

Enzymatic Assay of PEPSIN INSOLUBLE (EC 3.4.23.1)

PRINCIPLE:

Hemoglobin + H₂O $\xrightarrow{\text{Pepsin}}$ TCA-Soluble Peptides

CONDITIONS: T = 37°C, pH = 2.0, A_{280nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 10 mM Hydrochloric Acid Solution (Enzyme Diluent)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- B. 300 mM Hydrochloric Acid Solution
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- C. 2.5% (w/v) Hemoglobin Solution
(Prepare 100 ml in deionized water using Hemoglobin, Bovine, Sigma Prod. No. H-2625. Mix vigorously and filter through a glass wool filter.)
- D. 2.0% (w/v) Hemoglobin Substrate Solution (Hb)
(Prepare by adding 20 ml of Reagent B to 80 ml of Reagent C.)
- E. 5% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 100 ml in deionized water using Trichloroacetic Acid Solution, 6.1 N, approximately 100% (w/v), Sigma Stock. No. 490-10.)

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REAGENTS: (continued)

- F. Pepsin Insoluble Enzyme (Insol Enz)
(Weigh between 0.20-0.50 g of insoluble enzyme into a graduated cylinder containing 25 ml of deionized water. Allow the solution to swell for 4-12 hours. Transfer 0.50 ml of gel suspension to a graduated micro-column. Wash the sample with deionized water. The amount of deionized water used should be 50X the ml of suspension used. Resuspend the washed suspension to 15-25 units/ml suspension using Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent D (Hb)	5.00	5.00

Equilibrate at 37°C. Then add:

Reagent F (Enzyme Solution)	1.00	-----
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Incubate at 37°C for exactly 10 minutes in a shaker bath. Then add:

Reagent E (TCA)	10.00	10.00
Reagent F (Enzyme Solution)	-----	1.00

Mix by swirling and incubate at 37°C for 5 minutes. Filter the solutions through a Whatman #50 filter or a 0.8 µm syringe filter. Transfer the solutions to suitable quartz cuvettes and record the A_{280nm} for both the Test and Blank.

CALCULATION:

$$\text{Units/ml suspension} = \frac{(A_{280nm} \text{ Test} - A_{280nm} \text{ Blank})(df)}{(0.001)(10)(1)}$$

df = Dilution factor

0.001 = Change in Absorbance at 280 nm per unit of Pepsin (Unit Definition)

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

1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will produce a $\Delta A_{280\text{nm}}$ of 0.001 per minute at pH 2.0 at 37°C measured as TCA-soluble products using hemoglobin as substrate. (Final volume = 16 ml. Light path = 1 cm.)

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FINAL ASSAY CONCENTRATION:

In a 6.00 ml reaction mix, the final concentrations are 50 mM hydrochloric acid, 1.7% (w/v) hemoglobin and 5 - 15 units pepsin.

REFERENCE:

Anson, M.L. (1938) *Journal of General Physiology* **22**, 79-89

NOTE:

1. This assay is based on the cited reference.
2. 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.