Enzymatic Assay of CHONDRO-4-SULFATASE
(EC 3.1.6.9)

PRINCIPLE:

?Di-4S + H₂O $\xrightarrow{cs}$ Di-OS + SO₄

Abbreviations used:
?Di-4S = (4-Deoxy-β-D-Gluc-4-enuronosyl-[1→3]-N-acetyl-D-galactosamine-4-sulfate)
Di-OS = Unsaturated Disaccharide
CS = Chondro-4-Sulfatase

CONDITIONS:  T = 37°C, pH = 7.5, A₃₆₀nm, 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 or equivalent. 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.  1.47 mM Cetylpyridinium Chloride Solution (Cetyl Pyr)
(Prepare 100 ml in Reagent B (HCl) using Cetylpyridinium Chloride, 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.)
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PROCEDURE: (continued)

E. 20 mM Barium Chloride and 4.8% (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 or equivalent.)

G. 1% (w/v) ΔDi-4S Substrate Solution (ΔDi-4S)
(Prepare 1 ml in deionized water using ΔDi-4S, 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-4-Sulfatase Enzyme Solution
(Immediately before use, prepare a solution containing
0.1 - 0.3 unit/ml of Chondro-4-Sulfatase in cold
Reagent F.)

PROCEDURE:
Step 1:
Pipette (in milliliters) the following reagents into
suitable containers:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent I (Enzyme Solution)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (BSA)</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 37°C. Then add:
Reagent G (ΔDi-4S) | 0.05 | 0.05 |

Mix by swirling and incubate at 37°C 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.
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PROCEDURE:  (continued)

Step 2:
Pipette (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Mixture</td>
<td>0.30</td>
<td>------</td>
</tr>
<tr>
<td>Blank Mixture</td>
<td>------</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent E (BaCl₂/Gelatin)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Mix by swirling and let stand for 10 minutes at 25°C.
Transfer the solution to suitable cuvettes and record the A₃₆₀nm for the Test and Blank.

COLORIMETRIC ASSAY:

Standard Curve:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (K₂SO₄ Std)</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.07</td>
<td>0.10</td>
<td>---</td>
</tr>
<tr>
<td>Reagent E (BaCl₂/Gelatin)</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.28</td>
<td>0.27</td>
<td>0.25</td>
<td>0.23</td>
<td>0.20</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by swirling and let stand for 10 minutes at 25°C.
Transfer the Standards and Standard Blank to suitable cuvettes and record the A₃₆₀nm for Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA₃₆₀nm Standard = A₃₆₀nm Standard - A₃₆₀nm Standard Blank

Prepare a standard curve by plotting the ΔA₃₆₀nm Standard vs µmoles of potassium sulfate.

Sample Determination:

ΔA₃₆₀nm Sample = A₃₆₀nm Test - A₃₆₀nm Blank
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CALCULATIONS:

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

\[
\text{Units/mg enzyme} = \frac{(\mu\text{moles of inorganic sulfate liberated})(0.4)(df)}{(0.1)}
\]

0.4 = Total volume (in milliliters) of Step 1  
df = Dilution factor  
0.1 = Volume (in milliliter) of enzyme used in Step 1

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

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of inorganic sulfate from 4-deoxy-β-D-gluc-4-enuronosyl-[1→3]-N-acetyl-D-galactosamine 4-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→3]-N-acetyl-D-galactosamine 4-sulfate and 0.01 - 0.03 unit chondro-4-sulfatase.

REFERENCE:


NOTES:

1. This assay is based on the cited references.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
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This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.