3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CHITOSANASE
Product No. C 0794

PRINCIPLE:

$$\text{Chitosan} \xrightarrow{\text{Chitosanase}} \text{Reducing Sugars (D-Glucosamine equivalents)}$$

CONDITIONS: T = 37°C, pH = 5.5, A_{405nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 1000 mM Acetic Acid Solution
(Prepare 25 ml in deionized water using Acetic Acid, Aldrich Prod. No. 32,009-9.)

B. 55 mM Sodium Acetate Buffer, pH 5.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate Trihydrate, Sigma Prod. No. S 8625. Adjust pH to 5.5 at 37°C using 1 N NaOH.)

C. 0.1% (w/v) Chitosan Substrate Solution with 150 mM Acetate Buffer, pH 5.5 at 37°C (Chitosan).
(Dissolve 50 mg of Chitosan, Sigma Prod. No. C 3646, in 5 ml of Reagent A. Mix solution until completely dissolved (1 to 3 hours). Once dissolved, dilute the solution to 50 ml with Reagent B. Adjust pH of solution to 5.5 at 37°C using 1 N NaOH.)

D. 500 mM Sodium Hydroxide Solution
(Prepare 100 ml in deionized water using Sodium Hydroxide, Sigma Stock No. 930-65.)

E. 16.4 mM p-Hydroxy-Benzoic Hydrazide in 500 mM Sodium Hydroxide Color Reagent (PAHBAH)
(Dissolve 250 mg of p-Hydroxy-Benzoic Hydrazide, Sigma Prod. No. H9882, with 100 ml of Reagent D. Solution is stable for 2 hours.)

F. 1.5 mM D(+)-Glucosamine Standard Solution (Std. Soln.)
(Prepare 100 ml in deionized water using D(+)Glucosamine, Sigma Prod. No. G 4875.)

G. 50 mM Sodium Acetate Buffer, pH 5.5 at 37°C
(Dilute 50 ml of Reagent B to 55 ml with deionized water. Adjust to pH 5.5 at 37°C.)

H. Chitosanase Enzyme Solution
(Immediately before use, prepare a solution containing 0.4 to 2.0 units/ml of Chitosanase in cold Reagent G.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into microfuge tubes:

	Test	Blank
Reagent C (Chitosan)	0.975	0.975

Equilibrate sample solution to 37°C, place blank solution in an ice bath. Then add:

Reagent H (Enzyme Soln)	0.025	------

Immediately mix by inversion and incubate sample solution at 37°C for exactly 10 minutes. Stop the reaction by removing a 0.4 ml aliquot of test solution to a microfuge tube containing 0.8 ml of Reagent E (PAHBAH). Then add to blank solution in an ice bath:

Reagent H (Enzyme Soln)	------	0.025

Immediately stop the reaction by removing a 0.4 ml aliquot of test solution to a microfuge tube containing 0.8 ml of Reagent E (PAHBAH).

Place all tubes in a boiling water bath for exactly 5 minutes. Remove the tubes and place in an ice bath to cool to room temperature. Centrifuge all tubes in a microcentrifuge for 5 minutes. Remove the supernatants and record the A_{405nm} for the Sample and Blank.

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into microcentrifuge tubes:

	Std 1	Std 2	Std 3	Std 4	Std 5	Blank
Reagent F (Std Soln)	0.025	0.050	0.100	0.150	0.200	------
Milli-q Water	0.375	0.350	0.300	0.250	0.200	0.400
Reagent E (PAHBAH)	0.800	0.800	0.800	0.800	0.800	0.800

Cap and place all tubes in a boiling water bath for exactly 5 minutes. Remove the tubes and place in an ice bath to cool to room temperature. Centrifuge all tube in a microcentrifuge for 5 minutes. Remove the supernatants and record the A_{450nm} for the Standards ad Blank.

CALCULATIONS:

ΔA_{450nm} Standard = A_{450nm} Std - A_{450nm} Std Blank

Plot the ΔA_{450nm} Standards versus µmoles of D(+)Glucosamine.

CALCULATIONS: continued

ΔA_{450nm} Sample = A_{450nm} Sample - A_{450nm} Sample Blank

Determine the µmoles of D(+)Glucosamine equivalents liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of D(+)Glucosamine equivalents})(1)(df)}{(0.025)(10)(0.4)}$$

1 = Total volume (in milliliters) of assay
df = Dilution Factor
0.025 = Volume of enzyme (in milliliters) used in assay
10 = Time of assay as per the Unit Definition
0.40 = Volume (in milliliters) used in color development

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

$$\text{Units/mg protein} = \frac{\text{units/ml protein}}{\text{mg solid/ml protein}}$$

UNIT DEFINITION:

One unit will liberate 1.0 micromole of reducing sugars (D-Glucosamine equivalents) from chitosan per minute at pH 5.5 at 37 °C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 147.5 mM sodium acetate, 0.1% (2/v) chitosan, and 0.01-0.05 units chitosanase.

NOTE:

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