

Glucose Dehydrogenase FAD Dependent

Catalogue No. GLDE-70-1192
E.C number 1.1.99.10

ORIGIN

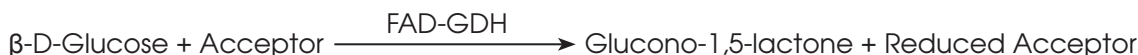
Aspergillus sp.

SPECIFICATIONS

Appearance Yellow freeze dried powder
 Activity >900 U/mg powder at 37°C
 Solubility Dissolves readily at 10 mg/ml in water
 Contaminants:
 o Glucoamylase <0.01%
 o Hexokinase <0.01%
 o 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:



APPLICATION

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

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 (available on request). Refer to Table 1 for guidance on factors to adjust units according to temperature of assay.

TABLE 1 Unit Conversion Factors

Assay Temp (°C)	Factor Relative to 37°C Result	Assay Temp (°C)	Factor Relative to 37°C Result
25°C	0.70	37°C	1.00
30°C	0.81	45°C	1.14

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.

Its main characteristics are as follows:

- Molecular Weight (by SDS-Page) 97kD diffuse band expected of a glycosylated protein
- Molecular Weight (by gel filtration) 130kD
- Isoelectric point. 4.4
- K_m (Calculated using Eadie-Hofstee) 5×10^{-2} M (D-Glucose)
- pH Optimum (**Fig. 1**) pH 7.0 to 8.0
- Temperature Optimum (**Fig. 2**) at least 50°C
- Stable pH range (**Fig. 3**) pH 5.0 to 7.5 (25°C for 20 hours)
- Thermal stability (**Fig. 4**) Stable up to 50°C (pH 6.0 for 15 mins)

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.

TABLE 2 Substrate Specificity

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).

Further investigations to be completed.

