Enzymatic Assay of GALACTOSE OXIDASE
(EC 1.1.3.9)

PRINCIPLE:

D(+)Galactose + O_2 $\xrightarrow{\text{Galactose Oxidase}}$ D-Galacto-hexodialdose + H_2O_2

H_2O_2 + o-Tolidine (reduced) $\xrightarrow{\text{Peroxidase}}$ o-Tolidine (oxidized) + H_2O

CONDITIONS: T = 25°C, pH = 6.0, A_{425nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 6.0 at 25°C with 1 M KOH.)

B. 0.5% (w/v) o-Tolidine[1] Solution
(Prepare 1 ml in Methanol using o-Tolidine, Sigma Prod. No. T-3501.)

C. Dye Buffer Solution (DB)
(Prepare by adding 0.2 ml of Reagent B to 24.0 ml of Reagent A. Mix thoroughly.)

D. 555 mM D(+)Galactose Solution (D-Gal)
(Prepare 15 ml in deionized water using D(+)Galactose, Sigma Prod. No. G-0750.[2])

E. Peroxidase Solution (POD)
(Immediately before use, prepare a solution containing 5 purpurogallin units/ml in cold deionized water using Peroxidase, Horseradish, Sigma Prod. No. P-8250.)

F. Galactose Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.50 unit/ml of Galactose Oxidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent C (DB)	1.70	1.70
Reagent D (D-Gal)	1.50	1.50
Reagent E (POD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{425nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	------	0.10
Reagent F (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{425nm} for approximately 5 minutes. Obtain the ΔA_{425nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{425nm}/\text{min Test} - \Delta A_{425nm}/\text{min Blank})(3.4)(df)}{(1.0)\,(0.1)}$$

3.4 = Volume (in milliliters)
df = Dilution factor
1.0 = The change in A_{425nm}/minute per unit Galactose Oxidase at pH 6.0 at 25°C in a 3.4 ml reaction mix
0.1 = volume (in milliliters) of assay

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

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

UNIT DEFINITION:

One unit will produce an ΔA_{425nm} of 1.0 per minute at pH 6.0 at 25°C, in a peroxidase and o-tolidine system.

FINAL ASSAY CONCENTRATION:

In a 3.40 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.002% (w/v) o-tolidine, 0.4% (v/v) methanol, 245 mM D(+)galactose, 0.5 unit peroxidase and 0.050 unit galactose oxidase.

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REFERENCES:

Yamamoto, K., Kondo, Y., Kumagai, H., and Tochikura, T. (1985) *Agric. Biol. Chem.* **49**, 2463 - 2464.

NOTES:

1. o-Tolidine is a possible carcinogen. **TAKE APPROPRIATE PRECAUTIONS**.

2. Allow to stand 2 hours at 25°C to allow for mutarotation.

3. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.