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ProductInformatio

β-GLUCURONIDASE, Bacterial
from Escherichia coli
Sigma Prod. No. G7896

CAS: 9001-45-0
ENZYME COMMISSION NUMBER: 3.2.1.31
SYNONYMS: β-D-Glucuronide glucuronosohydrolase; GUS

PHYSICAL DESCRIPTION:

Appearance: White to tan, lyophilized powder
Molecular weight: 68,259 (E. coli, monomer, 602 residues)[1]
Isoelectric Point: 4.8[2]
pH optimum: 6-7[2] The enzyme activity for this product is determined by Sigma at pH 6.8.
Salts present: Lyophilized from 10 mM potassium phosphate, 1 mM ethylenediamine tetraacetic acid and 1 mM dithiothreitol. Sucrose is present as a stabilizer
Specificity: Hydrolyzes a large number of glucuronides, but does not react with α-glucuronides or β-glucosides.[3]

STRUCTURE:

β-Glucuronidase is composed of four subunits. The tetramer has a molecular weight of approximately 290,000.[2]

ACTIVATORS:

Deoxyribonucleic acid and numerous diamines are activators.[3]

INHIBITORS:

Inhibitors of β-glucuronidase include certain divalent cations (Cu^{2+}, Zn^{2+})[4]; saccharic acid 1,4-lactone (Ki=170 nM), galacturonic acid (Ki= 4.3 mM) and glucuronic acid (Ki= 1.5 mM)[6].

SUBSTRATES:

Natural substrates include: Chondroitin Sulfate B (or Dermatan Sulfate), Heparan Sulfate, Estrone β-D-Glucuronide (Km = 0.02 mM), and Phenolphthalein Glucuronic Acid (Km = 0.018-3.08 mM).[5]

SUBSTRATES: (continued)

Synthetic substrates include: p-Nitrophenyl β-D-glucuronide (Km = 0.13-2.9 mM); 5-Bromo-4-chloro-3-indolyl-β-D-glucuronide; 5-Bromo-6-chloro-3-indolyl-β-D-glucuronide; 5-Bromo-3-indolyl-β-D-glucuronide; 6-Bromo-2-naphthyl-β-D-glucuronide; 6-Chloro-3-indolyl-β-D-glucuronide; Fluorescein mono-β-D-glucuronide; 8-Hydroxyquinoline glucuronide; 4-Methylumbelliferyl-β-D-glucuronide (MUG, Km = 0.041-1.3); Naphthol-AS-BI-β-D-glucuronic acid; 4-Trifluoromethylumbelliferyl glucuronide.[5]

APPLICATIONS:

β-Glucuronidase from E. coli has become the reporter enzyme of choice for genetic plant research. Coexpression of β-glucuronidase activity in recombinant plant cells has become a widely used technique for demonstration of exogenous gene fusions. Naleway gives extensive methodology for the GUS enzyme systems using both fluorogenic and chromogenic systems.[4] β-Glucuronidase from E. coli is effective in the hydrolysis of steroid glucuronides.[7]

β-Glucuronidase is highly sensitive to trace components, i.e. chloroform, which could result in as much as a three-fold variation in activity. For quantitative work, one should establish a base substrate activity and adjust subsequent analyses for the established activity.[8]

METHOD OF PREPARATION:

This product is prepared from E. coli. It is lyophilized from 10 mM potassium phosphate, 1 mM ethylenediamine tetraacetic acid and 1 mM dithiothreitol. Sucrose is added as a stabilizer.[8]

STABILITY / STORAGE AS SUPPLIED:

β-Glucuronidase from E. coli has exhibited full activity after five years when stored frozen.[8]

SOLUBILITY / SOLUTION STABILITY:

The enzyme can be reconstituted at 5 mg/mL in 75mM phosphate buffer, pH 6.8 giving a slightly hazy to a clear, colorless solution. It is best to add 1 mg/mL bovine serum albumin as a stabilizer. The 5 mg/mL solution can be frozen at -20°C for up to two months with no loss in activity.[8]

UNIT DEFINITION:

One Sigma or modified "Fishman" unit will liberate 1.0 µg of phenolphthalein from phenolphthalein glucuronide per hour at 37°C at pH 6.8 (30 minute assay).

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