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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PROTEINASE K[1]
(EC 3.4.21.64)
from Tritirachium album

PRINCIPLE:

$$\text{Hemoglobin} + H_2O \xrightarrow{\text{Proteinase K}} \text{TCA soluble peptides and amino acids}$$

CONDITIONS: T = 37°C, pH = 7.5, A_{750nm}, Light path = 1 cm

METHOD: Colorimetric

A. 1 M Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous (Sigma Product No. P 5379). Adjust to pH 7.5 at 37°C with 1 M KOH.)

B. 2.0% (w/v) Hemoglobin Solution with 6 M Urea, 100 mM Potassium Phosphate, pH 7.5 at 37°C. (Hemoglobin)
(Prepare 100 ml by dissolving 2.0 g of Hemoglobin (Sigma Product No. H 2652) in approximately 40 ml of deionized water. Allow to stand for 20 – 25 minutes at 37°C. Add 8.0 ml of 1 N NaOH (Sigma Stock No. 930-65) and stir for 10-15 minutes at 37°C. Add 36.0 g of Urea (Sigma Product No. U-1250) and equilibrate to 37°C. Stir for 45 minutes at 37°C. Add 10 ml of Reagent A. Adjust the pH to 7.5 at 37°C with 1 N HCl. Adjust the volume to 100 ml with deionized water.)

C. 20 mM Calcium Chloride Solution ($CaCl_2$)
(Prepare 200 ml in deionized water using Calcium Chloride, Sigma Prod. No. C-3881.)

D. 305 mM Trichloroacetic Acid Reagent (TCA)
(Dilute 20 ml of Trichloroacetic Acid, 6.1 N, Sigma Stock No. 490-10, to 400 ml with deionized water.)

E. 500 mM sodium Hydroxide Solution (NaOH)
(Prepare 400 ml in deionized water using Sigma Stock No. 930-65.)

F. Folin & Ciocalteu's Phenol Reagent (FC)
(Prepare 50 ml in deionized water using Folin & Ciocalteu's Phenol Reagent, Sigma Prod. No. F-9252.)

G. 1.1 mM L-Tyrosine Standard Solution (Std. Soln.)
(Prepare 100 ml in deionized water using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat gently (do not boil) until the tyrosine dissolves and cool to room temperature.)

H. 200 mM Hydrochloric Acid Solution (HCl)
(Prepare 100 ml using Hydrochloric Acid, Sigma Prod. No. H-7020.)

I. Proteinase K Enzyme Solution
(Immediately before use, prepare a solution containing 0.075-0.175 units/ml of Proteinase K in cold Reagent C.)

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PROCEDURE:

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

	Test	Blank
Reagent B (Hemoglobin)	2.50	2.50

Equilibrate to 37°C for 10 minutes. Then add:

Reagent D (TCA)	------	5.00
Reagent I (Enzyme Solution)	0.50	------

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

Reagent D (TCA)	5.00	------
Reagent I (Enzyme Solution)	------	0.50

Mix by swirling and incubate at room temperature for 20 minutes. Clarify by filtration through a 0.45 μm filter or by centrifugation.

COLOR DEVELOPMENT:

Sample:

Remove 2.50 ml of the filtrate or supernatant liquid from clarification step and place in a separate tube.

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

	Test	Blank
Filtrate/Supernatant	2.50	2.50
Reagent E (NaOH)	5.00	5.00

Mix by swirling. Proceed with standard curve preparation.

Standard Curve:

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

	Std 1	Std 2	Std 3	Std 4	Blank
Reagent G (Std Soln)	0.05	0.10	0.30	0.50	0.00
Reagent H (HCl)	2.45	2.40	2.20	2.00	2.50
Reagent E (NaOH)	5.00	5.00	5.00	5.00	5.00

Mix thoroughly by swirling. Then add 1.50 ml of Reagent F (FC) to all standards, tests, and blank tubes.

PROCEDURE: (continued)

Immediately mix thoroughly by swirling or vortexing and incubate at room temperature for 10-15 minutes. Transfer each test, blank, and standard solution into a suitable cuvette and measure the absorbance at 750 nm of all test, blank, and standard solutions. If the solutions are hazy, filter through a 0.45 µm filter immediately prior to measuring the absorbances.

CALCULATIONS:

Standard Curve:

ΔA_{750nm} Standard = A_{750nm} Standard - A_{750nm} Standard Blank

Plot the ΔA_{750nm} Standard versus µmoles of Tyrosine.

Sample Determination:

ΔA_{750nm} Sample = A_{750nm} Test - A_{750nm} Standard Blank

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

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole Tyrosine equivalents released }) (8.0) (DF)}{(0.50) (10) (2.5)}$$

8.0 = Total volume (in milliliters) of stopped reaction
10 = Time of assay (in minutes)
0.50 = Volume of enzyme (in milliliter) of enzyme used
2.5 = Volume (in milliliters) used in the Colorimeteric Determination

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

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

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentration are 83 mM Potassium Phosphate, 1.67% (w/v) Casein, 5.0 M Urea, 3.33 mM Calcium Chloride, and 0.037-0.088 unit Proteinase K.

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NOTE:

1. This assay is not to be used to assay Proteinase K-Acrylic Beads, P-0803, and Proteinase K-Agarose, P-9290.
2. This assay is based on the cited references.
3. Where Sigma Product Number or Stock Numbers are specified, equivalent Regents may be substituted.

REFERENCES:

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