



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of α -AMYLASE¹ (EC 3.2.1.1)

PRINCIPLE:

Starch + H₂O $\xrightarrow{\alpha\text{-Amylase}}$ Reducing Groups (Maltose)

CONDITIONS: T = 20°C, pH = 6.9, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 6.9 at 20°C with 1 M NaOH.)
- B. 1.0% (w/v) Soluble Starch Solution (Starch)
(Prepare 25 ml in Reagent A using Starch Potato Soluble, Sigma Prod. No. S-2630. Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 minutes. Allow the starch solution to cool to room temperature with stirring. Return the starch solution to its original volume (25 ml) by the addition of water and dispense samples for assay while stirring.)
- C. Sodium Potassium Tartrate Solution
(Dissolve 12.0 grams of Sodium Potassium Tartrate, Tetrahydrate, Sigma Prod. No. S-2377, in 8.0 ml of 2 M NaOH. Heat directly on a heating/stir plate using constant stirring to dissolve. **DO NOT BOIL.**)
- D. 96 mM 3,5-Dinitrosalicylic Acid Solution
(Prepare 20 ml in deionized water using 3,5-Dinitrosalicylic Acid, Sigma Prod. No. D-0550. Heat directly on a heating/stir plate using constant stirring to dissolve. **DO NOT BOIL.**)

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REAGENTS: (continued)

- E. Color Reagent Solution (Clr Rgt Soln)
(With stirring, slowly add Reagent C to Reagent D. Dilute to 40 ml with deionized water. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution is stable for 6 months.)
- F. 0.2% (w/v) Maltose Standard Solution
(Prepare 10 ml in deionized water using Maltose, Monohydrate, Sigma Prod. No. M-5885.)
- G. α -Amylase Solution
(Immediately before use, prepare a solution containing 1 unit/ml of α -Amylase in cold deionized water.)²

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Starch)	1.00	1.00

Mix by swirling and equilibrate to 20°C. Then add:

Reagent G (Enzyme Solution)	1.00	-----
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Mix by swirling and incubate for exactly 3.0 minutes at 20°C. Then add:

Reagent E (Clr Rgt Soln)	1.00	1.00
Reagent G (Enzyme Solution)	-----	1.00

Cap and place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature and add:

Deionized water	9.00	9.00
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Mix by inversion and record the $A_{540\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

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PROCEDURE: (continued)

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent F (Std Soln)	0.20	0.40	0.60	0.80	1.00	----
Deionized Water	1.80	1.60	1.40	1.20	1.00	2.00
Reagent E (Clr Rgt Soln)	1.00	1.00	1.00	1.00	1.00	1.00

Cap and place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature and add:

Deionized Water	9.00	9.00	9.00	9.00	9.00	9.00
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Mix by inversion and record the $A_{540\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{540\text{nm}} \text{ Standard} = A_{540\text{nm}} \text{ Std} - A_{540\text{nm}} \text{ Std Blank}$$

Plot the $\Delta A_{540\text{nm}}$ of the Standards vs milligrams of Maltose.

Sample Determination:

$$\Delta A_{540\text{nm}} \text{ Sample} = A_{540\text{nm}} \text{ Test} - A_{540\text{nm}} \text{ Blank}$$

Determine the milligrams of Maltose liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\text{mg of Maltose released}) (\text{df})}{(1)}$$

df = dilution factor

1 = Volume (in milliliter) of enzyme used

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.00 ml reaction mix, the final concentrations are 10 mM sodium phosphate, 0.50% (w/v) starch, 3.4 mM sodium chloride and 1 unit α -amylase.

REFERENCE:

Bernfeld, P. (1955) *Methods in Enzymology* **1**, 149-158

NOTES:

1. This enzyme assay is not to be used to assay α -Amylase, Insoluble, Sigma Prod. Nos. A-0909 and A-5386.
2. α -Amylase, Sigma Prod. No. A-4551 is diluted in Reagent A at 20°C rather than in water.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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