

**Enzymatic Assay of CHONDROITINASE C
(EC 4.2.2_)**

PRINCIPLE:

Chondroitin Sulfate C + H₂O $\xrightarrow{\text{Chondroitinase C}}$ Unsaturated Uronic Acid

CONDITIONS: T = 25°C, pH = 8.0, A_{232nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)
- B. 2.0% (w/v) Chondroitin Sulfate C Solution (CS)
(Immediately before use, prepare 1.0 ml in deionized water using Chondroitin Sulfate C, Sodium Salt, Sigma Prod. No. C-4384.)
- C. 50 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- D. Chondroitinase C Enzyme Solution
(Immediately before use, prepare a solution containing 80-120 units/ml of Chondroitinase C in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.40	0.40
Reagent D (Enz Sol)	0.05	-----

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

Reagent B (CS)	0.05	0.05
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PROCEDURE: (continued)

Immediately mix by swirling and incubate at 25°C for exactly 10 minutes. Then add:

	Test	Blank
Reagent C (HCl)	2.50	2.50
Reagent D (Enz Sol)	-----	0.05

Mix by swirling and centrifuge for 10 minutes at 13,000 rpm in a microcentrifuge. Transfer the supernatant to suitable quartz cuvettes and record the A_{232nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml} = \frac{(A_{232nm} \text{ Test} - A_{232nm} \text{ Blank})(3)(10)(6)(df)}{(5.50)(0.05)}$$

3 = Total volume (in milliliters) of stopped assay
 10 = Conversion factor from 1 μ mole to 0.1 μ mole as per the

Unit Definition

6 = Conversion factor from 10 minutes to 1 hour

df = Dilution factor

5.50 = Millimolar extinction coefficient of the unsaturated

uronic acid product at 232 nm.

0.05 = 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 μ mole of unsaturated uronic acid per hour at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 0.50 ml reaction mix, the final concentrations are 45 mM Tris, 0.2% (w/v) chondroitin sulfate C and 4-6 units chondroitinase c.

REFERENCES:

Ototani, N. and Yosizawa, Z. (1979) Carbohydrate Research,

70, 295

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NOTES:

1. This assay is based on 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.