Enzymatic Assay of HEPARINASE II

PRINCIPLE:

Heparin + H_2O $\xrightarrow{\text{Heparinase II}}$ Unsaturated Uronic Acid

CONDITIONS: T = 25°C, pH = 7.0, A_{235nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer, with 0.01% (w/v) Bovine Serum Albumin pH 7.0 at 25°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625 and Albumin Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.0 at 25°C with 0.1 M HCl.)

B. 5.0% (w/v) Heparin Solution (Heparin)
(Prepare 4 ml in deionized water using Heparin, Sodium Salt, Sigma Prod. No. H-3393.)

C. 10 mM Calcium Acetate Solution ($Ca(OAc)_2$)
(Prepare 10 ml in deionized water using Calcium Acetate, Sigma Prod. No. C-1000.)

D. 30 mM Hydrochloric Acid Solution
(Prepare 50 ml using Hydrochloric Acid, Sigma Prod. No. H-7020.)

E. Heparinase II Enzyme Solution
(Immediately before use, prepare a solution containing 30 - 40 units/ml of Heparinase II in cold Reagent A.)

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PROCEDURE:

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

	Test
Reagent A (Buffer)	0.13
Reagent B (Heparin)	0.04
Reagent C ($Ca(OAc)_2$)	0.03

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

Reagent E (Enzyme Solution)	0.10

Immediately mix by swirling and transfer 0.05 ml of the Test solution to 3.00 ml of Reagent D (T_i). Incubate at 25°C for exactly 60 minutes. Then transfer another aliquot (0.05 ml) of the Test solution to 3.00 ml of Reagent D (T_f).

Record the A_{235nm} for both the T_i and T_f solutions.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{235nm}\ T_f - A_{235nm}\ T_i)(0.3)(3.05)(10)(df)}{(5.50)(0.1)(0.05)}$$

0.05 = Aliquot from reaction mix used in the final volume
0.3 = Volume of reaction mix in assay
10 = 1 µmole to 0.1 µmole conversion according to unit definition
df = Dilution factor
5.50 = Millimolar extinction coefficient of the Unsaturated Uronic Acid products at 235 nm
3.05 = Final volume of assay
0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will form 0.1 µmole of unsaturated uronic acid per hour at pH 7.0 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 0.30 ml reaction mix, the final concentrations are 77 mM sodium acetate, 0.7% ((w/v) heparin, 1 mM calcium acetate, 0.008% (w/v) BSA and 3.0 - 4.0 units heparinase II.

REFERENCES:

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. This assay is a modification of the procedure described in the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.