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MBL
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MONOCLONAL ANTIBODY

Anti-β-galactosidase mAb

Code No.	Clone	Subclass	Quantity	Concentration
M094-3	5A3	Mouse IgG1	100 µL	1 mg/mL

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 hybridoma (clone 5A3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with full length *E. coli* β-galactosidase.

FORMULATION: 100 µg IgG in 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 µg/mL for chemiluminescence detection system
Immunoprecipitation; 1 µg
Immunohistochemistry; 10 µg/mL
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested*
*It is reported that clone 5A3 can be used in this application in the reference number 1).

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

INTENDED USE:
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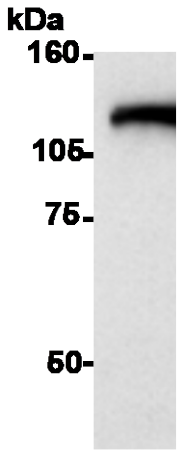
REFERENCE:
1) Sato, Y., *et al. Cell Biosci*. **1**, 7 (2011) [IC, FCM]



Western blot analysis of β-galactosidase expression in pcDNA3-LacZ/293T cells using M094-3.

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

PROTOCOLS:

SDS-PAGE & Western Blotting

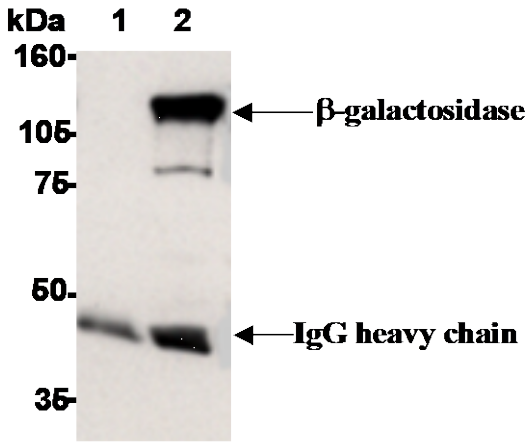
1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in 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 suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will be 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 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 6 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 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

Immunoprecipitation

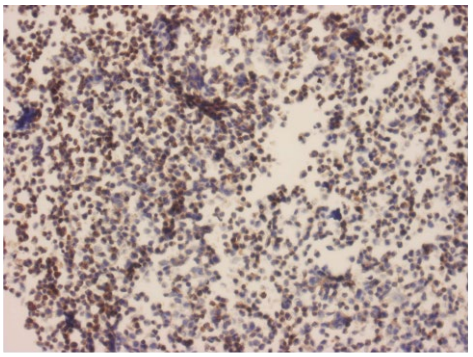
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge 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 suggest in the **APPLICATIONS** into 200 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**.)



Immunoprecipitation of β-galactosidase from pcDNA3-LacZ /293T with mouse IgG1 (1) or M094-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M094-3.*

Immunohistochemical staining for paraffin-embedded sections : SAB method

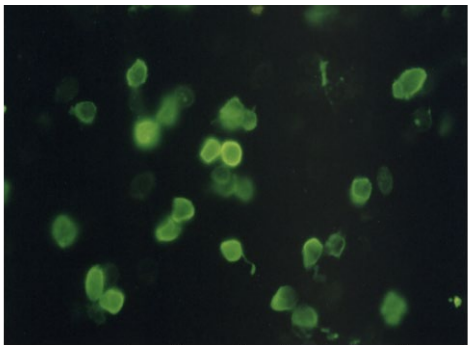
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous 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 Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) 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 PBS containing 1% BSA as suggest 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 Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) 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.
14) Now ready for mounting.



Immunohistochemical detection of β-galactosidase on paraffin embedded section of pcDNA3-LacZ/293T cells with M094-3.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
3) The glass slide was washed with PBS 3 times.
4) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
5) The glass slide was washed with PBS 3 times.
6) Add FITC conjugated anti-mouse IgG diluted with PBS onto the cells. Incubate for 20 minutes at room temperature. Keep out light by aluminum foil.
7) The glass slide was washed with PBS 3 times.
8) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
9) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of β-galactosidase on 4% PFA fixed pcDNA3-LacZ/293T cells with M094-3.

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