Enzymatic Assay of HEPARINASE II

PRINCIPLE:

Heparin + H₂O  $\rightarrow$  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.)
Enzymatic Assay of HEPARINASE II

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Test</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.13</td>
</tr>
<tr>
<td>Reagent B (Heparin)</td>
<td>0.04</td>
</tr>
<tr>
<td>Reagent C (Ca(OAc)₂)</td>
<td>0.03</td>
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</tbody>
</table>

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 (Tᵢ). 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 (Tᵢ).

Record the A₂₃₅nm for both the Tᵢ and Tᵢ solutions.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A₂₃₅nm \ Tᵢ - A₂₃₅nm \ Tᵢ)(0.3)(3.05)(10)(df)}{(5.50)(0.1)(0.05)}
\]

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:


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.

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