

**Suitability Assay for Starch Azure
as a Substrate for
α-AMYLASE**

PRINCIPLE:

Insoluble Starch Azure + H₂O $\xrightarrow{\alpha\text{-Amylase}}$ Soluble Colored Reaction Product

CONDITIONS: T = 37°C, pH = 7.0, A_{595nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 20 mM Sodium Phosphate Buffer with 50 mM Sodium Chloride, pH 7.0 at 37°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 7.0 at 37°C with 1 M NaOH.)
- B. 2.0% (w/v) Starch Azure Suspension (Starch Azure)
(Prepare 4.5 ml in Reagent A using Starch Azure, Sigma Prod. Nos. S-7629 or S-7776.)
- C. 2.75 M Acetic Acid Solution (Acetic Acid)
(Prepare 100 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-0808.)
- D. α-Amylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 mg/ml of α-Amylase, Sigma Prod. No. A-6255, in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Starch Azure)	4.50	4.50

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PROCEDURE: (continued)

Equilibrate to 37°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.50	-----

Mix by swirling and incubate at 37°C for exactly 15 minutes in a water bath where constant shaking can be maintained.

Then add:

Reagent C (Acetic Acid)		2.00
		2.00
Reagent D (Enzyme Solution)	-----	0.50

Mix by swirling. Filter the suspension through Whatman 54 filter paper. Then transfer the filtered solutions to suitable cuvettes and record the A_{595nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATION:

$$\Delta A_{595nm} = A_{595nm} \text{ Test} - A_{595nm} \text{ Blank}$$

$$\Delta A_{595nm} / 15 \text{ min/RM} = \frac{\Delta A_{595nm}}{(15)(RM)}$$

15 = Time (in minutes) of assay as per the Unit Definition
RM = Reaction Mixture

SPECIFICATION:

Compare the value of the $\Delta A_{595nm} / 15 \text{ min/RM}$ to that of a control sample. The values should be similar.

FINAL CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 18 mM sodium phosphate, 45 mM sodium chloride, 1.8% (w/v) starch azure, and 0.125 mg α-amylase.

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REFERENCE:

Rinderknecht, H., Wilding, P., and Haverback, B.J. (1967)
Experientia **23**, 805

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.