

# **ISOAMYLASE** from *Pseudomonas* sp. (Lot 130104b)

#### E-ISAMY

(EC 3.2.1.68) glycogen 6-alpha-D-glucanohydrolase CAZy: GH Family 13

## PROPERTIES

## I. ELECTROPHORETIC PURITY:

- Single major band on SDS-gel electrophoresis (MW = 71,500).

- Single major band on isoelectric focusing (pl = 5.0)

#### 2. SPECIFIC ACTIVITY:

All activities are at 40°C and pH 4.0. Isoamylase was assayed using oyster glycogen and using the Nelson/Somogyi reducing sugar procedure.  $\alpha$ -Amylase was measured using reduced maltoheptaose (10 mM) as substrate with measurement of reducing sugar increase, and by monitoring hydrolysis of maltoheptaose by HPLC using a Waters Sugar Pac<sup>®</sup> column. Incubation of 100 U of isoamylase with 0.2 mL of maltoheptaose (10 mg/mL) at pH 4.0 resulted in no production of low molecular weight oligosaccharides in 16 h. Maltase ( $\alpha$ -glucosidase) was measured with maltose (10 mg/mL) as substrate and exo- $\alpha$ -glucanase was measured with linear- $\alpha$ -1,4-maltodextrins (10 mg/mL) as substrate with measurement of released D-glucose.

Enzyme Activity	Substrate	Activity (U/mg protein)
Isoamylase	Oyster glycogen	260
$\alpha$ -amylase	Reduced Maltoheptaose	< 0.001
Maltase	Maltose	< 0.001
Exo- $\alpha$ -Glucanase	Linear- $\alpha$ -1,4-maltodextrins	< 0.000001

**One unit** of activity is the amount of enzyme required to release one micromole of reducing sugar equivalent from the defined substrate per min at pH 4.0 and  $40^{\circ}$ C. One Unit as defined here is approximately equal to 160 Sigma Isoamylase enzyme Units. Megazyme can supply an assay format based on glycogen/iodine if required. This allows the measurement of isoamylase in the presence of ammonium sulphate.

This enzyme is ideally suited for starch structural research.

## 3. PHYSICOCHEMICAL PROPERTIES:

pH Optima:	3.0 - 4.5
pH Stability:	3.5 - 5.5 (4 hr, 40°C )
Temperature Optima:	50°C
Temperature Stability:	< 45°C (pH 4.0, 30 min)

#### 4. **PRODUCT DETAILS:**

The enzyme is supplied as an ammonium sulphate suspension at 1000 U/mL in 0.02% sodium azide and should be stored at  $4^{\circ}$ C.

This enzyme is **very unstable** to freezing and thawing. **DO NOT FREEZE !** 

It is recommended that all buffers used for dilution contain BSA (I mg/mL).

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