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A JSR Life Sciences Company

Anti-β-galactosidase mAb

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

Western blotting	0.1 μg/mL
Immunoprecipitation	2 μg/sample
Immunocytochemistry	2 μg/mL
Immunohistochemistry	5 μg/mL (frozen section)

RELATED PRODUCTS

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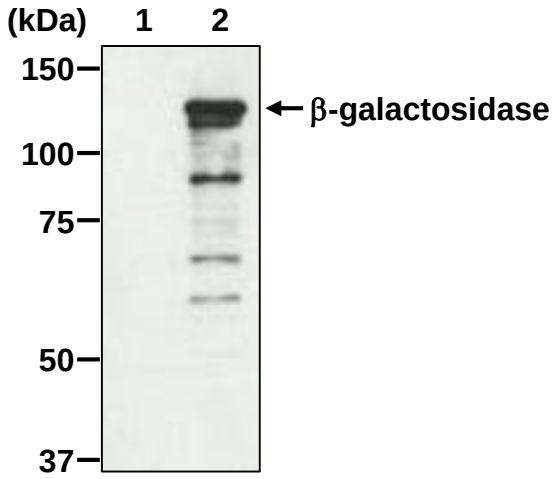
SDS-PAGE & Western blotting

1) Wash 1 x 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 x 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 mA/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

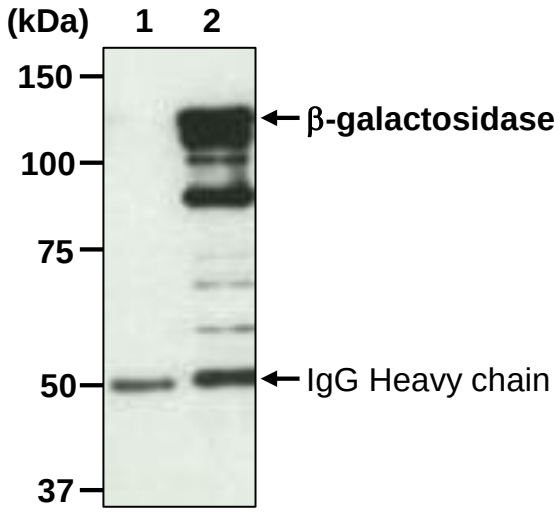
Lane 1: HEK293T
Lane 2: pcDNA-LacZ/HEK293T

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

Immunoprecipitation

1) Resuspend 1 x 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 µL of 50% protein A agarose beads slurry resuspended in 300 µL 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 µL 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 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
11) Load 10 µL 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/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.
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 µg/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)



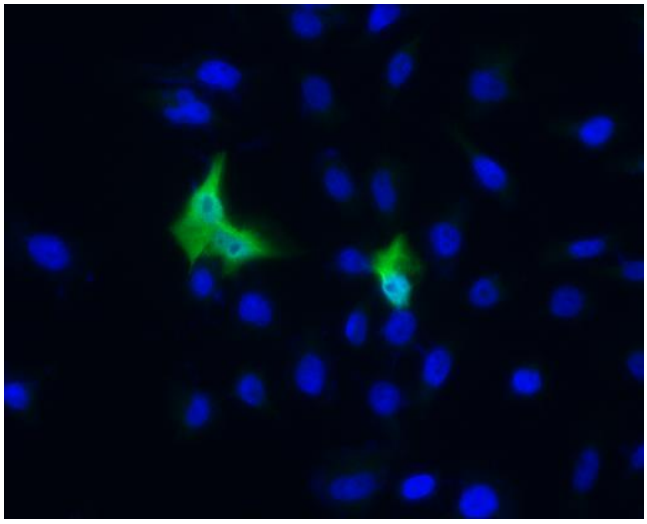
Immunoprecipitation of β-galactosidase from HEK293T transfectant

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 CO_2 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.



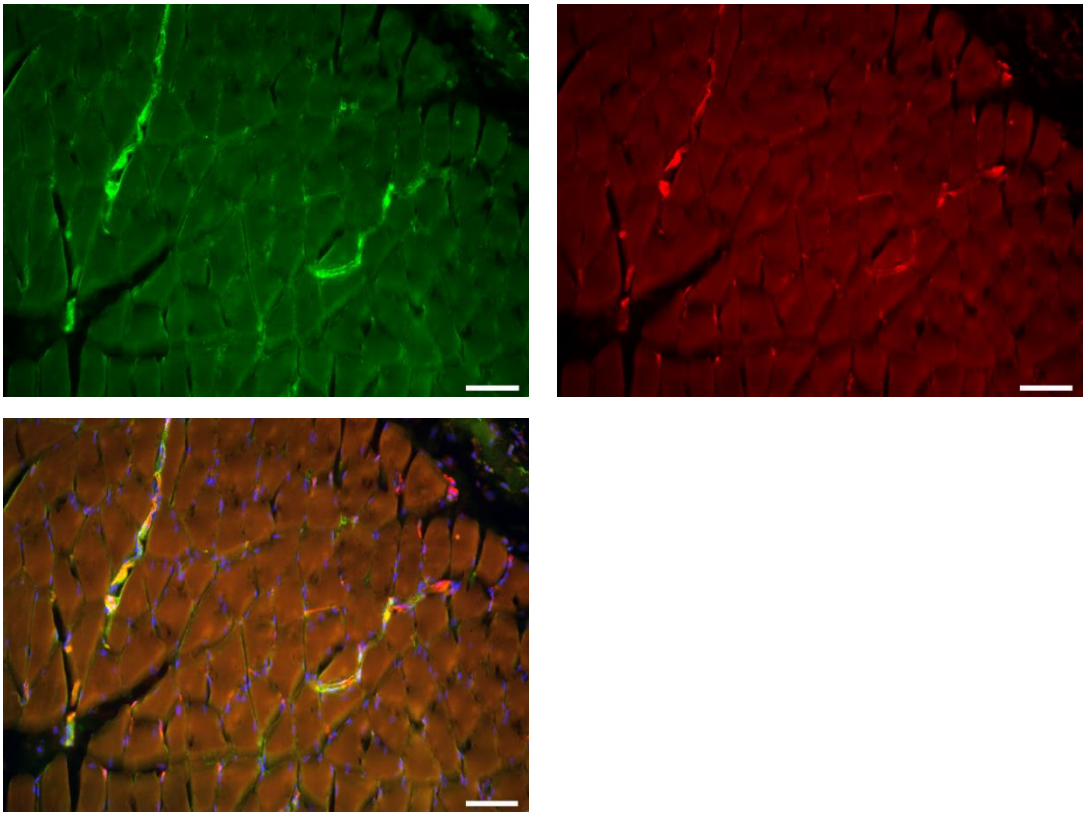
Immunocytochemical detection of β-galactosidase in HeLa transfectant

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)