ISOAMYLASE (Glycogen 6-glucanohydrolase) (*Flavobacterium odoratum*) (Lot 200601a)

Non-recombinant; Crystalline suspension. Purity (activity) ~ 100%.

E-ISAMYFO **06/20**

EC: 3.2.1.68
Synonyms: isoamylase; glycogen 6-alpha-D-glucanohydrolase
CAZy Family: GH13
CAS: 9067-73-6

PROPERTIES

1. ELECTROPHORETIC PURITY:

- Single major band on SDS-gel electrophoresis (MW = 83,000) with some minor bands.
- Single major band on isoelectric focusing (pI = 8.8).

2. SPECIFIC ACTIVITY:

140 U/mg protein (on oyster glycogen) at pH 6.0 (with 1 mM $CaCl_2$) and 40°C

One Unit of isoamylase activity is defined as the amount of enzyme required to release one µmole of glucose reducing sugar equivalents per minute from oyster glycogen (10 mg/mL) in MES buffer (100 mM, pH 6.0) plus 1 mM $CaCl_2$ at 40°C. One Unit as defined here is equivalent to ~ 67 KU of isoamylase as defined by Sigma Chemical Co.

3. SPECIFICITY:

Hydrolysis of (1,6)-α-D-glucosidic branch linkages in glycogen, amylopectin and their β-limit dextrins.

4. RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES:

Enzyme Activity	Substrate	Activity, %
Isoamylase	Oyster glycogen (10 mg/mL)	100
	Amylopectin (5 mg/mL)	35
α-Amylase	Reduced maltoheptaose	< 0.0004
Maltase	Maltose	< 0.0003
exo-α-Glucanase	Linear-α-1,4-maltodextrins	< 0.0004
α-glucosidase	*p*-NP-α-glucoside	< 0.0004
Ceralpha Reagent	Benzylidene blocked *p*NP maltoheptaoside	~ 0.25 [a]

[a] Isoamylase can remove terminal *p*NP from *p*NP-maltosaccharides of DP > 5. This should not be confused with *endo*-hydrolysis of maltodextrin chains by α-amylase.

Activities were measured at 40°C and pH 6.0 in the presence of 1 mM $CaCl_2$. α-Amylase was measured by monitoring hydrolysis of maltoheptaose by Dionex chromatography. Incubation of 20 U of isoamylase with 1.0 mL of maltoheptaose (10 mg/mL) at pH 6.0 (plus 1 mM $CaCl_2$) resulted in no production of low molecular weight oligosaccharides within 4 h. Maltase (α-glucosidase) was measured with maltose (10 mg/mL) as substrate and *exo*-α-glucanase was measured with linear-α-1,4-maltodextrins (10 mg/mL) as substrate with measurement of released D-glucose.

This enzyme is ideally suited for research on structure of starch and phytoglycogens.

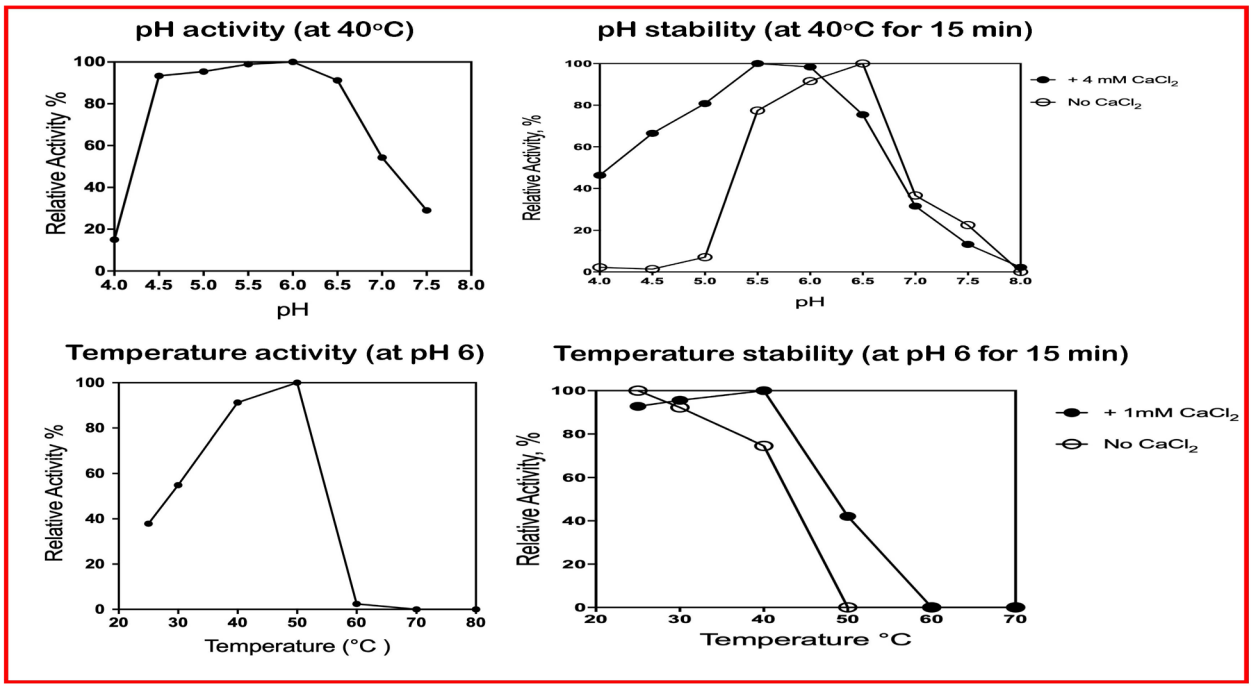
4. PHYSICOCHEMICAL PROPERTIES:

