



β-GALACTOSIDASE

SPECIFICATIONS

Product Code: BG13

Specific activity: >750 U/mg

Protein concentration: >20 mg/ml

Shipped with ice pack for next day delivery. Store at -20°C.

Stability: Enzyme is supplied in 50% glycerol, 25 mM sodium phosphate (pH 7.0). Stable at least 12 months when stored properly.

PROZYME® β-Galactosidase (β-D-galactoside galactohydrolase E.C. 3.2.1.23; lactase) is prepared from *Escherichia coli*. The enzyme catalyzes the hydrolysis of lactose and many β-D-galactopyranosides. The DNA sequence of the gene (LacZ) has been determined and encodes a 116,000 dalton polypeptide of 1023 amino acids in addition to the N-terminal f-met.

Wallenfels and Weil (1972) have reviewed the extensive literature on the enzymology of β-Galactosidase. The properties of the enzyme vary depending on both the substrate and buffer; what follows refers to cleavage of *o*-nitrophenyl-β-D-galactopyranoside (ONPG). Monovalent cations such as Na⁺ and K⁺ are stimulatory (Lederberg, 1950; Neville and Ling, 1967; Becker and Evans, 1969). Divalent cations such as Mg⁺⁺ are stimulatory under some conditions (Reithel and Kim,

1960; Wallenfels and Weil, 1972). Mg⁺⁺ is also capable of reversing the inhibition of β-galactosidase by EDTA (Reithel and Kim, 1960). Some divalent cations such as Cu⁺⁺, Pb⁺⁺ and Hg⁺⁺ are inhibitory (Wallenfels and Weil, 1972). The effects of 2-mercaptoethanol on β-galactosidase are complex: it is stimulatory (as are some other alcohols; Wallenfels and Weil, 1972), but inactivates the enzyme upon storage; although the presence of Mg⁺⁺ stabilizes the enzyme (Reithel *et al*, 1966; Shifrin *et al*, 1970), though perhaps not after long periods of exposure.

CHARACTERISTICS

Molecular weight: 465,000 daltons

Composition: The enzyme is a homotetramer with subunits of 116,000 daltons.

Extinction coefficient: $E_{280}^{1\%} = 20.9$ (Craven *et al*, 1965)

pH optimum: 6.8 (in the presence of Mg⁺⁺; Reithel and Kim, 1960).

Activators: Monovalent cations; Mg⁺⁺ and some divalent cations; some alcohols including 2-mercaptoethanol.

Inhibitors: α-galactosides (Kuby and Lardy, 1953); chelating agents; some heavy metals; organomercuric compounds.

Isoelectric point: 4.6 (Wallenfels and Weil, 1972)

Applications: β -galactosidase is often used as a reporter enzyme in enzyme immunoassays. The turnover number is higher than that of horseradish peroxidase and similar to that of alkaline phosphatase. Highly sensitive fluorometric assays have been developed using β -galactosidase and the synthetic substrate 4-methylumbelliferyl- β -D-galactopyranoside (Ishikawa and Kato, 1978; Imagawa *et al*, 1981; Labrousse *et al*, 1982). The enzyme is also used in colorimetric assays with ONPG (see ASSAY) as substrate. Conjugation procedures using the crosslinking agents N,N'-*o*-phenylenedimaleimide (Kato *et al*, 1976; Hamaguchi *et al*, 1979) or *m*-maleimidobenzoyl-N-hydroxysuccinimide ester (O'Sullivan *et al*, 1979; O'Sullivan and Marks, 1981) have been described.

Origin: USA

ASSAY

One unit of PROZYME β -galactosidase will hydrolyze 1 micromole of ONPG to *o*-nitrophenol and D-galactose per minute at pH 7.3 and 37°C.

Reagents

0.1 M sodium phosphate buffer (pH 7.3 at 37°C)
10 mg/ml ONPG in buffer
1M MgCl₂
2-mercaptoethanol

Procedure

Adjust spectrophotometer to 410 nm and 37°C.

Prepare the following reaction mix and prewarm to 37°C:

Buffer	27.4 ml
ONPG stock	2.3 ml
MgCl ₂ stock	0.03 ml
2-mercaptoethanol	0.260 ml

Prepare enzyme diluent as follows:

Buffer	3.0 ml
MgCl ₂ stock	0.003 ml
2-mercaptoethanol	0.026 ml

Prepare a dilution of the enzyme to contain 0.2 to 0.8 U/ml. Add 100 μ l of diluted enzyme to a 1.5 ml cuvette at 37°C, then add 1 ml of reaction mix. Record the increase in absorbance at 410 nm while keeping the temperature constant at 37°C.

Calculation

$$\text{Units/ml} = \frac{(\Delta A_{410}/\text{min})(V_r)(D)}{(V_e)(\epsilon_{410})}$$

where:

$\Delta A_{410}/\text{min}$ = change in absorbance per minute

D = dilution factor

V_r = assay volume (1.1 ml)

ϵ_{410} = millimolar extinction coefficient of *o*-nitrophenol (3.5 $\text{mM}^{-1}\text{cm}^{-1}$)

V_e = enzyme volume (0.1 ml)

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