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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CASEINASE
(Collagenase Products)

PRINCIPLE:

Casein + $H_2O \xrightarrow{\text{Protease}}$ Amino Acids

CONDITIONS: T = 37°C, pH = 7.5, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 50 mM Sodium Phosphate Buffer, pH 7.5 at 37°C.
(Prepare 200 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.5 at 37°C with 1 M HCl.)

B. 0.65% (w/v) Casein Solution (Casein)
(Prepare 125 ml in Reagent A using Casein,
Sigma Prod. No. C-7078. Heat gently to 80 - 85°C (do not boil) until a homogenous dispersion is obtained. Allow the solution to cool to 37°C. Adjust the pH to 7.5 at 37°C with 0.1 M HCl or 0.1 M NaOH, if necessary.)

C. 6.1 N Trichloroacetic Acid Reagent (TCA)
(Use Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

D. Folin & Ciocalteu's Phenol Reagent (F-C)
(Dilute 10 ml of Folin & Ciocalteu's Phenol Reagent, 2.0 N, Sigma Prod. No. F-9252, to 40 ml with deionized water.)

E. 500 mM Sodium Carbonate Solution (Na_2CO_3)
(Prepare 500 ml in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127.)

F. 50 mM TES Buffer with 0.36 mM Calcium Chloride, pH 7.4 at 37°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using TES Free Acid, Sigma Prod. No. T-1375, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust the pH to 7.4 at 37°C with 1 M NaOH.)

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REAGENTS: (continued)

G. 1.1 mM L-Tyrosine Standard (Std Soln)
(Prepare 100 ml in deionized water using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat gently until tyrosine dissolves and cool to room temperature.)

H. Collagenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.10 mg/ml of Collagenase in cold Reagent F.)

PROCEDURE:

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

	Test	Blank
Reagent B (Casein)	5.00	5.00

Equilibrate to 37°C. Then add:

Reagent H (Enzyme Solution)	1.00	------

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

Reagent C (TCA)	0.50	0.50
Reagent H (Enzyme Solution)	------	1.00

Filter through Whatman #50 filter paper or 0.8 µm syringe filters and use the filtrate in the color development.

COLOR DEVELOPMENT:

Standard Curve:

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

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent G (Std Soln)	0.05	0.10	0.20	0.40	0.50	------
Deionized Water	1.95	1.90	1.80	1.60	1.50	2.00
Reagent E (Na_2CO_3)	5.00	5.00	5.00	5.00	5.00	5.00
Reagent D (F-C)	1.00	1.00	1.00	1.00	1.00	1.00

Mix vigorously by inversion.

COLOR DEVELOPMENT: (continued)

Sample:

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

	Test	Blank
Test Filtrate	2.00	------
Blank Filtrate	------	2.00
Reagent E (Na_2CO_3)	5.00	5.00
Reagent D (F-C)	1.00	1.00

Mix vigorously by inversion. Incubate sample and standard vials at 37°C for 30 minutes. Remove and allow the vials to cool to room temperature. Transfer to suitable cuvettes. (If the solutions are hazy either centrifuge or filter through a 0.45 µm filter prior to determining the A_{660nm}.) Determine the A_{660nm} for the Test, Test Blank, Standards, and Standard Blank.

CALCULATIONS:

Standard Curve:

ΔA_{660nm} Standard = A_{660nm} Standard - A_{660nm} Standard Blank

Plot the ΔA_{660nm} Standard vs µmoles Tyrosine.

Sample Determination:

ΔA_{660nm} Sample = A_{660nm} Test - A_{660nm} Test Blank

Determine the µmoles of Tyrosine equivalents liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole Tyrosine equivalents released})\ (10)\ (6.5)\ (df)}{(1)\ (2)}$$

10 = Time conversion from 30 minutes to 5 hours (Unit Definition)
6.5 = Total volume (in milliliters) of stopped reaction
df = Dilution factor
2 = Volume (in milliliters) of sample used in Colorimetric Assay
1 = Volume (in milliliters) of enzyme used

CACLULATIONS: (continued)

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

UNIT DEFINITION:

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per 5 hours at pH 7.5 at 37°C (color by Folin & Ciocalteu reagent).

FINAL ASSAY CONCENTRATION:

In a 6.00 ml reaction mix, the final concentrations are 42 mM potassium phosphate, 0.54% (w/v) casein, 8.3 mM TES, 0.06 mM calcium chloride and 0.05 mg - 0.10 mg collagenase.

REFERENCES:

Anson, M.L. (1938) *J. Gen. Physiol.* **22**, 79-89

Folin, O. and Ciocalteu, V. (1927) *J. Biol. Chem.* **73**, 627-650

NOTES:

1. This assay is based on the cited references.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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