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# AM26630AF-N Monoclonal Antibody to Beta-galactosidase tag - Azide Free

**Alternate names:** Beta-Gal Fusion Protein, Beta-Gal tag, JW0335, Lactase, b0344, lacZ tag

**Quantity:** 0.1 mg

**Concentration:** 1.0 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 antibodies provide 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.

**Uniprot ID:** P00722

**NCBI:** AP_000996.1

**GeneID:** 945006

**Host / Isotype:** Mouse / IgG1

**Clone:** 5A3

**Immunogen:** E. coli full length β-galactosidase

**Format:**
**State:** Liquid Ig fraction
**Purification:** Protein A agarose
**Buffer System:** PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**Applications:**
**Western blot:** 1 µg/ml for chemiluminescence detection system.
**Immunoprecipitation:** 1 µg/200 µl of cell extract from 5x$10^6$ cells.
**Immunohistochemistry on paraffin sections:** 10 µg/ml.
**Immunocytochemistry:** 5 µg/ml.
For details see protocols below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:** This antibody reacts with β -galactosidase (116kDa).

**Species Reactivity:** **Tested:** E. coli

**Add. Information:** This product was originally produced by MBL International.

**Storage:** Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Shelf life: One year from despatch.

**Protocols:** SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 o 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 o C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make an 8 mg/mL solution.
3) Mix the sample with an equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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 specific transfer procedure.
6) 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 o C.
7) Incubate the membrane with primary antibody diluted with PBS, pH7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to X-ray film in a dark room for 10 minutes. Develop the film as usual. The conditions for exposure and development may vary.

Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 o 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 o C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the APPLICATIONS into 200 µ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 o C. Add 20 µ L of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 o 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 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)

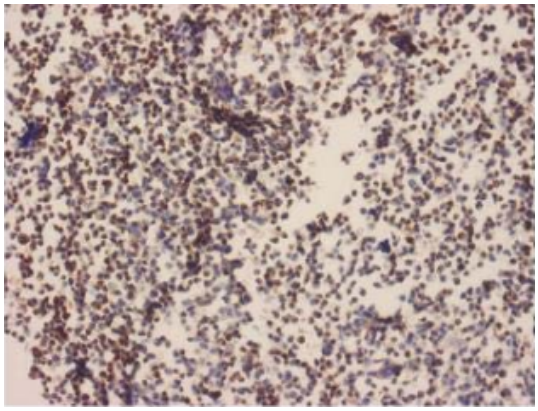
Immunohistochemical staining for paraffin-embedded sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes.
4) Remove the slides from PBS and cover each section with 3% H 2 O 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 nd cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody 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 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 Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temper ature. 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 2 O 2 in 150 mL PBS.
* DAB is a suspected carcinoge n 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.

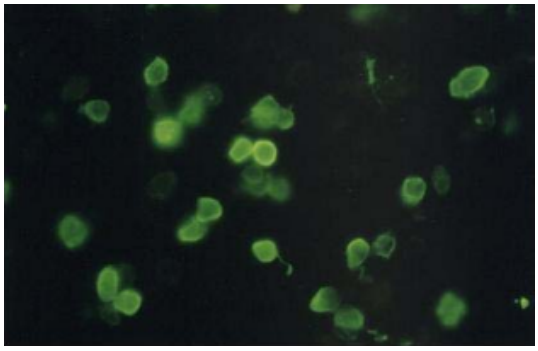
Immunofluorescence microscopy
1) Culture the cells in the appropriate condition on a glass slide (for example, spread 10 4 of pCDNA3-LacZ/293T cells for one slide, then incubate in a CO 2 incubator for one night.)
2) Fix the cells by immersing the slide in PBS containing 4% Formaldehyde for 10 minutes at room temperature.
3) The glass slides were 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 temperatur e (Optimization of antibody concentration or incubation condition are recommended if necessary.)
5) The glass slides were washed with PBS 3 times.
6) Add 50 µL of 1:40 FITC conjugated anti-mouse IgG uted with PBS onto the cells. Incubate for 20 minutes at room temperature. Keep out light by aluminum foil.
7) The glass slides were 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.

**Pictures:**

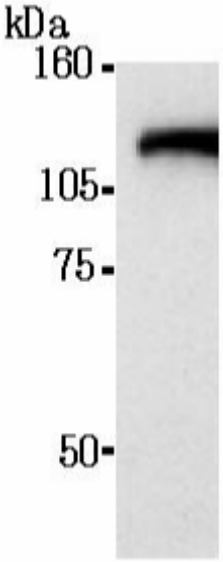
Immunohistochemical detection of beta-galactosidase on paraffin embedded section of pCDNA3-LacZ/293T cells with AM26630AF-N.



Immunohistochemical detection of beta-galactosidase on 4% PFA fixed pCDNA3-LacZ/293T cells with AM26630AF-N.



Western blot analysis of beta-galactosidase expression in pCDNA3-LacZ/293T cells using AM26630AF-N.



Immunopreciptation of beta-galactosidase from pCDNA3-LacZ/293T with mouse IgG1 (1) or AM26630AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26630AF-N.