

POLYCLONAL ANTIBODY

# Anti- $\beta$ -galactosidase pAb

Code No.	Quantity	Form
PM049	100 $\mu$ L	Rabbit IgG

**BACKGROUND:**  $\beta$ -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  $\beta$ -galactosidase (*lacZ*) gene, resulting in the expression of a fusion hybrid with  $\beta$ -galactosidase. Anti- $\beta$ -galactosidase antibody provides a simple method to isolate fusion proteins directly from crude bacterial lysates, using immunoaffinity chromatography or immunoprecipitation. Anti- $\beta$ -galactosidase can also be used for the immunocytochemical detection of  $\beta$ -galactosidase in cells and tissues that express transfected bacterial *lacZ* gene or  $\beta$ -galactosidase fusion protein.

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

**FORMULATION:** 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with  $\beta$ -galactosidase on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

## APPLICATIONS:

Western blotting: 1:1,000 for chemiluminescence detection system

Immunoprecipitation: 1  $\mu$ L/sample

Immunohistochemistry: 1:200

Immunocytochemistry: 1:100

Flow cytometry: Not tested

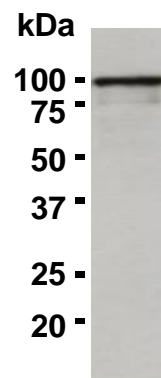
Detailed procedure is provided in the following **PROTOCOLS**.

## 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)

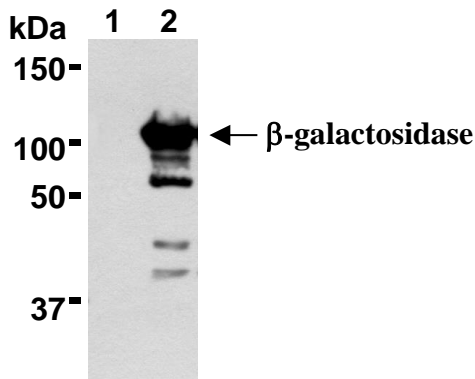


**Western blot analysis of  $\beta$ -galactosidase expression in transfectant using PM049.**

## PROTOCOLS:

### SDS-PAGE & Western Blotting

- 1) Wash the  $1 \times 10^6$  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  $\mu$ L 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<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer'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  $4^{\circ}\text{C}$ .
- 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  $\beta$ -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- $\beta$ -galactosidase monoclonal antibody (MBL; code no. M094-3).**

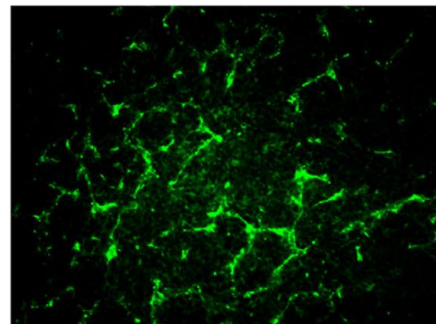
#### **Immunoprecipitation**

- 1) Wash the  $5 \times 10^6$  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  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
- 4) Add 20  $\mu$ L 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  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10  $\mu$ L/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<sub>2</sub>O<sub>2</sub> 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.



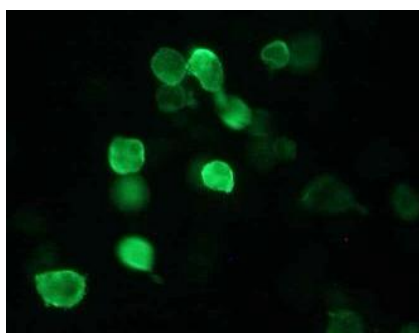
#### **Immunohistochemical detection of $\beta$ -galactosidase in frozen section of Lewis lung carcinoma xenograft with PM049.**

This data was provided by Dr. Minami (Laboratory for Systems Biology and Medicine at RCAST, University of Tokyo)

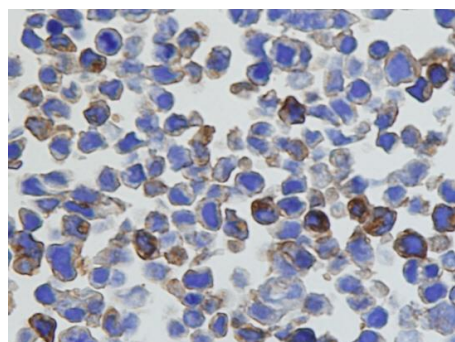
#### **Immunohistochemical staining for frozen sections: For acetone fixed section**

- 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  $\beta$ -galactosidase in transfectant with PM049.**



**Immunocytochemical detection of  $\beta$ -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  $1 \times 10^4$  cells for one slide, then incubate in a CO<sub>2</sub> 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  $\mu$ L of 1:500 Alexa Fluor<sup>®</sup> 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.

