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cis-Golgi Marker

Anti-GM130 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M179-3MS	5G8	Mouse IgG2a κ	20 µL	1 mg/mL

BACKGROUND: The Golgi apparatus is a eukaryotic organelle, which is mainly devoted to processing the proteins synthesized in the endoplasmic reticulum (ER). GM130 is a member of the golgin family of coiled-coil proteins that localizes predominantly to the cis-Golgi. GM130 might participate in ER-Golgi traffic.

SOURCE: This antibody was purified from hybridoma (clone 5G8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the synthetic peptide corresponding to C-terminal of human GM130.

FORMULATION: 20 µg IgG in 20 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 human GM130 on Western blotting, Immunoprecipitation, and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 2 µg/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Hela, 293T A549	Not Tested	Not Tested
Reactivity on WB	+		

INTENDED USE:

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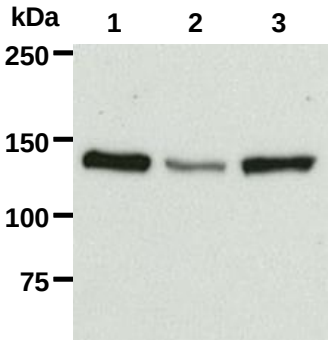
REFERENCES:

1) Diao, A., *et al.*, *J. Biol. Chem.* **283**, 6957-6967 (2008)
2) Alvarez, C., *et al.*, *J. Biol. Chem.* **276**, 2693-2700 (2001)



Western blotting analysis of GM130 in HeLa (1), 293T (2) and A549 (3) using M179-3.

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

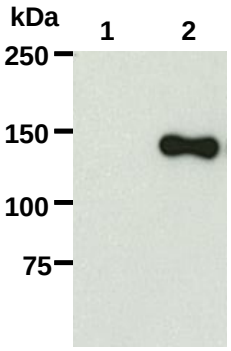
PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 20 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) 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 manufacturer's manual for precise transfer procedure.
4) 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.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.

8) Wash the membrane with PBS-T (5 minutes x 3).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 6 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; Hela, 293T, A549)

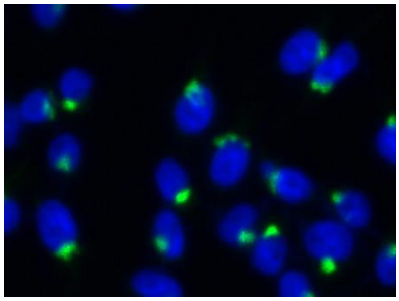


Immunoprecipitation of GM130 from HeLa with mouse IgG2a isotypic control, M076-3 (1) or M179-3 (2). *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with PM061.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 20 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A 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, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



Immunocytochemical detection of GM130 in HeLa using M179-3.
Green: anti-GM130
Blue: DAPI

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 cells of HeLa cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
4) Wash the glass slide 3 times with PBS.
5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide twice with PBS.
7) Add the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) Wash the glass slide twice with PBS.
9) Add 100 µL of 1:500 Alexa Fluor®488 conjugated anti-mouse IgG (Invitrogen; code no. 53818A) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the glass slide twice with PBS.
11) Counter stain with DAPI for 5 minutes at room temperature.
12) Wash the glass slide twice with PBS.
13) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

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