

MONOCLONAL ANTIBODY

cis-Golgi Marker

# Anti-GM130 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M179-3	5G8	Mouse IgG2a $\kappa$	100 $\mu$ L	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:** 100  $\mu$ g IgG in 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human GM130 on Western blotting, Immunoprecipitation, and Immunocytochemistry.

**APPLICATIONS:**

- Western blotting; 1  $\mu$ g/mL for chemiluminescence detection system
- Immunoprecipitation; 2  $\mu$ g/300  $\mu$ L of cell extract from  $3 \times 10^6$  cells
- Immunohistochemistry; Not tested
- Immunocytochemistry; 5  $\mu$ g/mL
- Flow cytometry; Not tested

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

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat	Monkey*
Cells	HeLa, 293T A549	Not tested	Not tested	
Reactivity on WB	+			

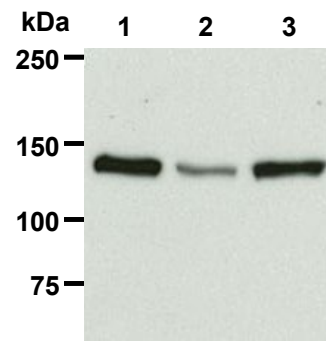
\*It is reported that this antibody can be used in COS-7 cells in Immunocytochemistry<sup>1)</sup>.

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**REFERENCES:**

- 1) Oh-hashii, K., *et al.*, *FEBS Lett.* **585**, 2481-7 (2011) [IC]
- 2) Diao, A., *et al.*, *J. Biol. Chem.* **283**, 6957-6967 (2008)
- 3) Alvarez, C., *et al.*, *J. Biol. Chem.* **276**, 2693-2700 (2001)



**Western blot 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.

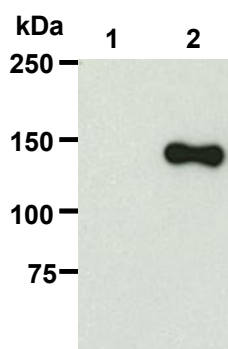
**PROTOCOLS:**

**SDS-PAGE & Western Blotting**

- 1) Wash cells (approximately  $1 \times 10^7$  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  $\mu$ L 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<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 times).

- 7) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (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 times).
- 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 and 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  $1 \times 10^7$  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  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20  $\mu$ L 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  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10  $\mu$ L/lane for the SDS-PAGE analysis.

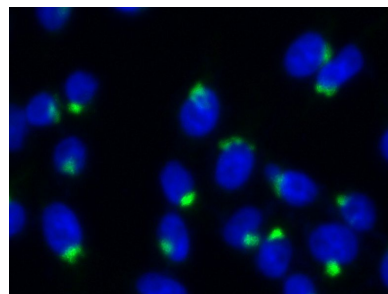
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

#### **Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread  $1 \times 10^4$  cells of HeLa cells for one slide, then incubate in a CO<sub>2</sub> 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 2 times 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 2 times with PBS.
- 9) Add 100  $\mu$ L of 1:500 Alexa Fluor<sup>®</sup>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 2 times with PBS.
- 11) Counter stain with DAPI for 5 minutes at room temperature.
- 12) Wash the glass slide 2 times 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)



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

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