B-GALACTOSIDASE GRADE VIII FROM ESCHERICHIA COLI
Sigma Prod. No. G5635

ProductInformation

CAS NUMBER: 9031-11-2
ENZYME COMMISSION NUMBER: 3.2.1.23
SYNONYMS: Lactase, β-D-Galactoside Galactohydrolase

PHYSICAL DESCRIPTION:

Appearance: White Powder
Molecular weight: The molecular weight is 465,396 based upon the most current amino acid sequence data.[1] A previous molecular weight that is often cited in the literature is 540,000.[2]
$E^{1\%}$ (280 nm) = 20.9[3]
Isoelectric point: The pI of β-Galactosidase is 4.61.[4]
pH Optimum: The pH optimum for this enzyme is 6-8. Sigma assays the β-galactosidase using o-nitrophenyl-β-D-galactoside as the substrate at pH 7.3.[4]
Salts present: The salt content of this product is approximately 15-20%. The composition of the buffer in prior to lyophilization, was 10 mM Tris HCl, 10 mM $MgCl_2$, and 1 mM β-mercaptoethanol.

STRUCTURE:

β-Galactosidase is a tetramer consisting of four equal subunits of 135,000 each. It is a sulfhydryl containing enzyme, with 19 cysteine residues per subunit.[3] Some of these are present in disulfide bridges. The SH groups are thought to be important in maintaining the active conformation of the enzyme. The number of active sites per molecule of enzyme is thought to be one per monomer of 135,000. Histidine residues are believed to be present in the active site of β-galactosidase.[4]

ACTIVATORS:

Both magnesium and sodium are activators of β-galactosidase when o-nitrophenyl-β-D-galactoside is used as the substrate. The optimal concentration of magnesium can range from 0.1 mM to 10 mM, depending upon the sodium concentration that is present.[5] Sigma assays this product at a magnesium concentration of 10 mM.

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INHIBITORS:

Inhibitors of β-galactosidase include heavy metals which complex sulfhydryl groups (such as copper, lead, and mercury.)[4] β-galactosidase can also be inhibited by EDTA, which chelates magnesium.[6]

SUBSTRATES:

β-Galactosidase will hydrolyze the natural disaccharide lactose as well as many chromogenic substrates: p-β-D-galactopyranoside, o-nitrophenyl-β-D-galactopyranoside, phenyl-β-D-galactoside, and 2,4-dinitrophenyl-β-D-galactoside, methylumbelliferyl-β-D-galactopyranoside (fluorogenic substrate) and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) which is used for histochemical staining.[4,7]
Km = 4.45 x 10^{-4}M (p-nitrophenyl-β-D-galactopyranoside)
Km = 3.85 x 10^{-3}M (lactose)

APPLICATIONS:

One of the most common uses of β-galactosidase is in detection systems. β-Galactosidase is conjugated to an antibody which specifically recognizes a target molecule (enzyme immunoassay or EIA). A method of conjugating β-galactosidase to a Fab fragment of IgG is described.[8] Another use of β-galactosidase is also used as a reporter enzyme to monitor the level of gene expression of a promoter. The product of most promoters is difficult to measure, so the promoter can be joined to a reporter gene which codes for a specific enzyme activity (such as β-galactosidase).[9]

METHOD OF PREPARATION:

A method of preparation for β-galactosidase (although not that necessarily used by Sigma) is described.[3]

STABILITY / STORAGE AS SUPPLIED:

This product is stable for at least one year when stored at -0°C.

SOLUBILITY / SOLUTION STABILITY:

Solutions of this enzyme should be prepared fresh. The presence of sulfhydryl groups makes it quite susceptible to oxidation. Oxidation may be minimized by preparing solutions in 50 mM Tris HCl, pH 7.3, 5-10 mM β-mercaptoethanol, and 10 mM $MgCl_2$. A clear and colorless solution is observed in this solvent. Thiol reducing reagents should only be used in the presence of magnesium chloride, since inhibition has been observed with β-mercaptoethanol when no magnesium chloride is present.[10]

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UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of o-nitrophenyl β-D-galactoside to o-nitrophenol and D-galactose per minute at pH 7.3 at 37°C.

REFERENCES:

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9. Young, D.C. et al., *Analytical Biochemistry*, 215, 24-30
10. Shifrin, S. et al., Nature, 227, 608-609 (1970) (1993).