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

PRINCIPLE:

\[ \text{?Di-6S} + \text{H}_2\text{O} \xrightleftharpoons{\text{CS}} \rightarrow \text{Di-OS} + \text{SO}_4 \]

Abbreviations used:

?Di-6S = 4-Deoxy-ß-D-Gluc-4-enuronosyl-[1→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  
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PROCEDURE:  (continued)

E. 20 mM Barium Chloride and 0.49% (w/v) Gelatin Solution  
(BaCl$_2$/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) ΔDi-6S Substrate Solution (ΔDi-6S)  
(Prepare 1 ml in deionized water using ΔDi-6S, ICN  
Immunobiologicals, Standard Disaccharide Kit,  
No. 320271.)

H. 5 mM Potassium Sulfate Solution (K$_2$SO$_4$ 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:

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

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°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 (From Step 1)</td>
<td>0.30</td>
<td>------</td>
</tr>
<tr>
<td>Blank Mixture (From Step 1)</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 solutions to suitable cuvettes and record the $A_{360\text{nm}}$ 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 $A_{360\text{nm}}$ for Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting the $\Delta A_{360\text{nm}}$ Standard vs µmoles of potassium sulfate.

Sample Determination:

$$\Delta A_{360\text{nm}} \text{ Sample} = A_{360\text{nm}} \text{ Test} - A_{360\text{nm}} \text{ Blank}$$
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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 µmole of inorganic sulfate from 4-deoxy-ß-D-gluc-4-enuronosyl-[1→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-ß-D-gluc-4-enuronosyl-[1→3]-N-acetyl-D-galactosamine 6-sulfate and 0.04 – 0.08 unit chondro-6-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.
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.