

Enzymatic Assay of PROTEINASE K¹
(EC 3.4.21.64)
from Tritirachium album

PRINCIPLE:

Casein + H₂O $\xrightarrow{\text{Proteinase K}}$ Amino Acids

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

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Potassium Phosphate buffer, pH 7.5 at 37°C.
(Prepare 200 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. 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 (do not boil) until a homogenous dispersion is obtained. Adjust the pH to 7.5 at 37°C if necessary with either 1 M NaOH or 1 M HCl.)
- C. 110 mM Trichloroacetic Acid Reagent (TCA)
(Dilute 9 ml of Trichloroacetic Acid, 6.1 N, approximately 100% (w/v), Sigma Stock No. 490-10, to 500 ml with deionized water.)
- D. Folin & Ciocalteu's Phenol Reagent (F-C)
(Dilute 10 ml of Folin & Ciocalteu's Phenol Reagent, Sigma Prod. No. F-9252, to 40 ml with deionized water.)
- E. 500 mM Sodium Carbonate Solution (Na₂CO₃)
(Prepare 500 ml in deionized water using Sodium Carbonate Anhydrous, Sigma Prod. No. S-2127.)
- F. 10 mM Sodium Acetate Buffer with 5 mM Calcium Acetate, pH 7.5 at 37°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, and Calcium Acetate, Sigma Prod. No. C-1000. Adjust the pH to 7.5 at 37°C with 0.1 M Acetic acid or 0.1 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 (do not boil) until the Tyrosine dissolves and cool to room temperature.)
- H. Proteinase K Enzyme Solution
 (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Proteinase K in cold Reagent F.)

PROCEDURE:

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

		<u>Test</u>	<u>Blank</u>
Reagent B (Casein)	5.00	5.00	

Equilibrate to 37°C. Then add:

Reagent H (Enzyme Solution)	1.00	-----
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Mix by swirling and incubate at 37°C for exactly 10 minutes. Then add:

Reagent C (TCA)	5.00	5.00
Reagent H (Enzyme Solution)	-----	1.00

Mix by swirling and incubate at 37°C for about 30 minutes. Filter through a Whatman #50 filter paper 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:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Reagent G(Std Soln)	0.05	0.10	0.20	0.40	0.00
Deionized Water	1.95	1.90	1.80	1.60	2.00
Reagent E(Na ₂ CO ₃)	5.00	5.00	5.00	5.00	5.00
Reagent D (F-C)	1.00	1.00	1.00	1.00	1.00

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COLOR DEVELOPMENT CONTINUED:

Sample:

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

	<u>Test</u>	<u>Blank</u>
Test Filtrate	2.00	-----
Blank Filtrate	-----	2.00
Reagent E (Na ₂ CO ₃)	5.00	5.00
Reagent D (F-C)	1.00	1.00

Mix by swirling and incubate at 37°C for 30 minutes. Remove the vials and allow them to cool to room temperature. If the solutions are hazy, filter through a 0.45 µm filter prior to measuring the absorbances. Transfer the solutions to suitable cuvettes and obtain the A_{660nm} for the Standard, Standard Blank, Test, and Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{660nm} \text{ Standard} = A_{660nm} \text{ Standard} - A_{660nm} \text{ Standard Blank}$$

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

Sample Determination:

$$\Delta A_{660nm} \text{ Sample} = A_{660nm} \text{ Test} - A_{660nm} \text{ Sample Blank}$$

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

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole Tyrosine equivalents released}) (11)}{(1) (10) (2)}$$

11 = Total volume (in milliliters) of stopped reaction

10 = Time of assay (in minutes) as per the Unit Definition

1 = Volume of enzyme (in milliliter) of enzyme used

2 = Volume (in milliliters) used in the Colorimetric Determination

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

In a 6.00 ml reaction mix, the final concentrations are 42 mM potassium phosphate, 0.54% (w/v) casein, 1.7 mM sodium acetate, 0.8 mM calcium acetate, and 0.1 - 0.2 unit protease.

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 not to be used to assay Proteinase K-Acrylic Beads, Sigma Prod. No. P-0803 and Proteinase K-Agarose, Sigma Prod. No. P-9290.
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.