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Enzymatic Assay of CHITINASE

REAGENTS:

A.200 mM Potassium Phosphate Buffer, pH 6.0 at 25°C with 2 mM Calcium Chloride. (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, and Calcium Chloride, Dihydrate. Adjust to pH 6.0 at 25°C with 1 M KOH.)

B.1.25% (w/v) Chitin Suspension (Chitin) (Prepare 25 ml in Reagent A using Chitin. Stir on a magnetic stirrer for approximately 15 minutes to ensure that large particles are broken apart.)

C.5.3 M Sodium Potassium Tartrate Solution (Dissolve 12.0 grams of Sodium Potassium Tartrate, Tetrahydrate, in 8.0 ml of 2 M NaOH. Heat in a boiling water bath to dissolve, but DO NOT BOIL.)

D.96 mM 3,5-Dinitrosalicylic Acid Solution (Dissolve 438 mg of 3,5-Dinitrosalicylic Acid, in 20 ml of deionized water. Heat in a boiling water bath to dissolve, but DO NOT BOIL.)

E.Color Reagent Solution (Clr Rgt Soln) (With stirring, slowly add Reagent C solution to Reagent D. Dilute to 40 ml with deionized water. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution is stable for approximately 6 months.)

F.Chitinase Enzyme Solution (Immediately before use, prepare a solution containing 1 unit/ml of Chitinase in cold Reagent A.)

G.β-N-Acetylglucosaminidase (NAGase) (Immediately before use, prepare a solution containing 35 units/ml in cold Reagent A using β-N-Acetylglucosaminidase .)

H.0.1% (w/v) N-Acetyl-D-Glucosamine Standard Solution (NAG) (Prepare 10 ml in deionized water using N-Acetyl-D-Glucosamine.)

PROCEDURE：

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

	Test	Blank
Reagent B (Chitin)	2.00	2.00
Deionized Water	-------	0.50
Reagent F (Enzyme Solution)	0.50	-------

Close each container securely with a cap. The vials should be placed on their sides on a rotary platform at a speed sufficient to keep the chitin in suspension. Incubate the Test and Blank for two hours at 25°C.

After two hours, place the vials into a boiling water bath for 5 minutes. Cool to room temperature by placing the vials in a cold water bath.

Add 1 unit of Reagent G (NAGase) to each vial. Incubate the vials for 30 minutes at 25°C with mixing as before. Centrifuge the suspensions and retain the supernatant liquid.

COLORIMETRIC ASSAY:

Standard Curve:

A standard curve is prepared by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent H (NAG Std Soln)	0.1	0.2	0.3	0.4	0.5	0
Deionized Water	2.9	2.8	2.7	2.6	2.5	3.0
Reagent E (Clr Rgt Soln)	1.5	1.5	1.5	1.5	1.5	1.5

Sample:

Pipet the following reagents into suitable vials:

	Test	Blank
Test Supernatant	1.0	---
Blank Supernatant	---	2.0
Deionized water	2.0	1.0
Reagent E (Clr Rgt Soln)	1.5	1.5

CALCULATIONS:

Standard Curve:

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

Plot the ΔA_{540nm} of the standards versus milligrams of NAG.

Sample Determination:

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

Determine the milligrams of NAG liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\text{mg NAG released})(2.5 + \text{Volume of NAGase3})}{(2)(1)(0.5)}$$

2.5 = Initial reaction volume of assay

2 = Conversion factor for converting 2 hours to 1 hour as per the Unit Definition

1 = Volume (in milliliter) of supernatant used in colorimetric determination

0.5 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will liberate 1.0 mg of N-acetyl-D-glucosamine from chitin per hour at pH 6.0 at 25°in a two step β-N-Acetylglucosaminidase from Aspergillus niger, (2 hour assay).