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Not for use in diagnostic procedures.



Anti- β -galactosidase mAb

CODE No.	M203-3
CLONALITY	Monoclonal
CLONE	6F4
ISOTYPE	Rat IgG2a κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Full length β -galactosidase from <i>E.coli</i>
FORMULATION	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.

APPLICATIONS-CONFIRMED

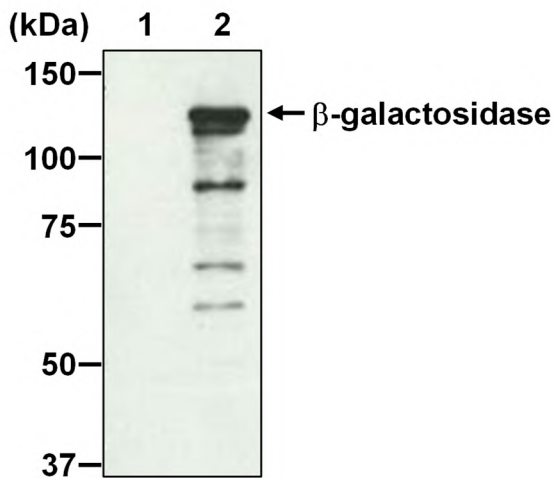
<u>Western blotting</u>	0.1 μ g/mL
<u>Immunoprecipitation</u>	2 μ g/sample
<u>Immunocytochemistry</u>	2 μ g/mL
<u>Immunohistochemistry</u>	5 μ g/mL (frozen section)

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SDS-PAGE & Western blotting

- 1) Wash 1×10^6 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.)
- 2) Centrifuge the tube at $12,000 \times g$ for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at $1 \text{ mA}/\text{cm}^2$ for 1 hr. 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.
- 5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C .
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T (5 min. x 3).
- 9) Incubate the membrane with HRP conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 10) Wash the membrane with PBS-T (5 min. x 3).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 3 min. develop the film as usual. The condition for exposure and development may vary.



Western blotting analysis of β -galactosidase

Lane 1: HEK293T

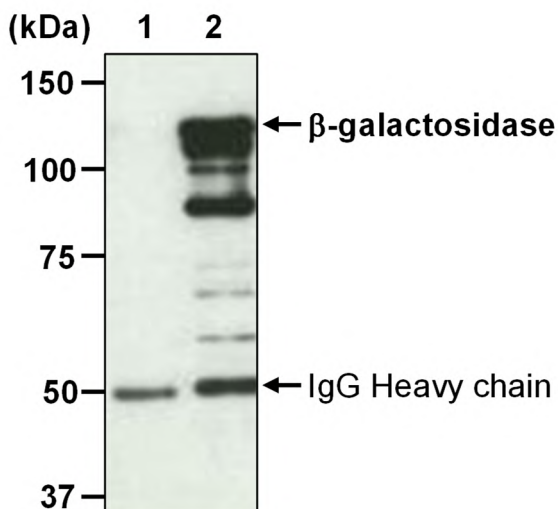
Lane 2: pcDNA-LacZ/HEK293T

Immunoblotted with Anti- β -galactosidase mAb (MBL, code no. M203-3)

Immunoprecipitation

- 1) Resuspend 1×10^7 cells with 1 mL of ice-cold Extraction buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors.
- 2) Centrifuge the tube at 12,000 x g for 10 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L of IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
- 4) Wash the beads once with 1 mL of IP buffer.
- 5) Add 250 μ L of cell lysate (prepared sample from step 2), then incubate with gentle agitation for 1 hr. at room temperature.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Resuspend the agarose with 1 mL of Extraction buffer.
- 8) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 9) Repeat steps 7)-8) 4 times.
- 10) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 11) Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 12) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 13) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 15) Incubate the membrane with 1 μ g/mL of Anti- β -galactosidase mAb (MBL, code no. M094-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 16) Wash the membrane with PBS-T (5 min. x 3).
- 17) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 18) Wash the membrane with PBS-T (5 min. x 3).
- 19) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
Expose to an X-ray film in a dark room for 3 min. develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Transfectant)



Immunoprecipitation of β -galactosidase from HEK293T transfectant

Cell: pcDNA-LacZ/HEK293T

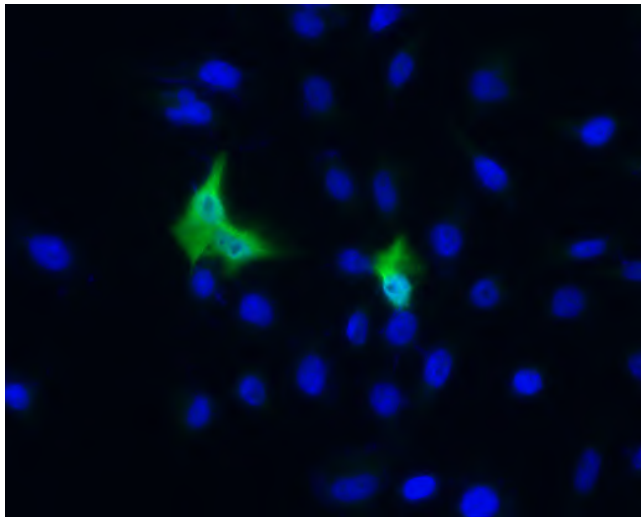
Lane 1: Rat IgG2a (isotype control) (MBL, code no. M081-3)

