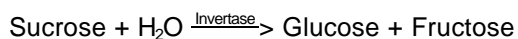


Enzymatic Assay of INVERTASE (EC 3.2.1.26)

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 4.6, A_{340nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 4.6 at 25EC
(Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.6 at 25°C with 1 M HCl.)
- B. 10.0% (w/v) Sucrose Substrate Solution (Sucrose)
(Prepare 100 ml in deionized water using Sucrose, Sigma Prod. No. S-7903. **PREPARE FRESH.**)
- C. 300 mM Trizma Base
(Prepare 100 ml in deionized water using Trizma Base, Reagent Grade, Sigma Prod. No. T-1503.)
- D. Invertase Enzyme Solution
(Immediately before use, prepare a solution containing 5-7 units/ml of Invertase in cold deionized water.)
- E. Glucose Assay Reagent (HK Kit)
(Prepare 50 ml in deionized water according to packaged insert instructions, using Sigma Stock No. 16-50.)

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PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable test tubes:

	<u>Test</u>	<u>Blank</u>
Reagent A	1.00	1.00
Reagent B (Sucrose)	0.50	0.50

Mix by swirling and equilibrate to 25°C. Then add:

Reagent D (Enzyme)	0.10	-----
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Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

Reagent C (Trizma)	0.40	0.40
Reagent D (Enzyme)	-----	0.10

Immediately mix by swirling and place on ice.

Step 2:

Add 3.00 ml of Reagent E (HK Reagent) to separate suitable 3 ml cuvettes (one for each blank and one for each test used in Step 1 above).

Measure and record the absorbance at 340 nm for each of the cuvettes with HK Reagent using a suitable spectrophotometer. (This is the Initial absorbance.)

Transfer 0.030 ml from each of the tubes in Step 1 to the corresponding cuvette in Step 2.

Monitor the absorbance at 340 nm until the absorbance is constant (approximately 10-15 minutes). (This is the endpoint of the HK reaction.)

Measure and record the absorbance at 340 nm for each of the cuvettes with HK Reagent using a suitable spectrophotometer. (This is the Final absorbance.)

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CALCULATIONS:

$$\Delta A_{340\text{nm}} \text{ Test} = A_{340\text{nm}} \text{ Test Final} - A_{340\text{nm}} \text{ Test Initial}$$

$$\Delta A_{340\text{nm}} \text{ Blank} = A_{340\text{nm}} \text{ Blank Final} - A_{340\text{nm}} \text{ Blank Initial}$$

$$\text{Corrected } \Delta A_{340\text{nm}} \text{ Test} = \Delta A_{340\text{nm}} \text{ Test} - \Delta A_{340\text{nm}} \text{ Blank}$$

$$\text{Units/ml enzyme} = \frac{(\text{Corrected } \Delta A_{340\text{nm}} \text{ Test})(2.0)(3.03)(\text{df})}{(6.22)(5)(0.10)(0.03)}$$

2.0 = Reaction mixture volume (in milliliters) of Step 1

3.03 = Total volume (in milliliters) of reaction mixture Step 2

df = Dilution factor

6.22 = Millimolar extinction coefficient of NADP to NADPH at 340 nm

5 = Time (in minutes) of assay

0.10 = Volume (in milliliter) of enzyme used

0.03 = Volume (in milliliter) of reaction mixture used in Step 2

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of sucrose to glucose and fructose per minute at pH 4.6 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.60 ml reaction mix, the final concentrations are 63 mM sodium acetate, 3.1% (w/v) sucrose and 0.5-0.70 unit invertase.

REFERENCE:

Bergmeyer (1974) *Methods of Enzymatic Analysis*, Volume I, 450-451

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NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.