APA196Ca62 100μg
Active Galactosidase Beta (GLb)
Organism Species: ***Canis familiaris; Canine (Dog)***
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Gln30~Val668
Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 6.9
Predicted Molecular Mass: 102.9kDa
Accurate Molecular Mass: 100kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8°C for one month.
Aliquot and store at -80°C for 12 months.
Stability Test: The thermal stability is described by the loss rate. The loss rate

was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

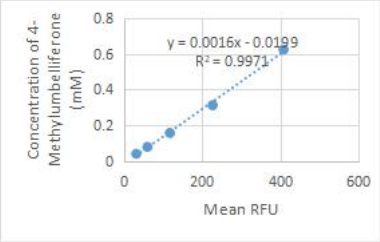
[SEQUENCE]

QRTFTIDYSHNRFLKDGQPFRYISGSIHYSRVPRFYWKDRLLKMKMAGLNAIQ
TYVPWNFHEPQPGQYQFSGEQDVEYFIKLAHELGLLVILRPGPYICAEWDMG
GLPAWLLLKESIILRSSDPDYLAAVDKWLGVLLPKMKPLLYQNGGPIITMQVEN
EYGSYFTCDYDYLRFLQKLFHHHLGNDVLLFTTDGANEKFLQCGALQGLYATVD
FGPGANITAAFQIQRKSEPKGPLVNSEFYTGWLDHWGQPHSTVRTEVVASSL
HDILAHGANVNLYMFIGGTNFAYWNGANMPYQAQPTSYDYDAPLSEAGDLT
EKYFALREVIRKFEKVPEGFIPPSTPKFAYGKVALKKLKTVEEALNVLCPPGPINSL
YPLTFIQVKQYFGFVMYRTTLPQDCSDPTPLSSPLSGVHDRAYVSVDGVPQGV
MERSNVITLNITGKAGATLDLLVENMGRVNYGRYINDFKGLISNLTLGSSILTN
WMIFPLNTEDAVRSHLGGWHGPNNGRHDKTFAHRSSNYTLPAFYMGNFSIP
SGIPDLPQDTFIQFPGWTKGQVWINGFNLGRYWPARGPQMTLFVPRHILVT
STPNTIMVLELEHAPCGDSGPEVCTVEFVDRPVIGAPPTPGHPPPDLSHRDLR
LDYV

[ACTIVITY]

GLB1 is a lysosomal beta -galactosidase that hydrolyzes the terminal beta -galactose from ganglioside and keratan sulfate. Defects in this gene are the causes of lysosomal storage diseases for GM1-gangliosidosis and Morquio B syndrome (also known as mucopolysaccharidosis IVB). In GM1 gangliosidosis, GM1 ganglioside accumulates in the neurons of the central nervous system, because of the deficiency of lysosomal beta -galactosidase activity. GM1 gangliosidosis demonstrates varying degrees of clinical severity but is invariably fatal, and children with the most common and severe form of GM1 gangliosidosis usually die within 3 years of birth. Morquio B syndrome patients are neurologically normal, but display severe skeletal dysostosis multiplex because of an accumulation of keratan sulfate.

The activity assay of GLB1 was measured by its ability to cleave a peptide substrate, 4-Methylumbelliferyl-beta -D-galactopyranoside. The reaction was performed in 50 mM Sodium Citrate, pH 3.5 (Assay Buffer), ainitiated by addition 50 μ L of 1.5 ug/ml GLB1 (diluted by Assay Buffer) to 50 μL of 1.2 mM substrate. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively, in kinetic mode for 5 minutes. The specific activity of recombinant dog GLB1 is >9700 pmol/min/μg.



RFU	4-Methylumbelliferone (mM)
407.3912	0.625
226.2912	0.3125
117.1912	0.15625
59.9312	0.078125
31.6012	0.0390625

Figure 1. The standard curve of 4-Methylumbelliferone

One unit of enzyme activity is defined as the 1 μg of enzyme required to convert 1 pmol of 4-Methylumbelliferyl-beta -D-galactopyranoside to 4-Methylumbelliferone in 1 min.

Specific Activity (pmol/min/μg)= $\frac{\Delta RFU * F}{T * N}$

△RFU=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of 4-Methylumbelliferone)

T= Time

N=Amount of enzyme

[IDENTIFICATION]

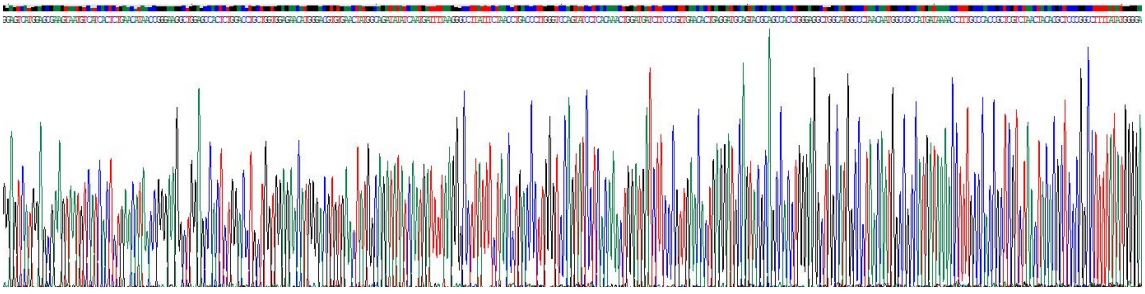


Figure 2. Gene Sequencing (extract)

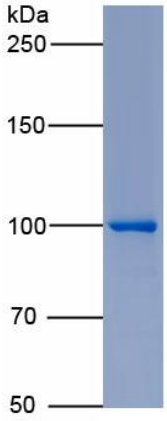


Figure 3. SDS-PAGE

Sample: Active recombinant GLb, Dog

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.