SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of AMYLOGLUCOSIDASE[1]
(EC 3.2.1.3)

PRINCIPLE:

Step 1:

Starch + H_2O $\xrightarrow{\text{Amyloglucosidase}}$ D-Glucose

Step 2:

D-Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose 6-Phosphate + ADP

Glucose 6-Phosphate + β-NADP $\xrightarrow{\text{G-6-PDH}}$ 6-PG + β-NADPH

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
G-6-PDH = Glucose-6-Phosphate Dehydrogenase
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
6-PG = 6-Phospho-D-Gluconate

CONDITIONS: T = 55°C, pH = 4.5, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 4.5 at 55°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 55°C with 1 M HCl.)

B. 1% (w/v) Starch Solution (Starch)
(Prepare 10 ml in Reagent A using Starch, Potato, Soluble, Sigma Prod. No. S-2630. Facilitate solubilization by heating. **DO NOT BOIL**.)

REAGENTS: (continued)

C. Amyloglucosidase Enzyme Solution (Amylogluc)
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Amyloglucosidase in cold deionized water.)

D. 50% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 5 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

E. Glucose (HK) Determination Vial (16-10)
(Immediately before use, dissolve the contents of one vial of Glucose (HK) 50, Sigma Stock No. 16-50 in 50 ml of deionized water.)

PROCEDURE:

Step 1:

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

	Test	Blank
Reagent B (Starch Solution)	1.00	1.00

Equilibrate to 55°C. Then add:

Reagent C (Amylogluc)	1.00	------

Immediately mix by swirling and incubate at 55°C for exactly 3 minutes. Then add:

Reagent D (TCA)	0.30	0.30
Reagent C (Amylogluc)	------	1.00

Mix by swirling and adjust to pH 7.0 with solid Sodium Bicarbonate, Sigma Prod. No. S-8875. Centrifuge the solutions to clarify and use the supernatant in Step 2.

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PROCEDURE: (continued)

Step 2:

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

	Test	Blank
Reagent E (16-10)	2.80	2.80

Equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record this value as the initial A_{340nm}. Then add:

Test Supernatant (Step 1)	0.20	------
Blank Supernatant (Step 2)	------	0.20

Immediately mix by inversion. Monitor the A_{340nm} until the A_{340nm} is constant (approximately 5 - 10 minutes at room temperature). Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS[1]:

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

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\ \text{Test} - \Delta A_{340nm}\ \text{Blank})(180)(2.3)(3.0)(df)}{(6.22)\ (1000)\ (1)\ (0.2)}$$

180 = Micrograms of glucose per micromole of glucose
2.3 = Total volume (in milliliters) of Step 1
3.0 = Total volume (in milliliters) of Step 2
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
1000 = Conversion factor from micrograms to milligrams
1 = Volume (in milliliter) of enzyme used in Step 1
0.2 = Volume (in milliliter) from Step 1 used in Step 2

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

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

CALCULATIONS[1]: (continued)

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

UNIT DEFINITION:

One unit will liberate 1.0 milligram of glucose from starch in three minutes at pH 4.5 at 55°C.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 25 mM sodium acetate, 0.5% (w/v) starch and 0.3 - 0.6 unit amyloglucosidase.

REFERENCE:

Bergmeyer, H. U., Gawehn K., and Grassl, M. (1974) *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Second Edition, Volume I, 434-435

NOTES:

1. The activities of Amyloglucosidase are expressed in the following manner: Sigma Prod. Nos. A-3514 and A-7420 (units/mg protein), Sigma Prod. No. A-3042 (units/ml), all from Aspergillus niger and Sigma Prod. No. A-7255, from Rhizopus mold (units/gram solid).

2. This assay is not to be used to assay Amyloglucosidase, Sigma Prod. No. A-2330.

2. This assay procedure is based on the cited reference.

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