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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GLUCOSE OXIDASE
(EC 1.1.3.4)

PRINCIPLE:

β-D-Glucose + O_2 + H_2O $\xrightarrow{GOD}$ D-Glucono-1,5-Lactone + H_2O_2

H_2O_2 + o-Dianisidine (reduced) $\xrightarrow{POD}$ o-Dianisidine (oxidized)

Abbreviations used:
GOD = Glucose Oxidase
POD = Peroxidase

CONDITIONS: T = 35°C, pH = 5.1, A_{500nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 5.1 at 35°C
(Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.1 at 35°C with 1 M HCl.)

B. 0.21 mM o-Dianisidine Solution
(Dissolve the contents of one 50 mg vial of o-Dianisidine Dihydrochloride, Sigma Stock No. 510-50, in 7.6 ml of deionized water. Dilute 1.0 ml to 100 ml with Reagent A.)

C. 10% (w/v) β-D(+)Glucose Substrate Solution
(Prepare 10 ml in deionized water using β-D(+)Glucose, Sigma Prod. No. G-5250.)

D. 0.17 mM o-Dianisidine and 1.72% (w/v) Glucose Solution (Reaction Cocktail)
(Immediately before use, prepare 29 ml by combining 24.0 ml of Reagent B with 5.0 ml of Reagent C. Equilibrate to 35°C and adjust to pH 5.1 if necessary with 1 M HCl or 1 M NaOH. **PREPARE FRESH.**)

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REAGENTS: (continued)

E. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 60 Purpurogallin units/ml of Peroxidase, Type II, Sigma Prod. No. P-8250, in cold deionized water.)

F. Glucose Oxidase Enzyme Solution
(For all Glucose Oxidase product numbers, except for crude products (Sigma Prod. Nos. G-6766 and G-1262) prepare an initial solution of 20 - 40 units/ml in cold Reagent A. Then immediately prior to use, further dilute to 0.4 - 0.8 unit in cold Reagent A. For crude products (Sigma Prod. Nos. G-6766 and G-1262), immediately prior to use prepare a solution of
0.4 - 2 units/ml in cold Reagent A.)[1]

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reaction Cocktail	2.90	2.90
Reagent E (POD)	0.10	0.10

Mix by inversion and equilibrate to 35°C. Monitor the A_{500nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and record the increase in A_{500nm} for approximately 5 minutes. Obtain the ΔA_{500nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{500nm}/\text{min Test} - \Delta A_{500nm}/\text{min Blank})(3.1)(df)}{(7.5)\,(0.1)}$$

3.1 = Volume (in milliliters) of assay
df = Dilution factor
7.5 = Millimolar extinction coefficient of oxidized o-Dianisidine at 500 nm
0.1 = Volume (in milliliters) of enzyme used

CALCULATIONS: (continued)

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of β-D-glucose to D-gluconolactone and H_2O_2 per minute at pH 5.1 at 35°C (equivalent to an O_2 uptake of 22.4 µl per minute). If the reaction mix is saturated with oxygen, the activity may increase by up to 100%.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 48 mM sodium acetate, 0.16 mM o-dianisidine, 1.61% (w/v) glucose, and 6 units peroxidase (concentration will vary as to which glucose oxidase is used.)

REFERENCE:

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) Volume I, Second Edition, 457-458, Academic Press Inc., New York, NY

NOTES:

1. a. Initial Enzyme Solutions: Prepare enzyme solutions in cold Reagent A in the concentrations indicated for the product numbers listed:
   Crude - Sigma Prod. Nos. G-1262 and G-6766, 0.2 mg solid/ml (no further dilutions are required)
   Type II - Sigma Prod. Nos. G-6125 and G-6641,
   1.0 mg solid/ml Solution - Sigma Prod. Nos. G-6891 and G-9010, 0.1 ml solution and 5.00 ml Reagent A
   Type VII - Sigma Prod. Nos. G-2133 and G-7016 0.2 mg solid/ml
   Type X - Sigma Prod. No. G-7141, 0.2 mg solid/ml

NOTES: (continued)

1. Continued

   b. Final Dilutions: Immediately prior to use dilute the initial enzyme solutions to the following concentrations:
   Type II - 0.1 ml of 1.0 mg solid/ml and 5.00 ml of Reagent A
   Solutions - 0.1 ml of initial dilution and 3.00 ml of Reagent A
   Type VII - 0.1 ml of 0.2 mg solid/ml and 5.00 ml of Reagent A
   Type X - 0.1 ml of 0.2 mg solid/ml and 5.00 ml of Reagent A

2. Peroxidase Unit Definition: One POD unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.

3. This assay is based on the cited reference.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.