Bio Academia
Manufacturer

AS ONE

# Anti-E. coli β-Galactosidase antibody, rabbit polyclonal

Cat.# 60-060 Size: 200µg

**Background:**

β-galactosidase is an exoglycosidase which hydrolyzes the β-glycosidic bond formed between agalactose and its organic moiety. It may also cleave fucosides and arabinosides but with much lower efficiency. It is an essential enzyme in the human body. Deficiencies in the protein can result ingalactosialidosis or Morquio B syndrome. In *E. coli*, the gene of β-galactosidase, the *lacZ* gene, is present as part of the inducible system *lac* operon which is activated in the presence of lactose when glucose level is low.

It is commonly used in molecular biology as a reporter marker to monitor gene expression. It also exhibits a phenomenon called α-complementation which forms the basis for the blue/white screening of recombinant clones. This enzyme can be split in two peptides, LacZα and LacZΩ, neither of which is active by itself but when both are present together, spontaneously reassemble into a functional enzyme. This property is exploited in many cloning vectors where the presence of the *lacZα* gene in a plasmid can complement in *trans* another mutant gene encoding the LacZΩ in specific laboratory strains of *E. coli*. However, when DNA fragments are inserted in the vector, the production of LacZα is disrupted, the cells therefore show no β-galactosidase activity. The presence or absence of an active β-galactosidase may be detected by X-gal, which produces a characteristic blue dye when cleaved by β-galactosidase, thereby providing an easy means of distinguishing the presence or absence of cloned product in a plasmid. *E. coli* β-Galactosidase consists of 1,024 amino acids with molecular mass of 116 kDa and functional form is a homotetramer.

**Specifications:**

**Product:** Protein A-purified IgG from rabbit anti-βGalactosidase serum.
2 mg/ml in PBS, 50% glycerol

**Storage:** Shipped at 4℃ and store at -20℃. Do not store below -20℃

**Reactivity:** *E. coli* β-Galactosidase and β-Gal Tagged proteins.

**Immunogen**: Full-size E. coli βGalactosidase

**Applications**

- Western blotting (1/ 1,000 ~1/2,000)
- Immunoprecipitation (1/200~1/500)
- Immunofluorescent staining (1/200~1/500)
- ELISA (1/2,000~1/3,000)
- Not tested for other application

**Data Link** UniProtKB B7UJI9 (BGAL_ECO27) Entrez Gene 945006 (*E. coli lacZ*)

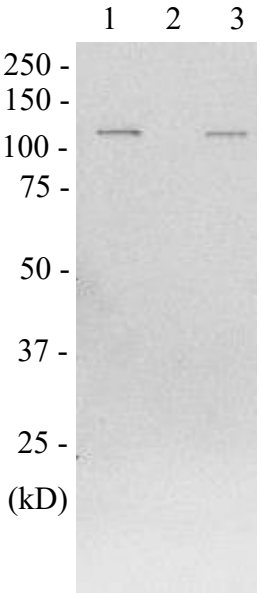


**Fig.1 Western blot analysis of β-galactosidase in E. coli crude extract.**

1. Purified β-galactosidase, 10 ng
2. Uninduced E. coli K12 cell extract (30 ug)
3. E.coli K12 cell extract induced by IPTG for β-galactosidase expression (30 ug)

The anti-β-galactosidase antibody was used at 1/1,000 dilution。Molecular mass of β-galactosidase is 116 kDa.

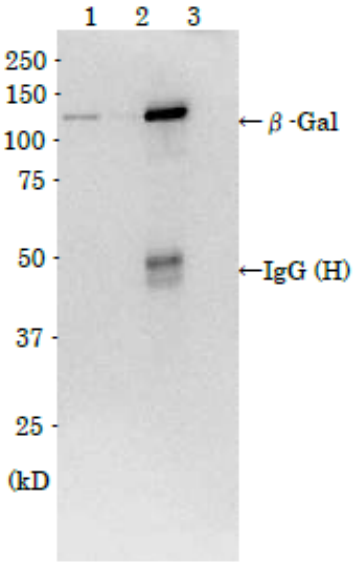


**Fig.2 Immunoprecipitation of β-galactosidase from E, coli crude extract**

Sample: Crude extract of E. coli K12 cells induced by IPTG for β-galactosidase expression.

