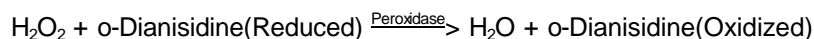
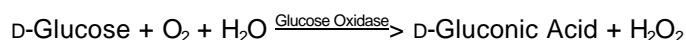
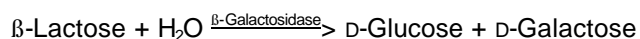


**Enzymatic Assay of β -GALACTOSIDASE
(EC 3.2.1.23)
from E. coli
 β -Lactose as Substrate**

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 7.3, $A_{510\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Potassium Phosphate Monobasic Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Anhydrous, Monobasic, Sigma Prod. No. P-5379.)
- B. 100 mM Potassium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.3 at 37°C with Reagent A.)
- C. 1.0% (w/v) β -Lactose Solution (Lac)
(Prepare 40 ml in Reagent A using β -Lactose, Sigma Prod. No. L-3750.)
- D. 100 mM Magnesium Sulfate Solution (MgSO_4)
(Prepare 5 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- E. 4.2% (v/v) Perchloric Acid Solution (PCA)
(Prepare 10 ml in deionized water using Perchloric Acid, Sigma Stock No. 24,425-2.)
- F. 6.3 mM o-Dianisidine Solution (o-Dianisidine)
(Prepare 1 ml in deionized water using o-Dianisidine, Dihydrochloride, Sigma Prod. No. D-3252.)

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REAGENTS: (continued)

- G. PGO Enzymes Solution (PGO)
(Immediately before use, dissolve 1 capsule of PGO Enzymes, Sigma Stock No. 510-6, in 100 ml of deionized water.)
- H. 5.6 mM Glucose Standard (Gluc Std)
(Use Glucose Standard Solutions, Sigma Stock No. 635-100.)
- I. β -Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing 3 - 5 units/ml of β -Galactosidase in cold Reagent B.)

PROCEDURE:

Step 1

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Buffer)	0.70	0.70
Reagent C (Lac)	4.00	4.00
Reagent D (MgSO ₄)	0.20	0.20

Mix by swirling and equilibrate to 37°C. Then add:

Reagent I (Enzyme Solution)	0.10	-----
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Mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent E (PCA)	1.00	1.00
Reagent I (Enzyme Solution)	-----	0.10

Mix by swirling and centrifuge to clarify, if the solution is turbid.

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COLORIMETRIC ASSAY:

Step 2:

Sample:

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

	<u>Test</u>	<u>Blank</u>
Test Supernatant	0.20	-----
Blank Supernatant	-----	0.20
Reagent F (o-Dianisidine)	0.10	0.10
Reagent G (PGO)	6.00	6.00

Mix by swirling and incubate at 37°C for 30 minutes. Transfer the Test and Blank solutions to suitable cuvettes and record the A_{510nm} for each using a suitable spectrophotometer.

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 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent H (Gluc Std)	0.005	0.01	0.05	0.10	0.20	-----
Deionized Water	0.195	0.19	0.15	0.10	-----	0.20
Reagent F (o-Dianisidine)	0.100	0.10	0.10	0.10	0.10	0.10
Reagent G (PGO)	6.000	6.00	6.00	6.00	6.00	6.00

Mix by swirling and incubate at 37°C for 30 minutes. Transfer the Standards and Standard Blank solutions to suitable cuvettes and record the A_{510nm} for the Standards and Standard Blank.

CALCULATIONS:

Standard Curve:

$$\Delta A_{510nm} \text{ Standard} = A_{510nm} \text{ Std} - A_{510nm} \text{ Std Blank}$$

Determine the μ moles of Glucose liberated using the standard curve.

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CALCULATIONS: (continued)

Sample Determination:

$$\Delta A_{510\text{nm}} \text{ Sample} = A_{510\text{nm}} \text{ Test} - A_{510\text{nm}} \text{ Test Blank}$$

Determine the μ moles of Glucose liberated using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles glucose released}) (6)}{(0.2) (30) (0.1)}$$

6 = Total volume (in milliliters) of Step 1

0.2 = Volume (in milliliter) used in Step 2

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

0.1 = Volume (in milliliter) 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 hydrolyze 1.0 μ mole of β -lactose to glucose and galactose per minute at pH 7.3 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 96 mM potassium phosphate, 0.8% (w/v) lactose, 4 mM magnesium sulfate and 0.3 - 0.5 unit β -galactosidase.

REFERENCES:

Bergmeyer, H.U. and Bernt, E. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume III, 2nd ed., 1205-1212, Academic Press, Inc., New York, NY

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume I, 2nd ed., 456, Academic, Press, Inc., New York, NY

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NOTES:

1. This assay is based on the cited references.
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.