ENZYMES

Glucose Dehydrogenase FAD Dependent

ORIGIN *Aspergillus sp.*

CAT# GLDE-70-1192
EC# 1.1.99.10

SPECIFICATIONS

Appearance	Yellow freeze dried powder
Activity	>900 U/mg powder at 37°C
Solubility	Dissolves readily at 10 mg/ml in water
Contaminants	**Glucoamylase** <0.01%
	Hexokinase <0.01%
	Glucose Dehydrogenase (NAD dependent) <0.01%

ASSAY PRINCIPLE

FAD dependent Glucose Dehydrogenase (FAD-GDH) catalyses the oxidation of glucose in the presence of an electron acceptor, such as 2,6-dichlorophenolindophenol or potassium ferricyanide:

β-D-Glucose + Acceptor $\xrightarrow{\text{FAD-GDH}}$ Glucono-1,5-lactone + Reduced Acceptor

UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will convert one micromole of glucose per minute at 37°C under the standard assay method conditions. Refer to Table 1 for guidance on factors to adjust units according to temperature of assay.

ASSAY TEMPERATURE (°C)	FACTOR RELATIVE TO 37°C RESULT	ASSAY TEMPERATURE (°C)	FACTOR RELATIVE TO 37°C RESULT
25°C	0.70	37°C	1.00
30°C	0.81	45°C	1.14

APPLICATION

Used in the determination of D-glucose in blood or urine. Suitable for use in glucose biosensors.

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CHARACTERISTICS

FAD-GDH is highly purified and suitable for use in glucose biosensors. The enzyme preparation is essentially free from impurities that are likely to cause interference by reacting with glucose in the sample.

Molecular Weight (SDS Page):	97kD diffuse band expected of a glycosylated protein
Molecular Weight (Gel Filtration):	130kD
Isoelectric Point:	4.4
$K_m$value (Eadie-Hofstee):	5 x 10^{-2} M (D-Glucose)
Optimum pH (Fig. 1):	pH 7.0 to 8.0
Optimum Temperature (Fig. 2):	at least 50°C
Stable pH Range (Fig. 3):	pH 5.0 - 7.5 (25°C for 20 hours)
Thermal Stability (Fig. 4):	Stable up to 50°C (pH 6.0 for 15 minutes)

SUBSTRATE SPECIFICITY

Substrate specificity was tested by substituting different sugars for glucose in the FAD-GDH assay. Assays were based on using a sugar concentration of 30 mM. See Table 2.

SUBSTRATE (30mM)	% OF D-GLUCOSE ACTIVITY	SUBSTRATE (30mM)	% OF D-GLUCOSE ACTIVITY
D-Glucose	100%	L-Glucose	<0.1%
2-Deoxy-D-glucose	25%	D-Mannitol	<0.1%
D-Xylose	11%	D-Lactose	<0.1%
D-Galactose	0.7%	D-Sorbitol	<0.1%
D-Mannose	0.4%	D-Ribose	<0.1%
D-Trehalose	0.2%	D-Maltose	<0.1%
D-Fructose	0.1%	Sucrose	<0.1%

INHIBITION

This section is still under evaluation, however it is known that the FAD-GDH reaction is inhibited by 1,10-phenanthroline (>80% inhibition with 50 mM concentration).

FIGURE 1: OPTIMUM pH

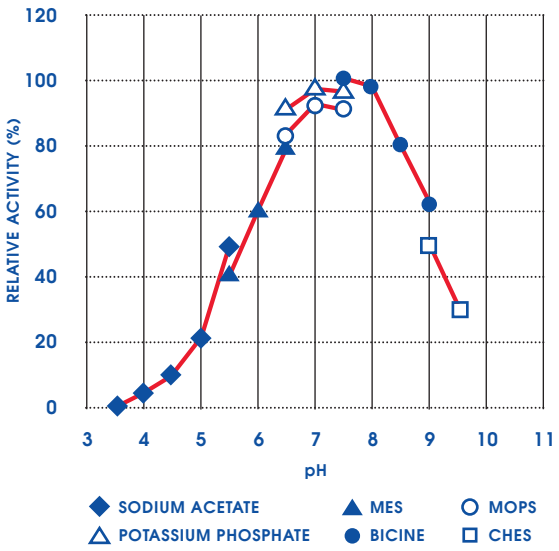


FIGURE 2: OPTIMUM TEMPERATURE

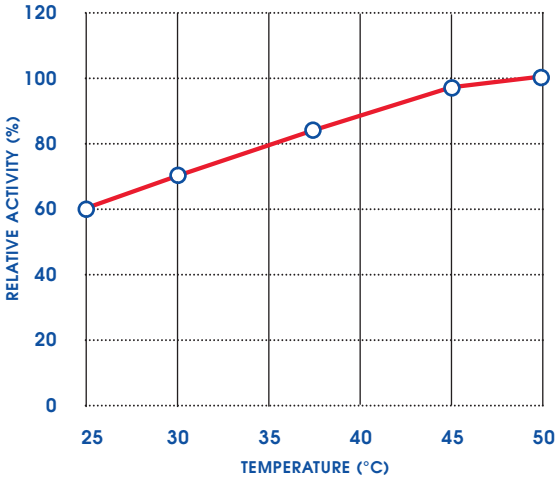


FIGURE 3: pH STABILITY (25°C FOR 20 HOURS)

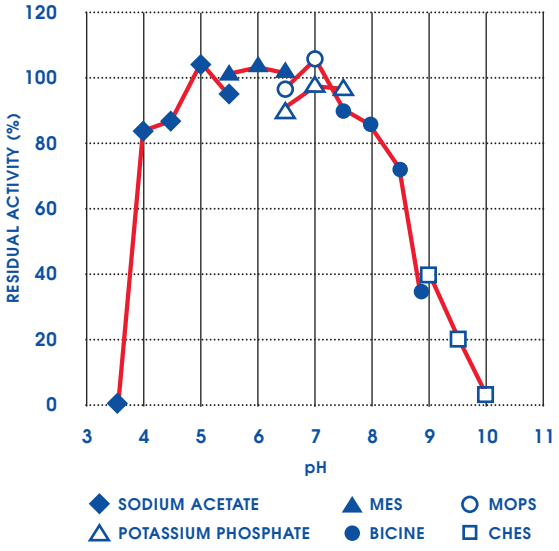
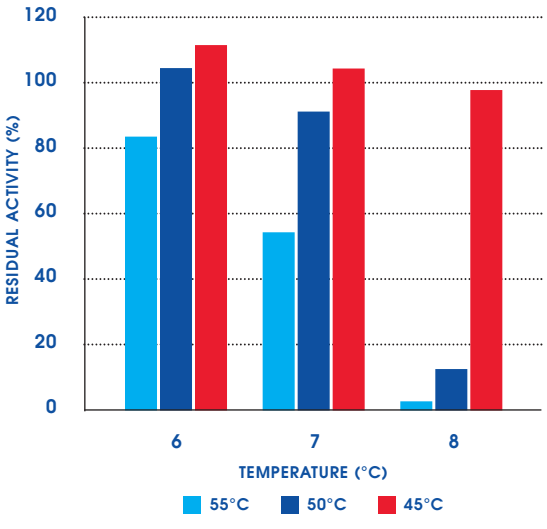


FIGURE 4: THERMAL STABILITY (pH 6.0 FOR 15 MINS.)



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