ENZYMES

Glucose Oxidase HPS300

ORIGIN *Aspergillus niger*

CAT# GLOX-70-6456
EC# 1.1.3.4

SPECIFICATIONS

Appearance	Yellow freeze dried powder
Powder Activity	>220 U/mg powder at 25°C
Specific Activity	>250 U/mg protein at 25°C
Solubility	Dissolves readily at 10 mg/mL in water to give a clear yellow solution essentially free from particulate matter
Contaminants	GO: Catalase Ratio >10,000:1
	Invertase< 0.005%
	Amylase< 0.001%
	Trehalase< 0.001%
	α-Glucosidase< 0.001%
	ß-Glucosidase< 0.001%

APPLICATION

Used in the determination of D-glucose in blood or urine.

UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will catalyse the oxidation of 1.0 micromole of glucose per minute at 25°C under the standard assay method conditions.

ASSAY PRINCIPLE

Glucose Oxidase catalyses the following reaction:

$$\text{ß-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{D-Glucono-}\partial\text{-lactone} + H_2O_{2a}$$

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CHARACTERISTICS

HPS300 is a highly purified grade of glucose oxidase for use in biosensors and clinical chemistry. HPS300 contains very low levels of specific contaminating activities that are likely to cause interference, either by reacting with other sugars in the sample, or by reacting with components of the biosensor and reagent formulation.

The Table below shows contaminating activities in a typical batch expressed as a % of Glucose Oxidase activity.

GO:Catalase Ratio:	66,892:1
Invertase:	0.0012%
Amylase:	0.00029%
Trehalase:	0.0000011%
α-Glucosidase:	0.000012%
ß-Glucosidase:	0.000021%

Molecular Weight[1]:	160kD
Structure[1]:	Glycoprotein with 2 equal subunits and 2 moles of FAD
Isoelectric point[2]:	4.2
K_m (Calculated in-house):	3.1 x 10^{-2} M
Optimum pH (Fig. 1):	pH 5.5 to 7.0
Optimum Temperature (Fig. 2):	30°C
pH Stability (Fig. 3):	pH 4.0 to 8.0 (25°C for 20 hours)
Thermal Stability (Fig. 4):	Stable at 50°C (pH 7.0 for 15 minutes)

TABLE 1: SUBSTRATE

Substrate specificity was tested in-house by substituting different sugars for glucose in the standard glucose oxidase assay procedure. Assays were based on a sugar concentration of 30mM.

SUBSTRATE	% OF D (+)-GLUCOSE ACTIVITY	SUBSTRATE	% OF D (+)-GLUCOSE ACTIVITY	SUBSTRATE	% OF D (+)-GLUCOSE ACTIVITY
D (+)-Glucose	100	D (+)-Maltose	0.004	D-Mannitol	<0.0001
2-Deoxy-D-Glucose	10.4	D (+)-Gluconic acid ∂--lactone	0.003	L (-)-Glucose	<0.0001
D (+)-Mannose	0.26	D (-)-Fructose	0.002	D (+)-Lactose	<0.0001
D (+)-Galactose	0.10	D-Sorbitol	0.0007	Sucrose	<0.0001
D (+)-Xylose	0.042	D-Ribose	<0.0001	D (+)-Trehalose	<0.0001

