Enzymatic Assay of CHONDROITINASE AC
(EC 4.2.2.5)

PRINCIPLE:

Chondroitin Sulfate + H_2O $\xrightarrow{\text{Chondroitinase AC}}$ Unsaturated Disaccharides

CONDITIONS: T = 37°C, pH = 7.3, A_{232nm}, Light path = 1 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, Prod. No. T-1503, and Sodium Acetate, Trihydrate, Prod. No S-8625. 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, Prod. No. A-4503, 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, Prod. No. C-0914.)

D. 0.5% (w/v) Chondroitin Sulfate B Solution (Chon B)
(Prepare 5 ml in Reagent B using Chondroitin Sulfate B, Sodium Salt, Prod. No. C-2413.)

E. 0.5% (w/v) Chondroitin Sulfate C Solution (Chon C)
(Prepare 5 ml in Reagent B using Chondroitin Sulfate C, Sodium Salt, Prod. No. C-4384.)

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:

	Test 1	Blank 1	Test 2	Blank 2	Test 3	Blank 3
Reagent A (Buffer)	2.40	2.40	2.40	2.40	2.40	2.40
Reagent B (BSA)	0.10	0.20	0.10	0.20	0.10	0.20
Reagent C (Chon A)	0.50	0.50	------	------	------	------
Reagent D (Chon B)	------	------	0.50	0.50	------	------
Reagent E (Chon C)	------	------	------	------	0.50	0.50

Mix by inversion and equilibrate to 37°C. Monitor the A_{232nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enz)	0.10	------	0.10	------	0.10	------

Immediately mix by inversion and record the increase in A_{232nm} for approximately 10 minutes. Obtain the $r\ A_{232nm}$/minute using the maximum linear rate for Tests and Blanks.

CALCULATIONS:

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

1.0 = Absorbance change per Unit Definition
RM = Reaction Mix

UNIT DEFINITION:

One unit will cause a $r\ A_{232nm}$ 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:

Saito, H., Yamagata, T., and Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1536.

Yamagata, T., Saito, H., Habuchi, O., and Suzuki, S., (1968) *J. Biol. Chem* **243**, 1523.

NOTES:

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

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.