

Enzymatic Assay of CHONDROITINASE B

PRINCIPLE:

Chondroitin Sulfate B + H₂O Chondroitinase B > Unsaturated Uronic Acid

CONDITIONS: T = 25°C, pH 7.5, A_{232nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 20 mM Tris HCl Buffer with 50 mM Sodium Chloride, 4 mM Calcium Chloride, and 0.01% (w/v) Bovine Serum Albumin, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Sodium Chloride, Sigma Prod. No. S-9625, Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881, and Albumin Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 25°C with 1 M HCl.)
- B. 2.0% (w/v) Chondroitin Sulfate B Solution (Chon B)
(Prepare 5 ml in deionized water using Chondroitin Sulfate B, Sodium Salt, Sigma Prod. No. C-2413.)
- C. 20 mM Hydrochloric Acid Solution (HCl)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- D. Chondroitinase B Enzyme Solution
(Immediately before use, prepare a solution containing 25 - 50 units/ml of Chondroitinase B in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.40	0.40
Reagent B (Chon B)	0.05	0.05

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PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.05	-----

Immediately mix by inversion and incubate at 25°C for exactly 20 minutes. Then add:

Reagent C (HCl)	2.50	2.50
Reagent D (Enzyme Solution)	-----	0.05

Mix by inversion. Centrifuge the solutions then transfer the Test and Blank to suitable cuvettes. Record the absorbance at 232 nm.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{232\text{nm}} \text{ Test} - A_{232\text{nm}} \text{ Blank})(3)(3)(\text{df})}{(0.55)(0.05)}$$

3 = Total volume (in milliliters) of assay

3 = Time factor to convert to 60 minutes as per the Unit Definition

df = Dilution factor

0.55 = Millimolar extinction coefficient of the reaction product multiplied by 0.1¹

0.05 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will form 0.1 μmole of unsaturated uronic acid per hour at pH 7.5 at 25°C using chondroitin sulfate B as substrate.

FINAL ASSAY CONCENTRATIONS:

In a 0.50 ml reaction mix, the final concentrations are 18 mM Tris, 45 mM sodium chloride, 4 mM calcium chloride, 0.009% (w/v) bovine serum albumin, 0.2% (w/v) chondroitin sulfate B and 1.25 - 2.5 units chondroitinase B.

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REFERENCE:

Saito, H., Yamagata, T. and Suzuki, S. (1968) *Journal of Biological Chemistry* **243**, 1536-1542.

Yamagata, T., Saito, H., Habuchi, O. and Suzuki, S. (1968) *Journal of Biological Chemistry* **243**, 1523-1535.

NOTES:

1. The millimolar extinction coefficient of the product as described in Yamagata, T. et al. (1988) is multiplied by 0.1 as per the Unit Definition.
2. This assay is based on the cited references.
3. 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.