

## Enzymatic Assay of PEPSINOGEN

### PRINCIPLE:

Pepsinogen  $\xrightarrow{\text{pH } 2}$  Pepsin

Hemoglobin + H<sub>2</sub>O  $\xrightarrow{\text{Pepsin}}$  TCA-Soluble Peptides

**CONDITIONS:** T = 37°C, pH = 2.0, A<sub>280nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

### REAGENTS:

- A. 10 mM Hydrochloric Acid Solution (Enz Dil)  
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020. Adjust to pH 2.0 at 37°C with either 100 mM HCl or 100 mM NaOH.)
- 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.)
- F. 1 μM Sodium Hydroxide Solution, pH 8.0 at 37°C (NaOH)  
(Prepare 10 ml in deionized water using 1 M NaOH. Adjust to pH 8.0 at 37°C with 1 mM HCl.)
- G. Pepsinogen  
(Use Pepsinogen, lyophilized powder.)

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**PROCEDURE:**

Step 1: Total Pepsinogen

- A. Inactivation and Denaturation of Free Pepsin:  
Dissolve 1 mg of Pepsinogen in 1 ml of Reagent F (NaOH) which has been equilibrated at 37°C. Incubate at 37°C for exactly 30 minutes.
- B. Activation of Pepsinogen to Pepsin  
After the 30 minute incubation in Step A, pipette (in milliliters) 0.20 ml of Step A solution into a 4 dram vial containing 10 ml of Reagent A (Enz Dil). Mix by swirling and let stand for 1 hour at 25°C.

Step 2: Total Available Pepsin

- A. Activation of Pepsinogen to Pepsin  
(Dissolve 2 mg of Pepsinogen in 6 ml of Reagent A (Enz Dil) and let stand for 1 hour at 25°C. Then pipette 1 ml into a suitable container containing 24 ml of Reagent A (Enz Dil).

Step 3: Pepsin Assay

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

	<u>Test1</u>	<u>Test 2</u>	<u>Blank 1</u>	<u>Blank2</u>
Reagent D (Hb) ~	5.00	5.00	5.00	5.00

Equilibrate at 37°C. Then add:

Pepsin (from Step 1)	1.00	-----	-----	-----
Pepsin (from Step 2)	-----	1.00	-----	-----

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

	<u>Test</u>	<u>Blank 1</u>	<u>Blank 2</u>
Reagent E (TCA)	10.00	10.00	10.00
Pepsin (from Step 1)	-----	1.00	-----
Pepsin (from Step 2)	-----	-----	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 Tests and Blanks.

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### CALCULATION:

The calculation shown below can be used to determine both the Total Pepsinogen (Step 1) and also Total Pepsin (Step 2)

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

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

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

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

### UNIT DEFINITION:

One unit will produce a r  $A_{280\text{nm}}$  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.)

### FINAL ASSAY CONCENTRATION:

In a 6.00 ml reaction mix, the final concentrations are 52 mM hydrochloric acid, 1.7% (w/v) hemoglobin and 0.01 mg pepsinogen (total available pepsin assay).

### REFERENCE:

Kassell, B. and Meitner, P.A. (1970) *Methods in Enzymology*, XIX, 337-347

### 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.**