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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PECTINASE
(EC 3.2.1.15)

PRINCIPLE:

Polygalacturonic Acid + $H_2O \xrightarrow{\text{Pectinase}}$ Galacturonic Acid

I_2 + Galacturonic Acid $\longrightarrow$ Oxidation products

$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$ (Titration of Excess Iodine)

CONDITIONS: T = 25°C, pH = 4.0

METHOD: Titrimetric

REAGENTS:

A. 0.5% (w/v) Polygalacturonic Acid Solution (Polygalacturonic Acid)
(Prepare 100 ml in deionized water using Polygalacturonic Acid, Sigma Prod. No. P-3889. Adjust to pH 4.0 at 25°C with 1 M NaOH.)

B. 50 mM Iodine with 200 mM Potassium Iodide (I_2/KI)
(Prepare 500 ml in deionized water using Iodine, Sigma Prod. No. I-3380 and Potassium Iodide, Sigma Prod. No. P-8256. Initially dissolve in 50 ml deionized water, allow to set at room temperature for 30 minutes and then QS to 500 ml with deionized water.)

C. 1 M Sodium Carbonate (Na_2CO_3)
(Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S-1641.)

D. 2.0 N Sulfuric Acid (H_2SO_4)
(Prepare 200 ml in deionized water by diluting Sulfuric Acid, ACS Reagent, Sigma Prod. No. S-1526 (1:17.8).)

E. 100 mM Sodium Thiosulfate, Standardized ($Na_2S_2O_3$)
(Prepare 100 ml in deionized water using Sodium Thiosulfate, Pentahydrate, Sigma Prod. No. S-8503. Standardize according to the ACS Reagent Procedure.)

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REAGENTS: (continued)

F. 1.0% (w/v) Starch Indicator
(Prepare 100 ml in deionized water using Starch Potato, Soluble, Sigma Prod. No. S-2630. **PREPARE FRESH**.)

G. Pectinase Enzyme Solution
(Immediately before use, prepare a solution containing 100 units/ml of Pectinase in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into 50 ml Erlenmeyer flasks:

	Test	Blank
Reagent A (Polygalacturonic Acid)	4.90	5.00

Equilibrate to 25°C and then add the following reagent:

Reagent G (Pectinase)	0.10	------

Mix by swirling and incubate at 25°C for exactly 5.0 minutes. At the end of 5 minutes incubation, add:

Reagent B (I_2/KI)	5.0	5.0
Reagent C (Na_2CO_3)	1.0	1.0

Mix by swirling and store in the dark for 20 minutes. Then add:

Reagent D (H_2SO_4)	2.0	2.0

Mix by swirling and titrate the Test and Blank with Reagent E ($Na_2S_2O_3$) until it is light yellow. Then add 1 drop of Reagent F (Indicator) and continue to titrate with Reagent E ($Na_2S_2O_3$) until solutions are colorless. (Record the volume of Reagent E used.)

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CALCULATIONS:

Units/ml enzyme =

$$\frac{(1)(100)(\text{ml of Reagent E for Blank - ml of Reagent E for Test})(df)}{(5)(0.1)(2)}$$

1 = One µmole galacturonic acid is oxidized by 1 microequivalent of I_2
100 = microequivalents of S_2O_3/ml of Reagent E
df = Dilution factor
5 = Time of reaction in minutes
0.1 = Volume (in milliliter) of enzyme used
2 = microequivalents of S_2O_3 oxidized per microequivalent of I_2 reduced

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

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per minute at pH 4.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 0.49% (w/v) polygalacturonic acid and 10 units pectinase.

REFERENCE:

Kertesz, Z. I. (1955) *Methods in Enzymology*, Volume I, 162-164.

NOTES:

1. The standardization procedure is described in Reagent Chemicals, (1981) 6th ed., American Chemical Society Specifications, 551-552.

2. This assay is based on the cited reference.

NOTES: (continued)

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