Lane 2: Anti- β -galactosidase mAb (MBL, code no. M203-3)

Immunoblotted with Anti- β -galactosidase mAb (MBL, code no. M094-3)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator overnight.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide twice with PBS.
- 4) Fix the cells with 4% paraformaldehyde/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide twice with PBS.
- 6) Permeabilize the cells with 200 µL of 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 7) Wash the slide twice with PBS.
- 8) Add 200 µL of the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 9) Wash the slide twice with PBS.
- 10) Add 200 µL of 1:500 Alexa Fluor® 488 Goat Anti-Rat IgG (Thermo Fisher Scientific, code no. A-11006) diluted with 2% FCS/PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide twice with PBS.
- 12) Counterstain with DAPI for 5 min. at room temperature.
- 13) Wash the slide twice with PBS.
- 14) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of β -galactosidase in HeLa transfectant

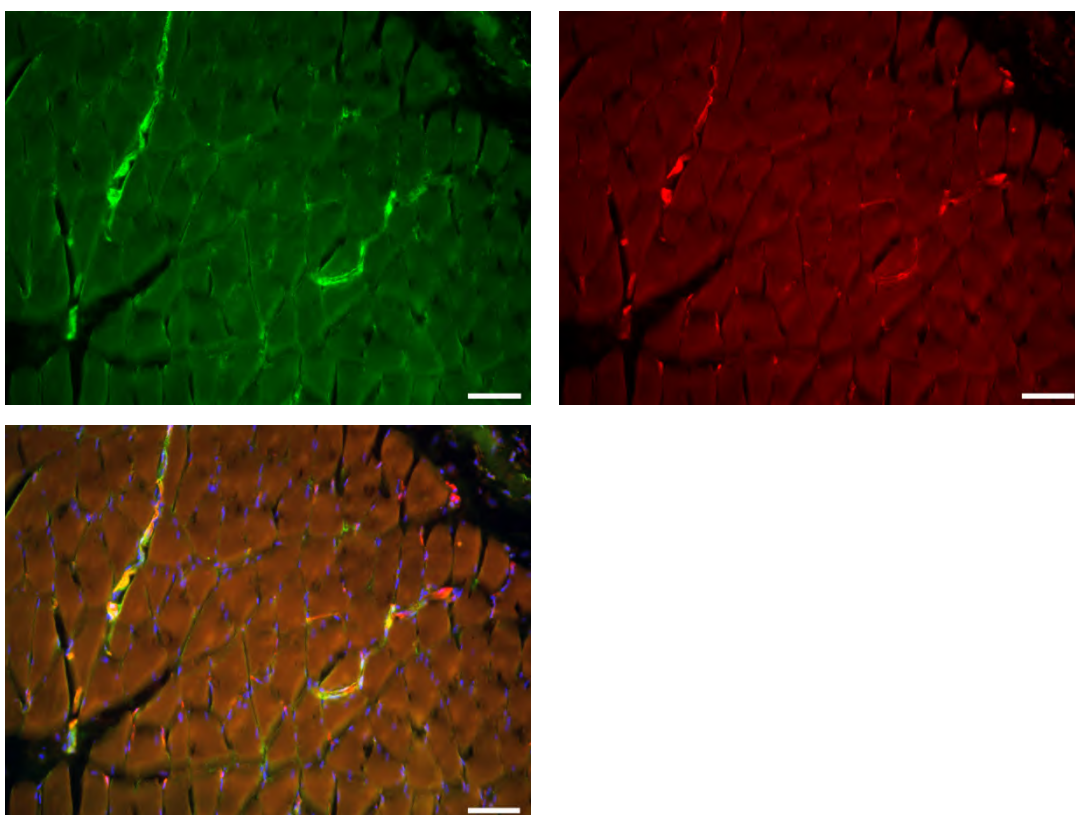
Cell: pcDNA-LacZ/HeLa

Green: Anti- β -galactosidase mAb (MBL, code no. M203-3)

Blue: DAPI

Immunohistochemistry (frozen section)

- 1) Wash the slide twice with PBS.
- 2) Wipe gently around the section and incubate with Protein Block, Serum-Free (Agilent, code no. X0909) for 30 min. at room temperature.
- 3) Incubate the slide with the primary antibody as suggested in the **APPLICATIONS** overnight at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 4) Wash twice for 5 min. each with PBS.
- 5) Incubate the slides with 1:100 diluted Alexa Fluor® 488 Anti-Rat IgG (Thermo Fisher Scientific, code no. A-11006) for 1 hr. at room temperature in dark chamber.
- 6) Wash twice for 5 min. each with PBS.
- 7) Counterstain with DAPI for 5 min. at room temperature.
- 8) Wash twice for 5 min. each with PBS.
- 9) Mount with FluorSave™ Reagent (MERCK, code no. 345789) and observe the slide using the fluorescent microscopy.



Immunohistochemical detection of β -galactosidase in endothelial cells

Tissue: Knock-in mouse skeletal muscle (β -galactosidase is expressed in endothelial cells)

Green: Anti- β -galactosidase mAb (MBL, code no. M203-3)

Red: Anti-mEndoglin pAb

Blue: DAPI

Data were kindly provided by Ms. Chihiro Makihara and Dr. Takashi Minami.
(Division of Vascular Biology, RCAST, The University of Tokyo)