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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-CD13 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M101-3	123H1	Rat IgG2b	100 µL	1 mg/mL

BACKGROUND: CD13 is a myeloid differentiation molecule expressed on committed myeloid progenitors, granulocytes, monocytes, and leukemic cells of myeloid origin. It is also expressed on non-hematopoietic cells including fibroblasts, renal proximal tubule, and small intestine brush-border membrane. The gene for CD13 has recently been cloned the cDNA sequence shows that CD13 is the metalloprotease aminopeptidase N. Biochemical studies have shown that CD13 is a 150 kDa glycoprotein, which exists as a 130 kDa intracellular precursor form. This 130 kDa precursor molecule is posttranslationally modified in the Golgi apparatus to produce the 150 kDa mature cell surface form of the molecule.

SOURCE: This antibody was purified from hybridoma (clone 123H1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Wister rat lymphocyte immunized with murine dendritic cells isolated from C57BL/6 mice.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse CD13 antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 5 µg/1000 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not tested	JAWS II, C2C12	Not tested
Reactivity on FCM		+	

INTENDED USE:

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REFERENCE:

1) Curnis, F., *et al.*, *Cancer Res.* **65**, 2906-2913 (2005)

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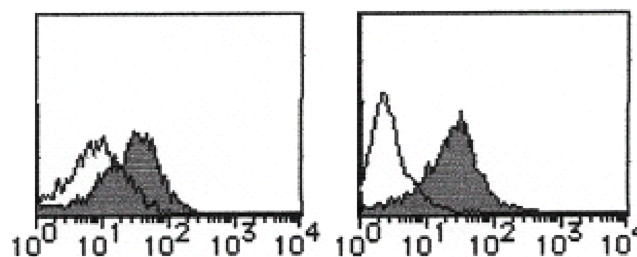


***Flow cytometric analysis of mouse CD13 expression on JAWS II (left) and C2C12 (right).** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of M101-3 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

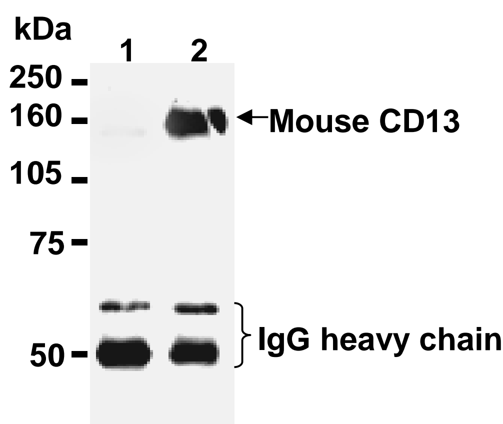
Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
 *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 30 µL of primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the

washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add FITC conjugated anti-rat IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; JAWS II, C2C12)



Immunoprecipitation of Mouse CD13 from JAWS II cells with Rat IgG2b (1) or M101-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M101-3.*

Immunoprecipitation

1) Wash the biotin labeled JAWS II cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 1000 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
8) Incubate the membrane with HRP-conjugated streptavidin diluted with 1% skimmed milk (in PBS, pH 7.2) for 15 minutes at room temperature.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; JAWS II)