MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 100289, 100330, 195196
Glucose Oxidase
Molecular Weight: ~160,000[1]
CAS #: 9001-34-0
Physical Description: Yellow lyophilized powder
Source: *Aspergillus niger*
Isoelectric Point: 4.2
Michaelis Constants: phosphate buffer, pH 5.6; 25°C, air:

Glucose:	3.3×10^{-2} mol/l[(2)]
Glucose:	1.1×10^{-1} mol/l[(3)]
2-Deoxyglucose:	2.5×10^{-2} mol/l
O_2:	2.0×10^{-4} mol/l

Structure: The enzyme contains 2 moles FAD/mole GOD.
Inhibitors: Ag^+, Hg^{2+}, Cu^{2+} [(4)], 4-chloromercuribenzoate, D-arabinose (50%). FAD binding is inhibited by several nucleotides.[5]
pH Optimum: 6.5
pH Stability: 8.0
pH Range: 4-7
Thermal Stability: Below 40°C
Description: Glucose oxidase is an FAD-containing glycoprotein. The enzyme is specific for b-D-glucose. O_2 can be replaced by hydrogen acceptors such as 2.6-dichlorophenol indophenol.
Relative Rates: D-glucose, 100; D-mannose, 20; 2-Deoxy-D-glucose, 20; negligible on other hexoses.
Solubility: Dissolves readily at 5 mg/ml in 0.1 M potassium phosphate pH 7.0, giving a clear, yellow solution, also soluble in water
Assay Procedure 1:
Unit Definition: One unit of glucose oxidase is the activity which causes the liberation of 1 micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions.
Reaction:

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

Reagents:

– 0.1 M Phosphate Buffer, pH 6.8: dissolve 6.8 g of potassium phosphate, monobasic, anhydrous and 7.1 g of sodium phosphate, dibasic, anhydrous in about 800 ml of deionized water. Adjust the pH to 6.8 ± 0.05 @ 25°C with 1 N HCl or 1N NaOH if necessary. Dilute to 1000 ml with deionized water and recheck the pH. Prepare this buffer fresh monthly and store at +4°C.
– 1.4 M Glucose/16.4 mM Benzoic Acid: dissolve 25.0 g of glucose and 0.2 g of benzoic acid in about 75 ml of hot deionized water. Cool to room temperature, then dilute the solution to 100 ml. Allow four hours for mutarotation to reach equilibrium before using. Store at +4°C. Use within two days of preparation.
– Substrate Solution: weigh 10 mg of 4-aminoantipyrine and 340 mg of phenol into a 100 ml volumetric flask. Add 10 ml of deionized water to dissolve the reagents. Then add 75 ml of glucose/benzoic acid solution (Reagent B) and dilute to 100 ml with deionized water. Store at +4°C. use within two days of preparation.
– Peroxidase Solution: dissolve 13,000 units of horseradish peroxidase (purpurogallin units) in 30 ml of 0.1 M phosphate buffer, pH 6.8. Dilute to 100 ml with deionized water. Store at +4°C. Use within two days of preparation.
– Reaction Mixture: immediately prior to the assay, mix equal volumes of the substrate solution (Reagent C) and the peroxidase solution (Reagent D). Keep at +4°C.
– Enzyme Solution Preparation: prepare a 10,001 dilution by adding 10 ul of the enzyme to a 100 ml volumetric flask containing 100 ml of deionized water.

Conditions:

Total Volume: 3.025 ml
Path Length: 1.00 cm
Wavelength: 510 nm

Temperature: 30°C

Procedure:

– Into a 1.0 cm^2 cuvette, pipet the following: 3.0 ml of reaction mixture (Reagent E).
– Place the cuvette into the spectrophotometer and allow the temperature of the reaction mixture to equilibrate to 25 ± 0.5°C. Subsequently, record the blank rate for 2 minutes.
– Add 0.025 ml of the enzyme (Solution F). Mix. Record the absorbance change for 5 minutes.
– Determine the absorbance change (DA) per minute over a 3 minute period. Subtract the blank rate if necessary.

Calculations:

$$U/ml = \frac{(DA'/min)(Vt)(dilution)}{(\varepsilon_{510})(Vs)}$$

Where:
DA' = Corrected absorbance change
Vt = Total assay volume, 3.025 ml
e_{510} = Millimolar extinction coefficient for quinoneimine dye, 6.584
Vs = Sample volume, 0.025 ml
Specific Activity
Calculate the specific activity as follows:

$$U/mg = \frac{U/ml}{mg/ml\ protein\ [per\ Biuret]}$$

Assay Procedure 2:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{GOD} \text{gluconate} + H_2O_2$$

$$H_2O_2 + DH_2 \xrightarrow{POD} 2\,H_2O + D$$

(D = o-dianisidine [o-dianisidine is carcinogenic])

