

**Enzymatic Assay of POLYGALACTURONASE
(EC 3.2.1.15)**

PRINCIPLE:

Polygalacturonic Acid + H₂O $\xrightarrow{\text{PG}}$ Reducing Sugars

Abbreviations:

PG = Polygalacturonase

CONDITIONS: T = 30°C, pH 5.0, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Acetate Buffer, pH 5.0 at 30°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 0.50% (w/v) Polygalacturonic Acid Solution (Poly Gal)
(Prepare 25 ml in Reagent A (Buffer) using Polygalacturonic Acid, Sodium Salt, Sigma Prod. No. P-1879.)
- C. 50 mM Sodium Acetate with 0.05% (w/v) Bovine Serum Albumin, pH 5.0 at 30°C (Enzyme Diluent)
(Prepare 25 ml in Reagent A (Buffer) using Albumin, Bovine, Sigma Prod. No. A-4503.)
- D. Polygalacturonase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 1.0 unit/ml Polygalacturonase in cold Reagent C)
- E. 16 mM Copper Sulfate, 1300 mM Sodium Sulfate, 226 mM Sodium Carbonate, 190 mM Sodium Bicarbonate and 43 mM Sodium Potassium Tartrate Solution (Copper Solution)
(Prepare 1 liter in deionized water using Cupric Sulfate Pentahydrate, Sigma Prod. No. C-7631, Sodium Bicarbonate, Sigma Prod. No. S-8875, Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627, Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127, and Sodium Potassium Tartrate Tetrahydrate, Sigma Prod. No S-2377.)²

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REAGENTS: (continued)

- F. 40 mM Molybdcic Acid, 19 mM Arsenic Acid and 756 mM Sulfuric Acid Solution (Arsenomolybdate Soln) (Prepare 1 liter in deionized water using Molybdcic Acid, Ammonium Salt, Tetrahydrate, Sigma Prod. No. M-0878, Arsenic Acid, Sodium Salt, Sigma Prod. No. A-6756, and Sulfuric Acid, Sigma Prod. No. S-1526.)
- G. 0.1% (w/v) D-Galacturonic Acid Standard Solution (Gal Std) (Prepare 10 ml in deionized water using D-Galacturonic Acid, Monohydrate, Sigma Prod. No. G-2125.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Poly Gal)	1.90	1.90

Equilibrate to 30°C. Then add:

Reagent D (Enzyme Soln)		0.10
Reagent C (Enzyme Diluent)	-----	----- 0.10

Immediately mix by inversion and incubate at 30°C for 10 minutes. Then add:

Reagent E (Copper Soln)		2.00 2.00
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Cover the tubes with glass marbles and place in a boiling water bath for 15 minutes. Cool to room temperature. Then add:

Reagent F (Arsenomolybdate Soln)	2.00	2.00
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Mix by inversion until the foaming stops or the precipitate is dissolved. Centrifuge the tubes to clarify the solution. Transfer the supernatant to suitable cuvettes 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 Blank</u>
Reagent G (Gal Std)	0.00	0.03	0.07	0.10
Reagent A (Buffer)	2.00	1.97	1.93	1.90

Mix by inversion and incubate at 30°C for 10 minutes.
Then add:

Reagent E (Copper Soln)	2.00	2.00	2.00	2.00
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Cover the tubes with glass marbles and place in a boiling water bath for 15 minutes. Cool to room temperature.
Then add:

Reagent F (Arsenomolybdate Soln)	2.00	2.00	2.00	2.00
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Mix by inversion until the foaming stops or the precipitate is dissolved. Centrifuge the tubes to clarify.

Transfer to suitable cuvettes and record the $A_{540\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting $\Delta A_{540\text{nm}}$ Standard vs μmoles of D-Galacturonic Acid.

Sample Determination:

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

Determine the μmoles of D-Galacturonic Acid liberated using the standard curve.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of D-Galacturonic Acid released}) (\text{df})}{(10) (0.1)}$$

df = Dilution factor

10 = Time of assay (in minutes) as per the Unit Definition

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will release 1.0 μmole of reducing sugar measured as D-galacturonic acid from polygalacturonic acid per minute at pH 5.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 0.5% (w/v) polygalacturonic acid, 50 mM sodium acetate, 0.002% (w/v) bovine serum albumin and 0.02 - 0.10 unit polygalacturonase.

REFERENCES:

Somogyi M. (1952) *J. Biol. Chem.* **195**, 19-23.

Somogyi M. (1945) *J. Biol. Chem.* **160**, 61-68.

Nelson, N. (1944) *J. Biol. Chem.* **153**, 375-380.

NOTES:

1. This assay is based on the cited references.
2. Sodium Sulfate, Sodium Carbonate, and Sodium Potassium Tartrate are dissolved in approximately 500 ml of deionized water. Cupric Sulfate is dissolved in approximately 100 ml of deionized water and slowly added to the above solution to avoid precipitation. Sodium Bicarbonate is dissolved first in deionized water and then added to the above solution. Dilute the solution to 1 liter. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. Store at room temperature.

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NOTES: (continued)

3. Molybdic Acid is dissolved in approximately 300 ml of deionized water. Add Sulfuric Acid slowly. Caution, this is an exothermic reaction! Arsenic Acid is dissolved in approximately 300 ml of deionized water and is added to the above solution. The solution is diluted to a total volume of 1 liter and incubated at 37°C for 48 - 72 hours. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. The solution expires six months after its preparation. Store at room temperature in an exhaust hood.
4. 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.