

Enzymatic Assay of PECTOLYASE
(EC 3.2.1.15 and EC 4.2.2.10)

PRINCIPLE:

Polygalacturonic Acid + H₂O $\xrightarrow{\text{Pectinase}}$ Galacturonic Acid

I₂ + 2Na₂S₂O₃ \longrightarrow 2NaI + Na₂S₄O₆ (Titration of Acid)

CONDITIONS: T = 25°C, pH = 5.5

METHOD: Titrimetric

REAGENTS:

- A. 0.5% (w/v) Polygalacturonic Acid Solution
(Prepare 100 ml in deionized water using Polygalacturonic Acid, Sigma Prod. No. P-3889. Heat to solubilize. Adjust to pH 5.5 at 25°C with 1 M NaOH.)
- B. 100 mM Iodine with 200 mM Potassium Iodide (I₂/KI)
(Prepare 500 ml in deionized water using Iodine, Sigma Prod. No. I-3380 and Potassium Iodide, Sigma Prod. No. P-8256. Stir the solution, at room temperature on a magnetic stir plate overnight to dissolve the iodide completely.)
- C. 1 M Sodium Carbonate (Na₂CO₃)
(Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S-1641.)
- D. 2.0 N Sulfuric Acid (H₂SO₄)
(Prepare 200 ml in deionized water by diluting Sulfuric Acid, ACS Reagent, Sigma Prod. No. S-1526.)
- E. 50 mM Sodium Thiosulfate, Standardized (Na₂S₂O₃)
(Prepare 100 ml in deionized water using Sodium Thiosulfate, Pentahydrate, Sigma Prod. No. S-8503. Standardize according to the ACS Reagent Procedure.¹)

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REAGENTS: (continued)

- F. 1.0% (w/v) Starch Indicator (Indicator)
(Prepare 100 ml in deionized water using Starch Potato, Soluble, Sigma Prod. No. S-2630. Heat to solubilize. **PREPARE FRESH.**)
- G. Pectinase Enzyme Solution
(Immediately before use, prepare a solution containing 50-100 units/ml of Pectinase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Polygalacturonic Acid)	4.90	5.00

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

	<u>Test</u>	<u>Blank</u>
Reagent G (Enzyme Solution)	0.10	-----

Mix by swirling and incubate at 25°C for exactly 5.0 minutes. Then add:

Reagent B (I ₂ /KI)	5.0	5.0
Reagent C (Na ₂ CO ₃)	1.0	1.0

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

Reagent D (H ₂ SO ₄)	2.0	2.0
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Mix by swirling and titrate the Test and Blank with Reagent E (Na₂S₂O₃) until it is light yellow. Then add 1 drop of Reagent F (Indicator) and continue to titrate with Reagent E (Na₂S₂O₃) until the solutions are colorless. (Record the volume of Reagent E used.)

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CALCULATION:

mMoles Galacturonic Acid =

$$\frac{(1 \text{ EqI}_2)(50 \text{ mEq/ml})(\text{ml of Reagent E for Blank} - \text{ml of Reagent E for Test})}{(2 \text{ EqS}_2\text{O}_3)(5)(\text{mg enzyme/RM})}$$

5 = Time of reaction in minutes

RM = Reaction Mix

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of galacturonic acid from polygalacturonic acid per minute at pH 5.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 0.5% (w/v) polygalacturonic acid and 5 - 10 units pectinase.

REFERENCE:

Kertesz, Z.I. (1955) *Methods in Enzymology*, Volume I, 158-164

(1993) *Reagent Chemicals ACS Specification*, 8th ed., 717-719

NOTES:

1. Standardize according to (1993) *Reagent Chemicals ACS Specification*, 8th ed., 717-719.
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.