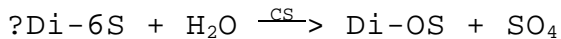


**Enzymatic Assay of CHONDRO-6-SULFATASE
(EC 3.1.6.10)**

PRINCIPLE:



Abbreviations used:

?Di-6S = 4-Deoxy- β -D-Gluc-4-enuronosyl-[1 \rightarrow 3]-N-acetyl-D-galactosamine-6-sulfate

Di-OS = Unsaturated Disaccharide

CS = Chondro-6-Sulfatase

CONDITIONS: T = 37°C, pH = 7.5, A_{360nm}, Light path = 1 cm

METHOD: Turbidimetric

REAGENTS:

- A. 200 mM Tris and 250 mM Sodium Acetate Buffer with 250 mM Sodium Chloride and 0.05% (w/v) Bovine Serum Albumin, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, Sodium Chloride, Sigma Prod. No. S-9625 and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 with either 1 M HCl or 1 M NaOH.)
- B. 300 mM Hydrochloric Acid Solution (HCl)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- C. 14.7 mM Cetylpyridinium Chloride Solution (Cetyl Pyr)
(Prepare 100 ml in Reagent B using Cetylpyridinium Chloride, Sigma Prod. No. C-9002. Clarify by warming before use. **PREPARE FRESH.**)
- D. 10.2 N Hydrochloric Acid Solution (HCl)
(Prepare 5 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)

**Enzymatic Assay of CHONDRO-4-SULFATASE
(EC 3.1.6.10)**

PROCEDURE: (continued)

- E. 20 mM Barium Chloride and 0.49% (w/v) Gelatin Solution (BaCl₂/Gelatin)
(Prepare by dissolving 0.5 g of Gelatin, Sigma Prod. No. G-9382 in 100 ml of deionized water with stirring at 60°C. Let stand overnight at 4°C. Then add 1.45 ml of Reagent D (HCl) and 0.5 g of Barium Chloride, Dihydrate, Sigma Prod. No. B-0750.)
- F. 0.01% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-4503.)
- G. 1% (w/v) ?Di-6S Substrate Solution (?Di-6S)
(Prepare 1 ml in deionized water using ?Di-6S, ICN Immunobiologicals, Standard Disaccharide Kit, No. 320271.)
- H. 5 mM Potassium Sulfate Solution (K₂SO₄ Std)
(Prepare 10 ml in deionized water using Potassium Sulfate, Sigma Prod. No. P-0772.)
- I. Chondro-6-Sulfatase Enzyme Solution
(Immediately before use, prepare a solution containing 0.75 - 1.5 unit/ml of Chondro-4-Sulfatase in cold Reagent F.)

PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.05	0.05
Reagent I (Enzyme Solution)	0.05	-----
Reagent F (BSA)	0.05	0.10

Mix by swirling and equilibrate to 37°C. Then add:

Reagent G (ΔDi-6S)	0.05	0.05
--------------------	------	------

Mix by swirling and incubate at 37° for exactly 5 minutes. Then add:

Reagent C (Cetyl Pyr)	0.20	0.20
-----------------------	------	------

Mix by swirling and let stand for 10 minutes at 37°C.

**Enzymatic Assay of CHONDRO-4-SULFATASE
(EC 3.1.6.10)**

PROCEDURE: (continued)

Step 2:

Pipette (in milliliters) the following reagents into a suitable container:

	<u>Test</u>	<u>Blank</u>
Test Mixture (From Step 1)	0.30	-----
Blank Mixture (From Step 1)	-----	0.30
Reagent E (BaCl ₂ /Gelatin)	0.70	0.70

Mix by swirling and let stand for 10 minutes at 25°C.
Transfer the solutions to suitable cuvettes and record the A_{360nm} for the Test and Blank.

COLORIMETRIC ASSAY:

Standard Curve:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent H (K ₂ SO ₄ Std)	0.02	0.03	0.05	0.07	0.10	---
Reagent E (BaCl ₂ /Gelatin)	0.70	0.70	0.70	0.70	0.70	0.70
Deionized Water	0.28	0.27	0.25	0.23	0.20	0.30

Mix by swirling and let stand for 10 minutes at 25°C.
Transfer the Standards and Standard Blank to suitable cuvettes and record the A_{360nm} for Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{360nm} \text{ Standard} = A_{360nm} \text{ Standard} - A_{360nm} \text{ Standard Blank}$$

Prepare a standard curve by plotting the ΔA_{360nm} Standard vs μmoles of potassium sulfate.

Sample Determination:

$$\Delta A_{360nm} \text{ Sample} = A_{360nm} \text{ Test} - A_{360nm} \text{ Blank}$$

**Enzymatic Assay of CHONDRO-4-SULFATASE
(EC 3.1.6.10)**

CALCULATIONS:

Determine the concentration of inorganic sulfate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of inorganic sulfate liberated})(0.4)(df)}{(0.05)}$$

0.4 = Total volume (in milliliter) of Step 1

df = Dilution factor

0.05 = Volume (in milliliter) of enzyme used in Step 1

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

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

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of inorganic sulfate from 4-deoxy- β -D-gluc-4-enuronosyl-[1 \rightarrow 3]-N-acetyl-D-galactosamine 6-sulfate per minute at pH 7.5 at 37°C.

FINAL CONCENTRATION:

In a 0.20 ml reaction mix, the final concentrations are 50 mM Tris, 62 mM sodium acetate, 62 mM sodium chloride, 0.02% (w/v) bovine serum albumin, 0.2% (w/v) 4-deoxy- β -D-gluc-4-enuronosyl-[1 \rightarrow 3]-N-acetyl-D-galactosamine 6-sulfate and 0.04 - 0.08 unit chondro-6-sulfatase.

REFERENCE:

Dodgson, K.S. (1961) *Biochemical Journal* **78**, 312-319

Yamagata, T. et al., (1968) *Journal of Biological Chemistry* **243**, 1523-1535

NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**Enzymatic Assay of CHONDRO-4-SULFATASE
(EC 3.1.6.10)**

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.