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Not for use in diagnostic procedures.

MBL
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POLYCLONAL ANTIBODY

Anti-β-galactosidase pAb

Code No.	Quantity	Form
PM049	100 µL	Rabbit IgG

BACKGROUND: β-galactosidase is a homo-tetrameric enzyme, with each subunit having a molecular weight of 116 kDa. Eukaryotic genes are often expressed as fusion protein by the β-galactosidase (*lacZ*) gene, resulting in the expression of a fusion hybrid with β-galactosidase. Anti-β-galactosidase antibody provides a simple method to isolate fusion proteins directly from crude bacterial lysates, using immunoaffinity chromatography or immunoprecipitation. Anti-β-galactosidase can also be used for the immunocytochemical detection of β-galactosidase in cells and tissues that express transfected bacterial *lacZ* gene or β-galactosidase fusion protein.

SOURCE: This antibody was purified from rabbit serum using protein A agarose. The rabbit was immunized with full length *E. coli* β-galactosidase.

FORMULATION: 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.

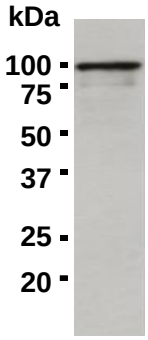
REACTIVITY: This antibody reacts with β-galactosidase on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 1 µL/sample
Immunohistochemistry; 1:200
Immunocytochemistry; 1:100
Flow cytometry; Not tested

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

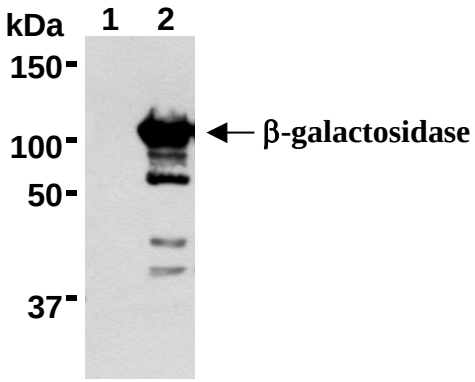
REFERENCES:
1) Otsu, K., *et al., J. Bone Miner. Res.* **31**, 1943-1954 (2016) [IHC]
2) Vaish, V., *et al., Genes Chromosomes Cancer*. **55**, 577-590 (2016)



Western blot analysis of β-galactosidase expression in transfectant using PM049.

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

PROTOCOLS:
SDS-PAGE & Western Blotting
1) Wash the $1x10^6$ transfectant cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) 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.
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% MeOH). See the manufacture'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 with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
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 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) 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 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.
10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.



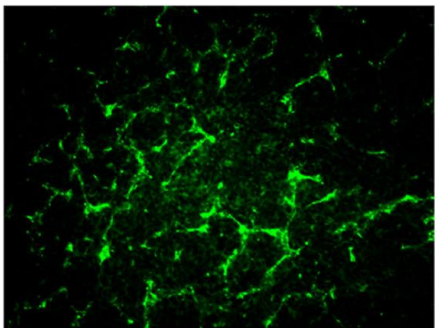
Immunoprecipitation of β-galactosidase from transfectant with normal rabbit IgG (1) or PM049 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-β-galactosidase monoclonal antibody (MBL; code no. M094-3).*

Immunoprecipitation

1) Wash the $5x10^6$ transfectant cells 3 times with PBS and suspend with 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 15 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 200 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
4) 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.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) 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**.)

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from the PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with ENVISION+Dual Link (DAKO; code no. K4063). Incubate for 1 hour at room temperature. Wash as in step 9).
10) Visualize by reacting for 10 minutes with DAB substrate solution (DAKO; code no. K3465). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
13) Now ready for mounting.

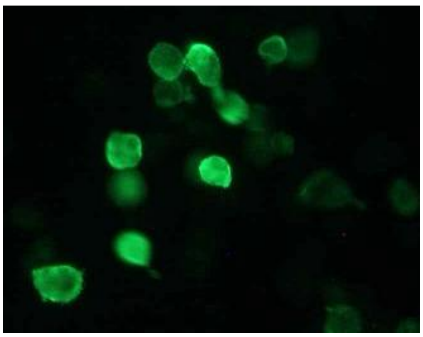


Immunohistochemical detection of β-galactosidase in frozen section of Lewis lung carcinoma xenograft with PM049.
This data was provided by Dr. Minami (Laboratory for Systems Biology and Medicine at RCAST, University of Tokyo)

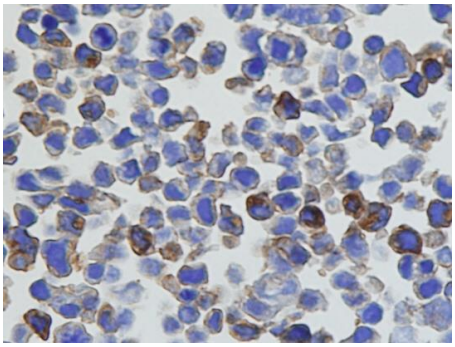
Immunohistochemical staining for frozen sections: For acetone fixed section

1) Wash the slide in PBS (5 minutes x 3 times).
2) Immerse the slide in Image iTTM FX™ for 30 minutes at room temperature
3) Wash the slide with PBS 3 times for 5 minutes each.
4) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Block (Dako; code no. X0909) for 20 minutes to block non-specific staining. Do not wash.
5) Tipp off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with Protein Block as suggested in the **APPLICATIONS**.
6) Incubate the sections overnight at 4°C.
7) Wash the slide with PBS 3 times for 5 minutes each.

8) Wipe gently around each section and cover tissues with 1:50 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A11008). Incubate for 1 hour at room temperature.
9) Wash the slide with PBS 3 times for 5 minutes each.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of β-galactosidase in transfectant with PM049.



Immunocytochemical detection of β-galactosidase in paraffin embedded section of transfectant with PM049.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ 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) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS.
7) Add the primary antibody diluted with 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) The glass slide was washed 3 times with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A11008) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

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