FIGURE 1: OPTIMUM pH

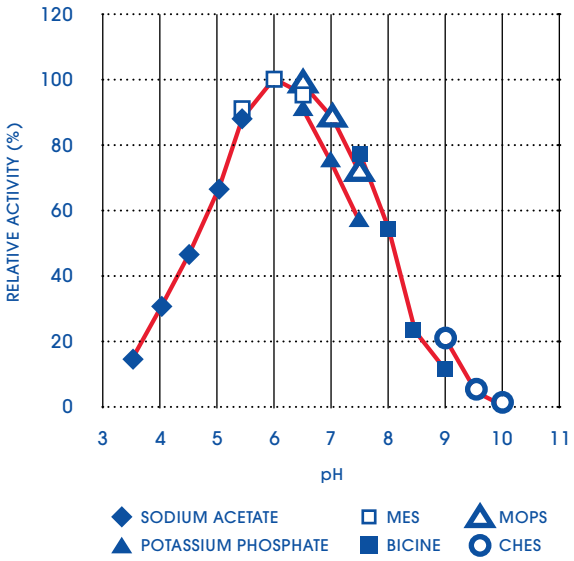


FIGURE 2: OPTIMUM TEMPERATURE

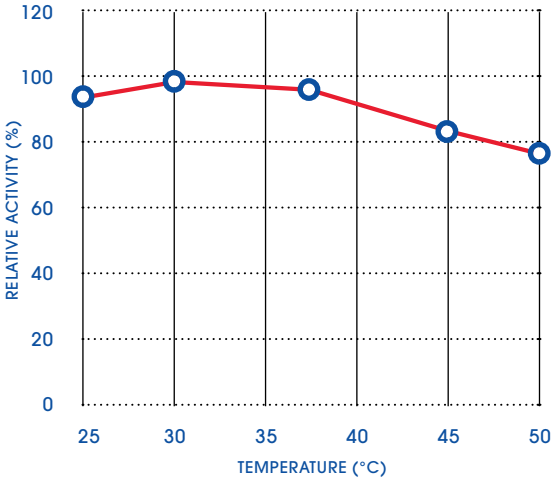


FIGURE 3: pH STABILITY (25°C FOR 20 HOURS)

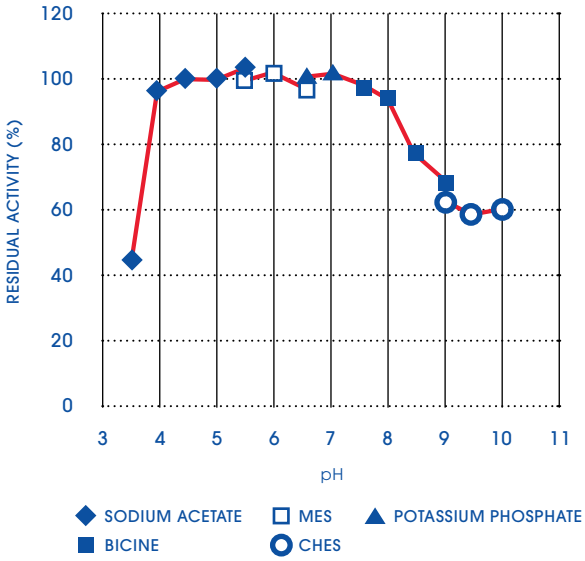
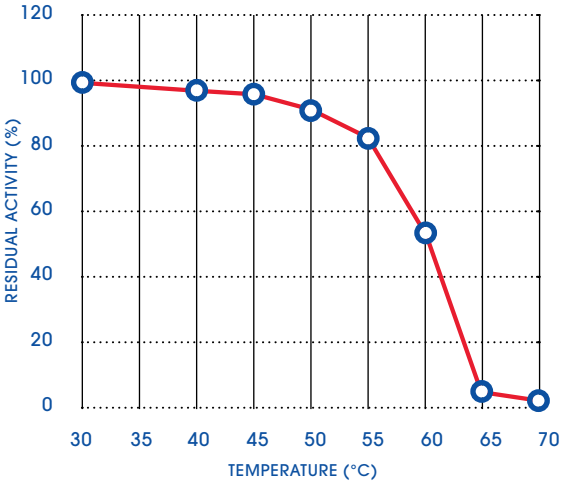


FIGURE 4: THERMAL STABILITY (pH 7.0 FOR 15 MINUTES)



(1) Tsuge, H.J., et al. Purification, properties, and molecular features of glucose oxidase from Aspergillus niger. J. Biochem., 78, 835-843 (1975).
(2) Pazar, J.H. and Kleppe, K. The oxidation of glucose and related compounds by the glucose oxidase from Aspergillus niger. Biochemistry 3: 578 – 583(1964).

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