

## Enzymatic Assay of HEPARINASE III (EC 4.2.2.8)

### PRINCIPLE:

Heparan Sulfate  $\xrightarrow{\text{Heparinase III}}$  Unsaturated Uronic Acid

**CONDITIONS:** T = 25°C, pH = 7.5, A<sub>235nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

### REAGENTS:

- A. 20 mM Tris HCl with 50 mM Sodium Chloride, 4 mM Calcium Chloride and 0.01% (w/v) Bovine Serum Albumin, pH 7.5 at 25°C  
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Sodium Chloride, Sigma Prod. No. S-9625, Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 25°C with 1 M HCl.)
- B. 1.0% (w/v) Heparan Sulfate Solution (HS)  
(Immediately before use, prepare 0.5 ml in deionized water using Heparan Sulfate, Sodium Salt, Sigma Prod. No. H-9637.)
- C. 50 mM Hydrochloric Acid Solution (HCl)  
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020)
- D. Heparinase III Enzyme Solution  
(Immediately before use, prepare a solution containing 75 - 100 units/ml of Heparinase III in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.23	0.23
Reagent B (HS)	0.05	0.05

Mix by swirling and equilibrate to 25°C. Then add:

Reagent D (Enzyme Solution)	0.02	-----
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Immediately mix by swirling and incubate at 25°C for exactly 30 minutes. Then add:

Reagent C (Hcl)	2.70	2.70
Reagent D (Enzyme Solution)	-----	0.02

Mix by swirling and centrifuge for 3 minutes. Transfer the supernatants to suitable quartz cuvettes. Record the  $A_{235nm}$  for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{235nm} \text{ Test} - A_{235nm} \text{ Blank})(2)(10)(3)(df)}{(5.50)(0.02)}$$

2 = Conversion factor from 30 minutes to 1 hour as per the Unit Definition

10 = 1 μmole to 0.1 μmole conversion factor as per the Unit Definition

3 = Total volume (in milliliters) of assay

df = Dilution factor

5.50 = Millimolar extinction coefficient<sup>1</sup> of the Unsaturated Uronic Acid products at 235 nm

0.02 = Volume (in milliliter) of enzyme used

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

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

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**UNIT DEFINITION:**

One unit will form 0.1  $\mu$ mole of unsaturated uronic acid per hour at pH 7.5 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 0.30 ml reaction mix, the final concentrations are 17 mM Tris, 42 mM sodium chloride, 3 mM calcium chloride, 0.008% (w/v) bovine serum albumin, 0.2% (w/v) heparan sulfate and 1.5 - 2 units heparinase III.

**REFERENCE:**

Linker, A. and Hovingh, P. (1972) *Methods in Enzymology* **28**, 902-911

Hovingh, P. and Linker, A. (1974) *Carbohydrate Research* **37**, 181-192

**NOTES:**

1. The millimolar extinction coefficient of the unsaturated uronic acid products is described in Hovingh, P. and Linker, A. (1974).
2. This assay is based on Linker, A. and Hovingh, P. (1972).
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.**