



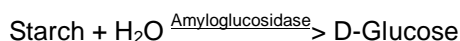
SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

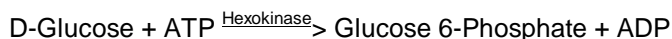
Enzymatic Assay of AMYLOGUCOSIDASE¹ (EC 3.2.1.3)

PRINCIPLE:

Step 1:



Step 2:



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.**)

Enzymatic Assay of AMYLOGUCOSIDASE¹
(EC 3.2.1.3)

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:

	<u>Test</u>	<u>Blank</u>
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.

Enzymatic Assay of AMYLOGLucosidase¹
(EC 3.2.1.3)

PROCEDURE: (continued)

Step 2:

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

	<u>Test</u>	<u>Blank</u>
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.

CALCULATIONS¹:

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

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

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

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

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

Enzymatic Assay of AMYLOGUCOSIDASE¹
(EC 3.2.1.3)

CALCULATIONS¹: (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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.