Enzymatic Assay of CHONDROITINASE AC  
(EC 4.2.2.5)

PRINCIPLE:
Chondroitin Sulfate + H₂O  \( \text{Chondroitinase AC} \) → Unsaturated Disaccharides

CONDITIONS:  \( T = 37^\circ C, \ pH = 7.3, \ A_{232nm}, \ \text{Light path} = \ 1 \ \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  250 mM Tris HCl and 75 mM Sodium Acetate Buffer, pH 7.3 at 37°C  
(Prepare 200 ml in deionized water using Trizma Base, and Sodium Acetate, Trihydrate, 
Adjust to pH 7.3 at 37°C with 1 M HCl.)

B.  0.06% (w/v) Bovine Serum Albumin Solution (BSA)  
(Prepare 50 ml in deionized water using Albumin, Bovine, or equivalent.)

C.  0.5% (w/v) Chondroitin Sulfate A Solution (Chon A)  
(Prepare 5 ml in Reagent B using Chondroitin Sulfate A, Sodium Salt.)

D.  0.5% (w/v) Chondroitin Sulfate B Solution (Chon B)  
(Prepare 5 ml in Reagent B using Chondroitin Sulfate B, Sodium Salt.)

E.  0.5% (w/v) Chondroitin Sulfate C Solution (Chon C)  
(Prepare 5 ml in Reagent B using Chondroitin Sulfate C, Sodium Salt.)

F.  Chondroitinase AC Enzyme Solution (Enz)  
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Chondroitinase AC in cold Reagent B.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Blank 1</th>
<th>Test 2</th>
<th>Blank 2</th>
<th>Test 3</th>
<th>Blank 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.40</td>
<td>2.40</td>
<td>2.40</td>
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<tr>
<td>Reagent B (BSA)</td>
<td>0.10</td>
<td>0.20</td>
<td>0.10</td>
<td>0.20</td>
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<tr>
<td>Reagent C (Chon A)</td>
<td>0.50</td>
<td>0.50</td>
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<tr>
<td>Reagent D (Chon B)</td>
<td>------</td>
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<td>0.50</td>
<td>0.50</td>
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<tr>
<td>Reagent E (Chon C)</td>
<td>------</td>
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<td>------</td>
<td>------</td>
<td>0.50</td>
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Mix by inversion and equilibrate to 37°C. Monitor the $A_{232\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

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<tbody>
<tr>
<td>Reagent E (Enz)</td>
<td>0.10</td>
<td>------</td>
<td>0.10</td>
<td>------</td>
<td>0.10</td>
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</tbody>
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Immediately mix by inversion and record the increase in $A_{232\text{nm}}$ for approximately 10 minutes. Obtain the $r\ A_{232\text{nm}}$/minute using the maximum linear rate for Tests and Blanks.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{r\ A_{232\text{nm}}/\text{min Test} - r\ A_{232\text{nm}}/\text{min Blank}}{(1.0) \ (\text{mg enzyme/RM})}
\]

1.0 = Absorbance change per Unit Definition  
RM = Reaction Mix

UNIT DEFINITION:

One unit will cause a $r\ A_{232\text{nm}}$ of 1.0 per minute due to the release of unsaturated disaccharides, from chondroitin sulfate A at pH 7.3 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.10 reaction mix, the final concentrations are 194 mM Tris, 58 mM sodium acetate, 0.08% (w/v) chondroitin sulfate A, B or C, 0.01% (w/v) BSA and 0.01 - 0.10 unit chondroitinase AC.
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REFERENCES:


NOTES:

1. This assay is a modification of that cited in the references.