### **RELATED PRODUCTS**

#### Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
D153-A48	Anti-GFP mAb- Alexa Fluor <sup>®</sup> 488 (RQ2)
D153-A59	Anti-GFP mAb- Alexa Fluor <sup>®</sup> 594 (RQ2)
D153-A64	Anti-GFP mAb- Alexa Fluor <sup>®</sup> 647 (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-Direct (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-Direct (1G9)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-Direct (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 $\mu$ L)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
M180-A48	Anti-HA-tag mAb- Alexa Fluor <sup>®</sup> 488 (TANA2)
M180-A59	Anti-HA-tag mAb- Alexa Fluor <sup>®</sup> 594 (TANA2)
M180-A64	Anti-HA-tag mAb- Alexa Fluor <sup>®</sup> 647 (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-7	Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-6	Anti-DDDDK-tag mAb-Biotin (FLA-1)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
M185-A48	Anti-DDDDK mAb- Alexa Fluor <sup>®</sup> 488 (FLA-1)
M185-A59	Anti-DDDDK mAb- Alexa Fluor <sup>®</sup> 594 (FLA-1)
M185-A64	Anti-DDDDK mAb- Alexa Fluor <sup>®</sup> 647 (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 $\mu$ L)
M192-6	Anti-Myc-tag mAb-Biotin (My3)
M047-3	Anti-Myc-tag mAb (PL14)
M047-7	Anti-Myc-tag mAb-HRP-Direct (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
M047-A48	Anti-Myc-tag mAb- Alexa Fluor <sup>®</sup> 488 (PL14)
M047-A59	Anti-Myc-tag mAb- Alexa Fluor <sup>®</sup> 594 (PL14)
M047-A64	Anti-Myc-tag mAb- Alexa Fluor <sup>®</sup> 647 (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 $\mu$ L)
D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-Direct (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
D291-A48	Anti-His-tag mAb-Alexa Fluor <sup>®</sup> 488 (OGHis)
D291-A59	Anti-His-tag mAb-Alexa Fluor <sup>®</sup> 594 (OGHis)
D291-A64	Anti-His-tag mAb-Alexa Fluor <sup>®</sup> 647 (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)

M167-3	Anti-V5-tag mAb (1H6)	3320-205	HA-tag peptide (2 mg x 5)
M214-3	Anti-V5-tag mAb (OZA3)	3342	HA-tagged Protein Magnetic PURIFICATION KIT
M214-6	Anti-V5-tag mAb-Biotin (OZA3)	3325	DDDDK-tagged Protein PURIFICATION KIT
M214-7	Anti-V5-tag mAb-HRP-DirecT (OZA3)	3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
PM003	Anti-V5-tag pAb (polyclonal)	3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
PM003-7	Anti-V5-tag pAb-HRP-DirecT (polyclonal)	3325-205	DDDDK-tag peptide (1 mg x 5)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)	3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE
PM021	Anti-S-tag pAb (polyclonal)	3343	DDDDK-tagged Protein Magnetic PURIFICATION KIT
PM070	Anti-E-tag pAb (polyclonal)	3305	c-Myc-tagged Protein MILD PURIFICATION KIT Ver. 2
PM022	Anti-T7-tag pAb (polyclonal)	3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
563	Anti-VSV-G-tag pAb (polyclonal)	3300-205	c-Myc tag peptide (EQKLISEEDL) (1 mg x 5)
M071-3	Anti-GST-tag mAb (3B2)	3306K	c-Myc-tagged Protein PURIFICATION CARTRIDGE
PM022	Anti-GST-tag pAb (polyclonal)	3340	c-Myc-tagged Protein Magnetic PURIFICATION KIT
M095-3	Anti-Luciferase mAb (2D4)	3310	His-tagged Protein PURIFICATION KIT
PM016	Anti-Luciferase pAb (polyclonal)	3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
PM047	Anti-Renilla Luciferase pAb (polyclonal)	3317	V5-tagged Protein PURIFICATION KIT Ver.2
M094-3	Anti-β-galactosidase mAb (5A3)	3318	V5-tagged Protein PURIFICATION GEL Ver.2 (1 mL)
M203-3	Anti-β-galactosidase mAb (6F4)	3315-205	V5-tag peptide (2 mg x 5)
PM049	Anti-β-galactosidase pAb (polyclonal)	3341	V5-tagged Protein Magnetic PURIFICATION KIT
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)		
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)		
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)		
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)		
M211-3	Anti-Strep-tag II mAb (4F1)		
M214-3	Anti-mini-AID-tag mAb (1E4)		
M214-7	Anti-mini-AID-tag mAb-HRP-DirecT (1E4)		

Other related antibodies and kits are also available.  
Please visit our website at <http://ruo.mbl.co.jp/>

#### Smart-IP series

3190	Magnetic Rack
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
M214-11	Anti-V5-tag mAb-Magnetic Beads (OZA3)
D058-11	Anti-Multi Ubiquitin mAb-Magnetic Beads (FK2)
M075-11	Mouse IgG1 (isotype control)-MagneticBeads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)
M201-10	Anti-Phosphotyrosine mAb-Magnetic Agarose (PT4)

#### Protein Purification Kits

3320	HA-tagged Protein PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)