The increase in absorbance is measured at 436 nm.
Reagents:
1. Potassium phosphate buffer (0.1 mol/l; pH 7.0):
a. 1.36 g KH_2PO_4/100 ml distilled water.
b. 2.28 g $K_2HPO_4 \cdot 3H_2O$/100 ml distilled water. Adjust the pH of (1b) to 7.0 by adding (1a).
2. o-Dianisidine (23 mmol/l): saturate 100 ml phosphate buffer (1) for 10 minutes with O_2, then add 1.0 ml o-dianisidine solution: 6.6 mg o-dianisidine hydrochloride/1 ml distilled water.
3. Glucose (55 mmol/l): 100 mg glucose/1 ml.
4. Horseradish peroxidase: 10 mg/ml, 250 U/mg; dilute 1:5 with ammonium sulfate solution, 3.2 mol/l or distilled water.
Sample:

– Volume activity should be 0.1 to 0.15 U/ml.
– For lyophilized enzyme: dissolve ~25 mg lyophilizate in 25 ml buffer (1), allow solution to stand 1 hour at room temperature, then cool to +4°C. Dilute to above volume activity with buffer (1).
– For enzyme solution: dilute solution 1:50000 with buffer (1).

Conditions:
Wavelength: 436 nm; e_{436} = 8.3 [$mmol^{-1}$ x 1 x cm^{-1}]
Light Path:1 cmTotal Volume: 3.06 ml
Temperature:25°CSample Volume: 0.05 ml
Pipette into cuvette:
o-dianisidine(2)2.50 ml
glucose(3)0.50 ml
POD(4)0.01 mlmix, check the temperature, start reaction by addition of
sample0.05 mlmix, read the increase in absorbance (DA) per minute
using the linear portion of the curve.
DA/min should be < 0.020.
Calculation:
One unit is the enzyme activity, which oxidizes 1 umole of D-glucose under the assay conditions (25°C, pH 7.0).

$$\text{Volume Activity} = \frac{3.06}{8.3 \times 1.0 \times 0.050} \times \Delta A/min\ [U/ml\ sample\ solution]$$

$Activity_{lyo.}$ = volume activity x dilution factor [U/mg lyophilizate]
$Activity_{sol.}$ = volume activity x 50000 [U/ml solution]
Availability:

Catalog Number	Description	Size

100289	Glucose Oxidase, Activity: 15-20 units/mg solid. Unit Definition: One unit will liberate 1.0 umole of H_2O_2 per minute at pH 7.0 and 25°C	10 KU 50 KU 250 KU 500 KU 1000 KU
100330	Glucose Oxidase, Activity: $\geq$ 40 units/mg solid	10 KU 50 KU 250 KU 500 KU
195196	Glucose Oxidase, Type X, Activity > 100 units/mg.	10 KU 50 KU 250 KU 500 KU

References:

– Tsuge, H., Natsuaki, O., Ohashi, K., *J. Biochem.*, **v. 78**, 835 (1975).
– Swoboda, B.E.P., Massey, V., *J. Biol. Chem.*, **v. 240**, 2209 (1965).
– Gibson, H.Q., Swoboda, B.E.P., Massey, V., "Kinetics and Mechanism of Action of Glucose Oxidase." *J. Biol. Chem.*, **v. 239**, 3927 (1964).
– Nakamura, S., Ogura, O., *J. Biochem.*, **v. 63**, 308 (1968).
– Swoboda, B.E.P., *Biochim. Biophys. Acta*, **v. 175**, 365 (1969).
– Underkofler, L.A., *Proc. Internat. Symp. Enzyme Chem.*, 1957, Ichihara, K. (ed.), pg. 486. Academic Press: New York (1958).
– Bentley, R., *Methods in Enzymology*, **Vol. 1** (Colowick, S.P. and Kaplan, N.O., eds.) pg. 340. Academic Press: New York (1955).
– Scott, D., *J. Agr. Food Chem.*, **v. 1**, 727 (1953).
– Keilin and Hartee, *Biochem. J.*, **v. 50**, 331 (1952).
– Kleppe, K., "The effect of hydrogen peroxide on glucose oxidase from *Aspergillus niger*." *Biochem.*, **v. 5**, 139 (1966).
– Nicol, M.J. and Duke, F.R., "Substrate inhibition with glucose oxidase." *J. Biol. Chem.*, **v. 241**, 4292 (1966).
– Pazur, J.H. and Kleppe, K., "The oxidation of glucose and related compounds by glucose oxidase from *Aspergillus niger*." *Biochem.*, **v. 3**, 578 (1964).
– Pazur, J.H., Kleppe, K., and Cepure, A., "A glycoprotein structure for glucose oxidase from *Aspergillus niger*." *Arch. Biochem. Biophys.*, **v. 111**, 351 (1965).
– Swoboda, B.E.P. and Massey, V., "On the Reaction of the glucose oxidase from *Aspergillus niger* with bisulfite." *J. Biol. Chem.*, **v. 241**, 3409 (1966).