

**Enzymatic Assay of β -GLUCOSIDASE
(EC 3.2.1.21)**

PRINCIPLE:

β -D-Glucoside + H₂O $\xrightarrow{\beta\text{-Glucosidase}}$ D-Glucose + an Alcohol

METHOD: Stopped Spectrophotometric Endpoint Determination

CONDITIONS: T = 37°C, pH = 5.0, A_{340nm}, Light path = 1 cm

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 5.0 at 37°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. 1.0% (w/v) Salicin Substrate Solution (Salicin)
(Prepare 50 ml in Reagent A using Salicin, Sigma Prod. No. S-0625.)
- C. β -Glucosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 unit/ml of β -glucosidase in cold deionized water.)
- D. Glucose (HK) Reagent Solution (HK)
(Prepare 50 ml in deionized water using Glucose (HK) 50 Reagent Vials, Sigma Stock No. 16-50.)
- E. 500 mM Carbonate-Bicarbonate Buffer Solution (Carb/Biocarb)
(Prepare 100 ml by dissolving the contents of 10 Carbonate-Bicarbonate Buffer Capsules, Sigma Prod. No. C-3041 in 100 ml of deionized water or by dissolving 2.95 g of Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127 and 1.90 g of Sodium Bicarbonate, Sigma Prod. No. S-8875 into 100 ml of deionized water.)

**Enzymatic Assay of β -GLUCOSIDASE
(EC 3.2.1.21)**

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into 4 dram vials:

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	1.00
Reagent B (Salicin)	4.00	4.00

Incubate at 37°C for 5 minutes. Then add:

Reagent C (Enz Sol)	1.00	-----
---------------------	------	-------

Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

Reagent E (Carb/Bicarb)		5.00
		5.00

This stopped reaction mixture is then used in Step 2.

Step 2:

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

	<u>Test</u>	<u>Blank</u>
Reagent D (HK)	1.80	1.80

Obtain the initial $A_{340\text{nm}}$ using a suitable spectrophotometer. Then add:

Blank (Stopped reaction from Step 1)	-----	0.20
Test (Stopped reaction from Step 2)	0.20	-----

Mix by inversion and monitor the reaction at $A_{340\text{nm}}$. Once it has reached the endpoint (approximately 5 - 10 minutes at room temperature) record the final $A_{340\text{nm}}$ using a suitable spectrophotometer.

**Enzymatic Assay of β -GLUCOSIDASE
(EC 3.2.1.21)**

CALCULATIONS:

$$\Delta A_{340\text{nm}} = A_{340\text{nm}} \text{ Final} - A_{340\text{nm}} \text{ Initial}$$

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}} \text{ Test} - \Delta A_{340\text{nm}} \text{ Blank})(2)(10)(\text{df})}{(6.22)(10)(0.2)(1)}$$

2 = Final assay volume (in milliliters) of Step 2
10 = Initial assay volume (in milliliters) of Step 1
df = Dilution factor
6.22 = Millimolar extinction coefficient of β -NADH at $A_{340\text{nm}}$
10 = Time of assay in minutes (as per the Unit Definition)
0.2 = Volume (in milliliters) of Test used in Step 2
1 = Volume (in milliliter) of enzyme used in Step 1

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of glucose from salicin per minute at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 80 mM sodium acetate, 0.8% (w/v) salicin, and 0.5 unit β -glucosidase.

NOTES:

1. This method of enzymatic assay was developed at Sigma.
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.