MACS molecular

Anti-c-myc antibodies

Anti-c-myc-FITC	130-092-472
Anti-c-myc-Biotin	130-092-471

Contents

1. Description

Components	1 mL Anti-c-myc antibodies: monoclonal mouse Anti-c-myc antibodies conjugated to fluorescein isothiocyanate (FITC) or biotin.
Clone	SH1-26E7.1.3 (isotype: IgG1).
Capacity	For up to 100 extracellular or 200 intracellular stainings, respectively. For details see corresponding protocols below.
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 Anti-c-myc conjugates detect fusion proteins with the human c-myc proto-oncogene tag (EQKLISEEDL) expressed in prokaryotic and eukaryotic cells.

1.2 Applications

- Immunofluorescence (IF) of adherent cells expressing c-myc-tagged proteins by fluorescence microscopy.
- Flow cytometry (FACS) of cells expressing c-myc-tagged proteins on the cell surface.
- Biotin-conjugate can also be used for Western blot detection.

1.3 Recommended antibody dilution

For antibody labeling of cell lines.

Anti-c-myc conjugate	FITC	Biotin
Flow cytometry[a]		
- extracellular staining	1:11	1:11
- intracellular staining	n.r.	n.r.
Immunofluorescent staining (intracellular staining)	1:11	1:11
Western blot		1:400–1:800

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 µL of buffer.
n. r.: not recommended

1.4 Reagent requirements

Fixation and intracellular staining of adherent cells (protocol 2.1)

- Phosphate-buffered saline (PBS), pH 7.5.
- Fixative: 4% Paraformaldehyde (PFA) in PBS; pH 7.4.

  To prepare a volume of 100 mL, add 4 g PFA to 90 mL PBS. Heat to 60–65 °C with continuous stirring to dissolve the PFA. Do not heat above 70 °C, as PFA will degrade. Allow solution to cool down to room temperature, check and correct pH if needed to pH 7.4, and adjust with PBS to final volume. Filter through paper filter to remove insoluble aggregates and store fixative in aliquots at –20 °C.
- Permeabilization buffer: PBS supplemented with 0.1% Triton® X-100. Prepare just prior to use. It is recommendable to make a 10% stock solution and store it at –20 °C.
- Blocking solution: 10% normal goat serum in PBS. Prepare freshly from frozen aliquots prior to use.
- Antibody diluent: 1% BSA in PBS. Prepare and store in suitable aliquots at –20 °C.
- Humid chamber, e.g., put a moistened piece of absorptive filter paper into a large square plastic dish and place a layer of Parafilm® M onto the moist filter.
- (Optional) DAPI: 5–10 µg/mL in PBS, pH 7.5; TOTO-3® dye.
- (Optional) Anti-Biotin-FITC (# 130-090-857) as secondary antibody reagent in combination with Anti-c-myc-Biotin.

Extracellular staining in suspension (protocol 2.2)

- 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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Mouse IgG1-FITC isotype control antibody (# 130-092-213).

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- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-c-myc-Biotin.
- (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 fixation and intracellular staining of adherent cells

▲ Volumes given below are for adherent cells grown on standard round glass cover slips submerged in 24-well cell culture plates. Please note that volumes may need to be adjusted when cells are cultured and stained using other cell culture plates or cover slips of other sizes.

Transfection of cells

1. Plate cells on round glass cover slips submerged in 24-well cell culture plates. Incubate cells overnight at 37 °C in medium containing serum.

   ▲ **Note:** Alternatively, 6-well plates or standard petri dishes can be used to accomodate several cover slips. For fixing the cells, transfer the cover slips into 24-well plates.

2. Transiently transfect cells with c-myc expression vector including construct of interest. Incubate cells in appropriate cell culture medium to allow transient expression of the recombinant protein comprising the c-myc epitope tag.

   ▲ **Note:** For stable expression of the recombinant protein of interest the protocol has to be adapted and a specific selection process for stably transfected cells has to be employed. For an antibiotics-free protocol for the specific selection of transfected cells (MACSelect™ Transfected Cell Selection Kits), see www.miltenyibiotec.com.

Fixation of cells

1. Remove medium from cells and incubate for 10 minutes in 500 µL fixative.
2. Wash fixed cells 3 times for 5 minutes with PBS.
3. (Optional) Store cells in PBS/0.05% azide at 4 °C.

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

Permeabilization and staining of cells

1. Prepare a humid chamber, e.g., by putting a moistened piece of absorptive filter paper into a large square plastic dish and placing a layer of Parafilm® M onto the moist filter.
2. Remove PBS from the 24-well cell culture plate and incubate cells for 1 minute at room temperature in 500 µL permeabilization buffer.
3. Wash fixed cells 3 times for 5 minutes with PBS.
4. First spot a drop of about 50 µL 10% normal goat serum onto the Parafilm layer in the humid chamber. Then, using forceps, place cover slips with cell side down onto the drop. Incubate for 20 minutes.
5. In order to minimize loss of cells when moving the coverslips after each incubation step, pipette a small amount of PBS right at the edge of each coverslip immediately prior to lifting the coverslip.

   ▲ **Note:** Perform all subsequent blocking and immunolabeling steps as described in steps 4 and 5.

6. Prepare antibody-staining solution by adding 5 µL Anti-c-myc to 50 µL antibody diluent and incubate for 1 hour in humid chamber in the dark as described above.

   ▲ **Note:** As expression levels in stable transfectants might be lower than in cells transiently expressing the protein of interest, it might be necessary to decrease the antibody dilution factor for stable transfectants.

7. Wash stained cells 3 times for 5 minutes with 50 µL PBS in humid chamber.
8. (Optional) If Anti-c-myc-Biotin was used, prepare antibody-staining solution by adding 5 µL Anti-Biotin-FITC to 50 µL antibody diluent and continue as described in steps 5 and 6.
9. (Optional) Incubate cells with DAPI or TOTO3 dye in PBS for 10 minutes at room temperature to stain cell nuclei and subsequently wash 3 times in PBS for 5 minutes in humid chamber.
10. (Optional) Pipette fluorescence mounting medium (antifade) onto slides and place cover slips with cell side down onto the drop (according to the manufacturer's instruction) and store at 4 °C.

2.2 Protocol for extracellular staining in suspension

▲ Volumes 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-c-myc antibody.

   ▲ **Note:** See table for exceptions.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-c-myc-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC # 130-090-857, Anti-Biotin-PE # 130-090-756, or Anti-Biotin-APC # 130-090-856), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

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3. Examples of immunofluorescent staining with Anti-c-myc antibodies

Chinese hamster ovary (CHO) cells transiently transfected with c-myc-tagged CD4 were stained intracellularly with Anti-c-myc-FITC (A) or Anti-c-myc-Biotin and Anti-Biotin-FITC (B) and analyzed by confocal light microscopy to control the expression of the gene of interest.

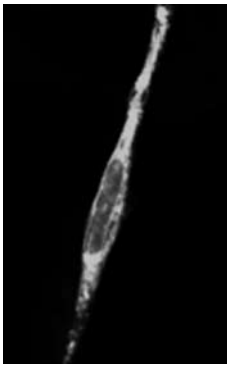
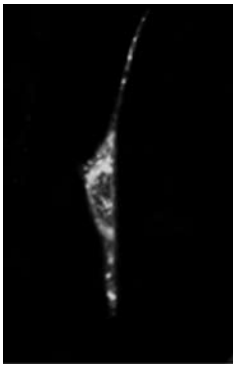
A)  B) 

Figure 1: CHO cells transiently expressing c-myc detected with FITC-conjugated antibody (A) and with biotin-labeled antibody (B).

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

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.

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