Enzymatic Assay of CHONDROITINASE ABC
(EC 4.2.2.4)

PRINCIPLE:

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

CONDITIONS: T = 37°C, pH = 8.0, A_{232nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 250 mM Tris HCl and 300 mM Sodium Acetate Buffer with 0.05% (w/v) Bovine Serum Albumin, pH 8.0 at 37°C (Prepare 200 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 8.0 at 37°C with 1 M HCl or 1 M NaOH.)

B. 0.5% (w/v) Chondroitin Sulfate A Solution (Chon A) (Prepare 5 ml in Reagent A using Chondroitin Sulfate A, Sodium Salt, Sigma Prod. No. C-7571.)

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

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

E. 0.01% (w/v) Bovine Serum Albumin Solution (BSA) (Prepare 20 ml in deionized water using Albumin Bovine Serum, Sigma Prod. No. A-4503.)

F. 50 mM Potassium Chloride Solution, pH 1.8 at 25°C (KCl) (Prepare 50 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 1.8 at 25°C with 1 M HCl.)

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REAGENTS: (continued)

G. Chondroitinase ABC Enzyme Solution
(Immediately before use, prepare a solution containing 0.06 - 0.10 unit/ml of Chondroitinase ABC in cold Reagent E.)

PROCEDURE:

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

	Test 1	Test 2	Test 3
Reagent G (Enzy Soln)	0.80	0.80	0.80
Equilibrate to 37°C for 2 minutes. Then add:			
Reagent B (Chon A)	0.20	----	----
Reagent C (Chon B)	----	0.20	----
Reagent D (Chon C)	----	----	0.20

Mix by inversion. Incubate at 37°C for 21 minutes. At 0, 3, 6, 9, 12, 15, 18, and 21 minutes (T_T), transfer 0.10 ml from each Test to separate tubes containing 0.90 ml of Reagent F (KCl). The Blank for this assay is T_0

Incubate each tube for an additional 10 minutes at 37°C. Centrifuge for 10 minutes and transfer the Test and Blank supernatants to suitable quartz cuvettes. Record the absorbance at 232 nm for each tube.

CALCULATIONS:

T_T = Time intervals (minutes) for enzyme assay incubation

$r\ A_{232nm}$ Test (T_T) = A_{232nm} Test (T_T) - A_{232nm} Blank (T_0)

Plot the $r\ A_{232nm}$ Test (T_T) vs. Time. Determine the $r\ A_{232nm}$/min Test from a linear portion of the graph.

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CALCULATIONS:

$$\text{Units/ml} = \frac{(\text{r } A_{232nm}/\text{min Test})\ (1.0)\ (df)}{(E_{mM})\ (0.10)\ (0.8)}$$

df = Dilution factor
E_{mM} = Millimolar extinction coefficient of unsaturated disaccharides: 5.1 for products from Chondroitin A and Chondroitin B, and 5.5 for products from Chondroitin C.
0.10 = Volume (in milliliter) of reaction mix used
T = Time (in minutes) of the assay as per the Unit Definition
1.0 = Total volume (in milliliter) of assay
0.8 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of 2-acetamido-2-deoxy-3-O- (ß-D-gluc-4-ene-pyranosyluronic acid)-4-O-sulfo-D-galactose from chondroitin sulfate A or 1.0 µmole of 2-acetamido-2-deoxy-3-O-(ß-D-gluc-4-ene-pyranosyluronic acid)-6-O-sulfo-D-galactose from chondroitin sulfate C per minute at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 50 mM Tris, 60 mM sodium acetate, 0.02% (w/v) bovine serum albumin, 0.1% (w/v) chondroitin sulfate A, B or C, and 0.048 - 0.08 unit chondroitinase ABC.

REFERENCE:

Saito, H., Yamagata, T. and Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1536-1542

Yamagata, T., Saito, H., Habuchi, O., and Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1523-1535

NOTES:

1. This assay is based on the cited references.

NOTES: (continued)

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.