

**Enzymatic Assay of β -GALACTOSIDASE
(EC 3.2.1.23)
 β -Lactose as Substrate**

PRINCIPLE:

β -Lactose + H₂O $\xrightarrow{\beta\text{-Galactosidase}}$ D-Glucose + D-Galactose

D-Glucose + O₂ + H₂O $\xrightarrow{\text{Glucose Oxidase}}$ D-Gluconic Acid + H₂O₂

H₂O₂ + o-Dianisidine (Reduced) $\xrightarrow{\text{Peroxidase}}$ H₂O + o-Dianisidine (Oxidized)

CONDITIONS: T= 30°C, pH = 4.5, A_{510nm}, Light path = 1 cm

METHOD: Colorimetric

- A. 10 mM Citric Acid
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759.)
- B. 20 mM Sodium Phosphate Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876.)
- C. Phosphate Citrate Buffer, pH 4.5 at 30°C
(Phos-Cit Buffer)
(Prepare 100 ml using Reagent B. Adjust to pH 4.5 at 30°C with Reagent A.)
- D. 5.0% (w/v) β -Lactose Solution (Lac)
(Prepare 10 ml in Reagent C using β -Lactose, Sigma Prod. No. L-3750.)
- 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 (Dian)
(Prepare by dissolving one 50 mg preweighed vial of o-Dianisidine, Hydrochloride, Sigma Stock No. 510-50, in the appropriate volume of deionized water.)
- 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.)

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REAGENTS: (continued)

- H. 2.0 mM Glucose Solution (Gluc Std)
(Prepare 10 ml in deionized water using β -D(+)-Glucose, Sigma Prod. No. G-5250.)
- I. β -Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing 5.0 - 10 units/ml β -Galactosidase in cold deionized water.)

PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Phos-Cit Buffer)	0.20	0.20
Reagent D (Lac)	4.70	4.70

Mix by swirling and equilibrate to 30°C . Then add:

Reagent I (Enzyme Solution)	0.10	-----
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Mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

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

(If solution is hazy, centrifuge to clarify.)

COLORIMETRIC ASSAY:

Step 2:

Sample:

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

Blank Supernatant	-----	0.20
Test Supernatant	0.20	-----
Reagent F (Dian)	0.10	0.10
Reagent G (PGO)	6.00	6.00

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COLORIMETRIC ASSAY: (continued)

Mix by swirling and incubate at 30°C for 30 minutes. Transfer the Test and Blank solutions to suitable cuvettes and record the A_{510nm} for the Test and Blank 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</u> <u>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 (Dian)	0.10	0.10	0.10	0.10	0.10	0.10
Reagent G (PGO)	6.00	6.00	6.00	6.00	6.00	6.00

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

CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting the ΔA_{510nm} of the Standards vs μ moles of Glucose.

Sample Determination:

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

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

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles glucose released}) (6) (df)}{(0.2) (10) (0.1)}$$

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CALCULATIONS: (continued)

6 = Total volume (in milliliters) of assay in Step 1
df = Dilution factor
0.2 = Volume (in milliliter) used in Colorimetric
Determination
10 = 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 4.5 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 4.7% (w/v) lactose, and 0.5 - 1.0 unit β -galactosidase. The concentration of citric acid and sodium phosphate are not exactly known.

REFERENCES:

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

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

NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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