Datasheet for ABIN5541540

anti-beta-Galactosidase Tag antibody

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Overview

Quantity:	0.1 mg
Target:	beta-Galactosidase Tag
Reactivity:	Please inquire
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This beta-Galactosidase Tag antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunocytochemistry (ICC), Immunofluorescence (IF), Immunoprecipitation (IP)

Product Details

Immunogen:	E. coli full length beta-galactosidase
Clone:	5A3
Isotype:	IgG1
Specificity:	This antibody reacts with β -galactosidase (116 kDa).
Purification:	Protein A agarose

Target Details

Target:	beta-Galactosidase Tag
Abstract:	beta-Galactosidase Tag Products
Target Type:	Tag

Target Details

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. < div dir=ltr data-font-name=g_font_p0_2 data-canvas-width=6.720080307006836>
UniProt:	P00722

Application Details

Application Notes:	Western blot: 1 µg/mL for chemiluminescence detection system. Immunoprecipitation: 1 µ g/200 µL of cell extract from 5x10 6 cells. Immunohistochemistry on paraffin sections: 10 µ g/mL. Immunocytochemistry: 5 µg/mL. For details see protocols below.
Protocol:	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, pH 7.2 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

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	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.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS containing 50 % glycerol, pH 7.2. No preservative is contained.
Preservative:	Azide free
Storage:	-20 °C
Storage Comment:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.