Resuspend the cell pellet in 100 µL of Inside Perm and add 10 µL of anti-biotin antibody.

    ▲ **Note:** For more than 10^6 cells, scale up the volume of buffer and anti-biotin antibody to a final concentration of 10^7 cells/mL and a final dilution of anti-biotin antibody of 1:11.

    ii) Mix well and incubate for 5–10 minutes in the dark at room temperature.

    iii) Wash cells by adding 10–20 volumes of Inside Perm, centrifuge, and carefully remove the supernatant completely.
14. 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

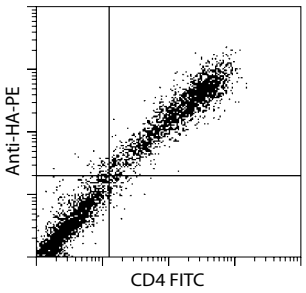
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated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

1. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend 10^7 cells in 100 µL of buffer.
3. Add 10 µL of the Anti-HA antibody.
4. Mix well and refrigerate for 10 minutes in the dark (2–8 °C).

   ▲ **Note:** Higher temperatures and/or longer incubation times lead to non-specific cell labeling. Working on ice requires increased incubation times.
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. (Optional) If Anti-HA-Biotin was used, resuspend 10^7 cells in 100 µL of buffer, add 10 µL of anti-biotin antibody, and continue as described in steps 3 and 4.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

3. Examples of immunofluorescent staining with Anti-HA antibodies

293HEK cells transiently transfected with HA-tagged CD4 were stained intracellularly with Anti-HA-PE and CD4-FITC (# 130-080-501), and analyzed by flow cytometry to control gene-of-interest and MACSelect™ System surface marker expression.



All protocols and data sheets are available at **www.miltenyibiotec.com**.

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

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