3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-57
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of β-GLUCOSIDASE

(EC 3.2.1.21)

PRINCIPLE:

β-D-Glucoside + H_2O $\xrightarrow{\beta\text{-Glucosidase}}$ D-Glucose + an Alcohol

CONDITIONS: T = 37°C, pH = 5.0, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric[1]

REAGENTS:

A. 100 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 200 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% (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 1.2 - 2.4 units/ml of β-Glucosidase in cold deionized water.)

D. 16 mM Copper Sulfate, 1300 mM Sodium Sulfate,
226 mM Sodium Carbonate, 190 mM Sodium Bicarbonate, and 43 mM Sodium Potassium Tartrate Solution (Copper Soln)
(Prepare 1 liter in deionized water using Cupric Sulfate Pentahydrate, Sigma Prod. No. C-7631, Sodium Bicarbonate, Sigma Prod. No. S-8875, Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627, Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127, and Sodium Potassium Tartrate Tetrahydrate, Sigma Prod. No. S-2377.[2])

E. 40 mM Molybdic Acid, 19 mM Arsenic Acid, and
756 mM Sulfuric Acid Solution (Ars-Mol Soln)
(Prepare 1 liter in deionized water using Molybdic Acid, Ammonium Salt Tetrahydrate, Sigma Prod. No. M-0878, Arsenic Acid, Sodium Salt, Sigma Prod. No. A-6756, and Sulfuric Acid, Sigma Prod. No. S-1526.[3])

REAGENTS: (continued)

F. Glucose Standard Solution (Glucose)
(Use Glucose Standard Solution, Sigma Stock No. 635-100.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable test tubes:

	Test	Blank
Reagent B (Salicin)	4.00	4.00

Equilibrate to 37°C. Then add:

Deionized Water	------	1.00
Reagent C (Enzyme)	1.00	------

Mix by inversion and incubate at 37°C for exactly 10 minutes.
Immediately transfer 1 ml of reaction mixture into a suitable container containing 1 ml of Reagent D as indicated below and proceed with Somogyi's method[2] of assaying reducing sugars.
Pipette (in milliliters) the following reagents into suitable containers:

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Test Solution	1.00	---	---	---	---	---	---	---
Test Blank Solution	---	1.00	---	---	---	---	---	---
Deionized Water	---	---	0.97	0.95	0.93	0.90	0.80	1.00
Reagent F (Glucose)	---	---	0.03	0.05	0.07	0.10	0.20	---
Reagent D (Copper Soln)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Immediately mix by inversion. Place a marble over the top of the tube and transfer the tubes to a boiling water bath. Incubate for 10 minutes. Remove from the boiling water bath and allow to cool to room temperature. Then add:

Reagent E (Ars-Mol Soln)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

PROCEDURE: (continued)

Shake or vortex until foaming stops and any precipitate present is dissolved. Then add:

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Deionized Water	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Mix and transfer to suitable cuvettes. Obtain the A_{540nm} for Test, Blank and Standards, using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA_{540nm} Std = A_{540nm} Std - A_{540nm} Blank

Prepare a standard curve by plotting the ΔA_{540nm} of the Standard versus the µmoles of glucose liberated.

Sample Determination:

ΔA_{540nm} Sample = A_{540nm} Test - A_{540nm} Test Blank

Determine the µmoles of glucose liberated using the Standard curve.

$$\text{Units/mg enzyme} = \frac{(\mu\text{moles of glucose liberated})\ (5)}{(10)\ (1)\ (\text{mg enzyme/RM})}$$

5 = Volume of Reaction Mix
10 = Time of assay (in minutes) as per Unit Definition
1 = Volume of enzyme assay used in Colorimetric Determination

UNIT DEFINITION:

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

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FINAL ASSAY CONCENTRATIONS:

In a 5.00 ml reaction mix, the final concentrations are 80 mM sodium acetate, 0.8% (w/v) salicin and 1.2 - 2.4 units β-glucosidase.

REFERENCES:

Somogyi M., (1952) *J. Biol. Chem.* **195**, 19-23.
Somogyi M., (1945) *J. Biol. Chem.* **160**, 61-68.
Nelson N., (1944) *J. Biol. Chem.* **153**, 375-380.

NOTES:

1. The method of assaying for the presence of reducing sugars, described here, is that of Somogyi.

2. Sodium Sulfate, Sodium Carbonate, and Sodium Potassium Tartrate are dissolved in approximately 500 ml of deionized water. Cupric Sulfate is dissolved in approximately 100 ml of deionized water and slowly added to the above solution to avoid precipitation. Sodium Bicarbonate is dissolved first in deionized water and then added to the above solution. Dilute the solution to 1 liter. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. Store at room temperature.

3. Molybdic Acid is dissolved in approximately 300 ml of deionized water. Add Sulfuric Acid slowly. Caution, this is an exothermic reaction! A solution of arsenic acid is dissolved in approximately 300 ml of deionized water and is added to the above solution. The solution is diluted to a total volume of 1 liter and incubated at 37°C for 48 - 72 hours. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. The solution expires six months after preparation. Store at room temperature in an exhaust hood.

NOTES: (continued)

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