1. Crude cell extract
2. Supernatant of the immuno-precipitated E. coli crude extract
3. Immuno-precipitate of crude E. coli extract

The anti-β-galactosidase antibody was used at 1/500 dilution for immune-precipitation and 1/1,000 dilution for western blotting.

IgG (H) is heavy chain of IgG

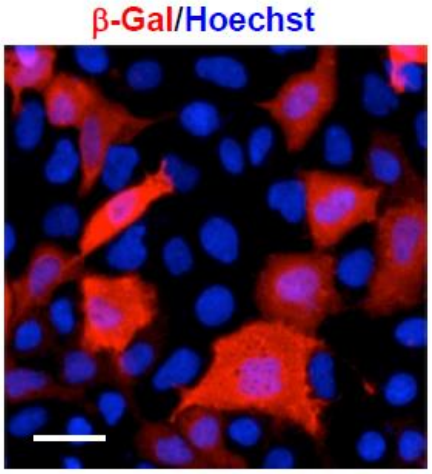


**Fig.3 Immunofluorescence staining of β-galactosidase expressed in HEK293A cells.**
HEK293A cells were transfected with β-Gal cDNA, fixed with 4% paraformaldehyde 24 hrs later, permeabilized with methanol, and immunostained with anti-β-Gal antibody (1:500) and Alexa 555-conjugated rabbit IgG (1/500). Chromosomal DNA was stained with Hoechst 33342. Scale bar, 50 mm.
Note that the antibody reacts only transfected cells.

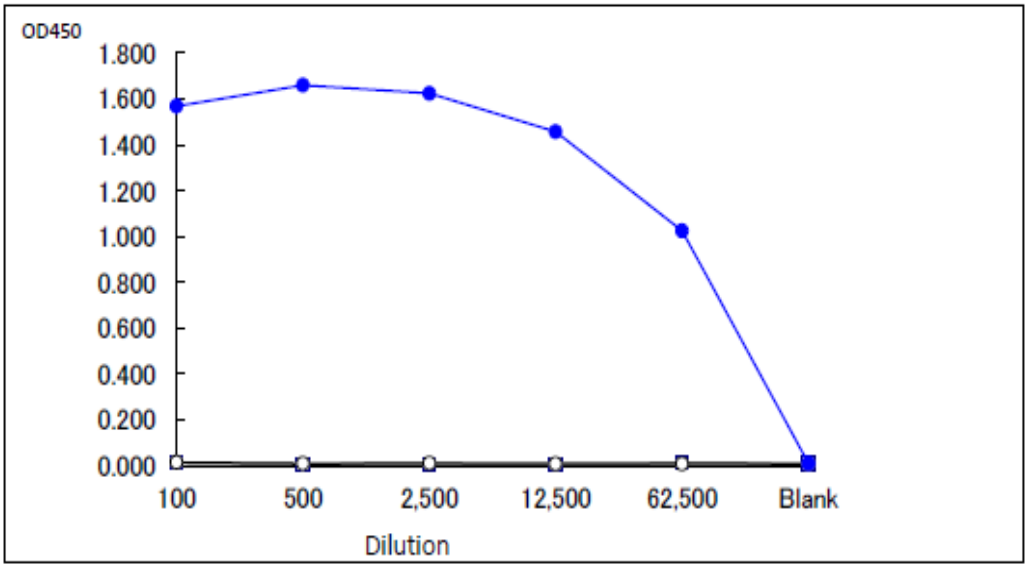


**Fig.4 Titration of antibody reactivity of anti-β-galactosidase antiserum by ELISA**
Plate was coated with 100 ng of β-galactosidase per well and 100 µl of the antiserum at the indicated dilution was added to each well and incubated. After washing, goat anti-rabbit-IgG conjugated with HRP was added as 2nd antibody. Color was developed with TMB as substrate.