Enzymatic Assay of b-GLUCURONIDASE
(EC 3.2.1.31)

PRINCIPLE:

$$\text{PheP-Gluc} + H_2O \xrightarrow{\beta\text{-Glucuronidase}} \text{D-Glucuronate} + \text{Phenolphthalein}$$

Abbreviation:
PheP-Gluc = Phenolphthalein Glucuronide

CONDITIONS: T = 37°C, pH = 3.8, A_{540nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer, pH 3.8 at 37°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 3.8 at 37°C with 1 M HCl.)

B. 1.2 mM Phenolphthalein Glucuronide Substrate Solution (PheP-Gluc)
(Prepare 10 ml in deionized water using Phenolphthalein Glucuronic Acid, Sodium Salt, Sigma Prod. No. P-0376.)

C. 200 mM Glycine Buffer, pH 10.4.
(Use Glycine Buffer Solution, Sigma Stock No. 105-2, or prepare 100 ml in deionized water using Glycine Free Base, Sigma Prod. No. G-7126. Adjust to pH 10.4 at 37°C with 1 M NaOH.)

D. 0.2% (w/v) Sodium Chloride with 0.1% (w/v) Bovine Serum Albumin
(Prepare 20 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625 and Albumin, Bovine Serum, Sigma Prod. No. A-4503.)

E. β-Glucuronidase Enzyme Solution
(Immediately before use, prepare a solution containing 300 units/ml of β-Glucuronidase in cold Reagent D.)

REAGENTS: (continued)

F. 95% (v/v) Ethanol
(Prepare 20 ml in deionized water using 200 Proof, USP Ethyl Alcohol, Quantum Chemical Corporation.)

G. 0.05% (w/v) Phenolphthalein Standard Solution (Std Soln)
(Prepare 5 ml by dissolving 2.5 mg of Phenolphthalein, Sigma Prod. No. P-9750 in 5 ml of Reagent F.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.70	0.70
Reagent B (PheP-Gluc)	0.70	0.70
Reagent C (Glycine Buffer)	------	0.10

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

Reagent E (Enzyme Solution)	0.10	------

Mix by inversion and incubate at 37°C for exactly 30 minutes. Then add:

Reagent C (Glycine Buffer)	5.00	5.00
Reagent E (Enzyme Solution)	------	0.10

Immediately mix by inversion. Transfer the solutions to suitable cuvettes and determine the A_{540nm} for both the Test and Blank using a suitably thermostatted spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Blank
Reagent A (Buffer)	0.70	0.70	0.70	0.70	0.70
Reagent B (PheP-Gluc)	0.70	0.70	0.70	0.70	0.70
Reagent F (Ethanol)	0.07	0.05	0.03	----	0.10
Reagent G (Std Soln)	0.03	0.05	0.07	0.10	----
Reagent C (Glycine Buffer)	5.00	5.00	5.00	5.00	5.00

COLORIMETRIC ASSAY: (continued)

Mix by inversion and transfer the standards to suitable cuvettes. Determine the A_{540nm} for each standard using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA_{540} Standard = A_{540} Standard - A_{540} Standard blank

Prepare a standard curve by plotting ΔA_{540} Standard vs micrograms of Phenolphthalein.

Sample Determination:

ΔA_{540} Sample = A_{540} Sample - A_{540} Sample blank

Determine the total micrograms of phenolphthalein liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu g \text{ phenolphthalein liberated}) (2) (df)}{0.1}$$

2 = Time correction of assay (Unit Definition = 1 hour)
df = Dilution factor
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One Sigma or modified "Fishman" unit will liberate 1.0 microgram of phenolphthalein from phenolphthalein glucuronide per hour at pH 3.8 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 1.50 ml reaction mix, the final concentrations are 47 mM sodium acetate, 0.56 mM phenolphthalein glucuronic acid, 30 units β-glucuronidase, 0.01% (w/v) sodium chloride, and 0.007% (w/v) bovine serum albumin.

NOTES:

Fishman, W.H. and Bernfeld, P. (1955) *Methods in Enzymology*, Volume I, 262-269

Combie, J., Blake, J.W., Nugent, T.E., and Tobin, T. (1982) *Clin. Chem.* **28**, 83-86

Fishman, W.H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 2nd ed., Volume II, 929-932, Academic Press, New York

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.