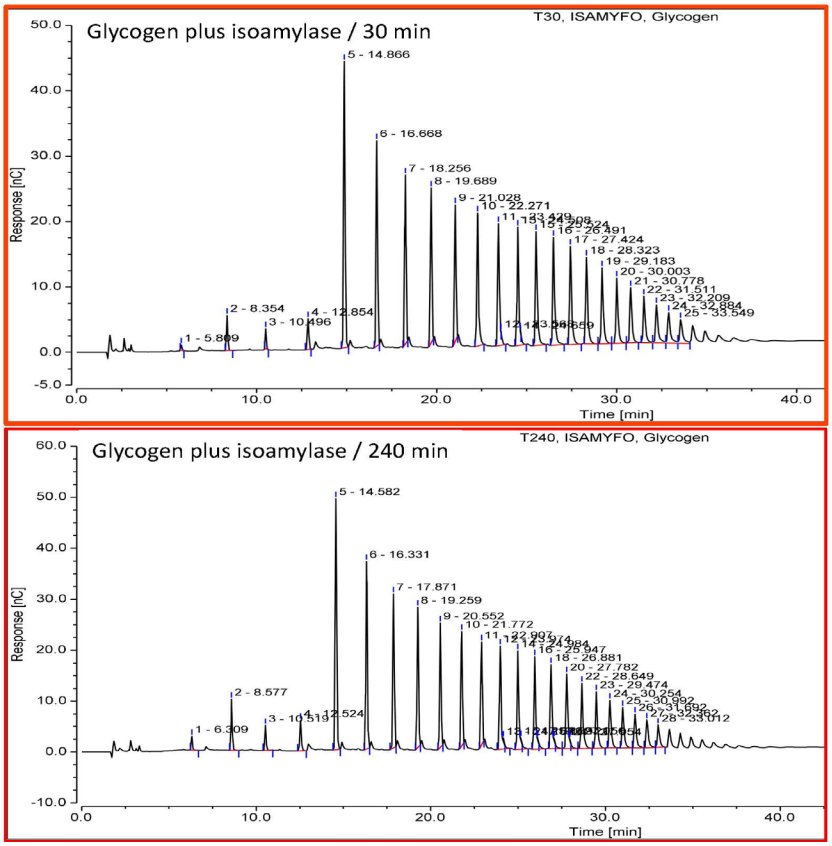
Recommended conditions of use are at pH 6.0 in the presence of 1 mM $CaCl_2$ and at up to 40°C.

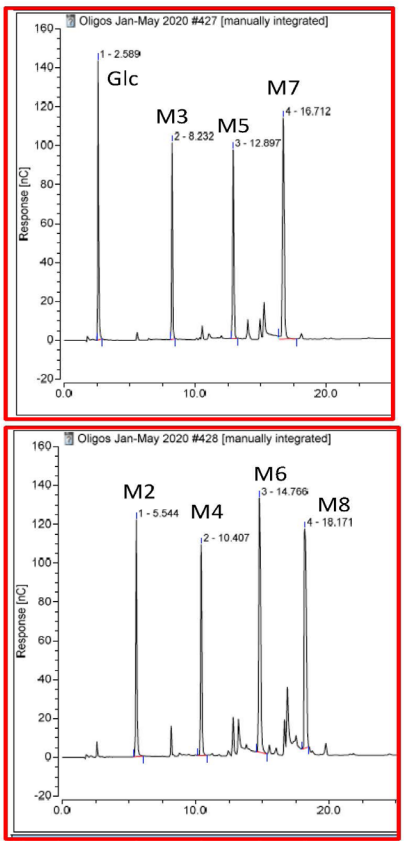
pH Optima (in presence of 1 mM calcium): 4.5-6.5.
pH Stability (in presence of 1 mM calcium): 3.5-6.0 (16 h, 4°C)
Temperature Optima (in presence of 1 mM $CaCl_2$): 50°C (assayed at pH 6 for 10 min)
Temperature Stability (in presence of 1 mM $CaCl_2$): < 50°C (incubated at pH 6 for 15 min)

5. PRODUCT DETAILS:

The enzyme is supplied as a crystalline suspension at ~ 200 U/mL in 3.2 M ammonium sulphate solution containing 0.02% sodium azide. Store at 4°C. **Swirl to mix the enzyme before dispensing.**







Hydrolysis of oyster liver glycogen by Flavobacterium sp. isoamylase. Incubation for 30 and 240 min to demonstrate complete debranching of the glycogen as well as the purity of the isoamylase (lack of production of glucose and limited increase in level of maltose).

INCUBATION CONDITIONS:

Oyster liver glycogen (20 mL, 5 mg/mL) in MES buffer (100 mM, pH 6) containing $CaCl_2$ (1 mM) incubated with 20 U of **E-ISAMYFO** at 40°C for either 30 or 240 min. Reaction terminated by heating the reaction solution in a boiling water bath for 5 min. Sample filtered and analysed using HPAEC-PAD (Dionex ICS5000 + DP system and Chromeleon 7 software) with CarboPac PA200 guard and analytical columns DIONEX ion chromatography.