# Table of Contents

**Structure of Glycosaminoglycans** ................................................................. 03
- Systemic Analysis of Glycosaminoglycan (GAG) Structure ................. 04
- Structure of the Unsaturated Disaccharides .................................... 04
- Disaccharide Composition of Heparan Sulfate and Heparin .......... 05
- Disaccharide Composition of Chondroitin Sulfate and Dermatan Sulfate .... 05

**Proteoglycan (GAG Side Chain) Degrading Enzymes** ................................... 06
- Chondroitinase Enzymes ........................................................................... 06
  - Perineuronal Net Removal by Chondroitinase ABC .................. 07
  - Chondroitinase ABC Citations ......................................................... 07
  - Immunoprecipitation for Chondroitin Sulfate Proteoglycan .......... 05
- Heparinase Enzymes ................................................................................. 09
- K5 Heparan Lyase Enzyme ...................................................................... 10
- Hyaluronidase .......................................................................................... 10
- Keratanase Enzymes ............................................................................... 11

**Glycobiology Antibodies** ........................................................................ 12
- Proteoglycan Antibodies ........................................................................ 12
- Glycosaminoglycan (GAG) Antibodies .................................................. 12
- Reactivity of antibodies to Glycosaminoglycans .............................. 13
- Chondroitin Sulfate "stub" Monoclonal Antibodies ............................ 13
- Hyaluronic Acid Binding Protein ............................................................. 14
- Glycobiology Antibodies Reactivity ....................................................... 15
- Glycobiology Antibodies IHC and IF ..................................................... 16
- Glycobiology Antibodies Flow Cytometry ............................................. 23
- Glycobiology Antibodies Western Blot & Immunoprecipitation ........ 24
- Glycobiology Antibodies Citations ....................................................... 25

**Glycobiology Kits** ..................................................................................... 28
- Glycobiology ELISA Kits .......................................................................... 28
- Proteoglycan Detection Kit .................................................................... 28
- Heparanase Assay Kit .............................................................................. 29
- Hyaluronidase Assay Kit .......................................................................... 29

**Substrates & Standards:** ........................................................................... 30
- Polysaccharides ....................................................................................... 30
- Oligosaccharides ...................................................................................... 31
- Disaccharides .......................................................................................... 32
- Fluorescence labeled GAGs ................................................................... 33
- Hyalose: Size Standards for Glycobiology Research ........................... 34
Proteoglycans are glycosylated proteins which have covalently attached highly anionic glycosaminoglycans (GAGs). Proteoglycans are present in different forms within different types of extracellular matrices and connective tissues. Heparan sulfate proteoglycans (HSPGs) are composed of a core protein with heparan sulfate (HS) GAG chains attached.

Example structure of a proteoglycan with GAG chains linking from a core protein.

![Proteoglycan structure diagram](image-url)
Systematic Analysis of Glycosaminoglycan (GAG) Structure

Proteoglycans

Glycosaminoglycans

GAG-degrading enzymes
Enzymes with appropriate specificity are available

Digest I

Gel permeation Chromatography

Di-saccharides
Tetra-saccharides
Hexa-saccharides
Oligo-/Poly-saccharides

Digest II
Digest II’
Digest II “

HPLC Analysis I

Sulfatases
Glycuronidases

HPLC Analysis II

Structure of the Unsaturated Disaccharides

Chondroitin Sulfate Family

<table>
<thead>
<tr>
<th>ΔDiHS</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OS</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>-6S</td>
<td>H</td>
<td>SO³</td>
<td>H</td>
<td>A</td>
</tr>
<tr>
<td>-4S</td>
<td>H</td>
<td>SO³</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>-US</td>
<td>H</td>
<td>H</td>
<td>SO³</td>
<td></td>
</tr>
<tr>
<td>-(L,L)S</td>
<td>H</td>
<td>SO³</td>
<td>SO³</td>
<td></td>
</tr>
<tr>
<td>-(L,U)S</td>
<td>H</td>
<td>SO³</td>
<td>SO³</td>
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<td></td>
</tr>
<tr>
<td>-(U,U)S</td>
<td>H</td>
<td>SO³</td>
<td>SO³</td>
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</tr>
<tr>
<td>-(L,U,L)S</td>
<td>H</td>
<td>SO³</td>
<td>SO³</td>
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</tr>
</tbody>
</table>

Heparan Sulfate Family

<table>
<thead>
<tr>
<th>ΔDiHS</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
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<tr>
<td>-OS</td>
<td>H</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td>-NS</td>
<td>H</td>
<td>SO³</td>
<td>H</td>
</tr>
<tr>
<td>-6S</td>
<td>SO³</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td>-US</td>
<td>H</td>
<td>Ac</td>
<td>SO³</td>
</tr>
<tr>
<td>-(L,L)S</td>
<td>SO³</td>
<td>SO³</td>
<td>H</td>
</tr>
<tr>
<td>-(L,U)S</td>
<td>SO³</td>
<td>SO³</td>
<td>SO³</td>
</tr>
<tr>
<td>-(U,L)S</td>
<td>SO³</td>
<td>SO³</td>
<td>SO³</td>
</tr>
<tr>
<td>-(U,U)S</td>
<td>SO³</td>
<td>SO³</td>
<td>SO³</td>
</tr>
</tbody>
</table>
Disaccharide Composition of Heparan Sulfate and Heparin


Disaccharide Composition of Chondroitin Sulfate and Dermatan Sulfate
**Proteoglycan (GAG Side Chain) Degrading Enzymes**

### Chondroitinase Enzymes

Chondroitinase catalyzes the removal of Chondroitin Sulfate and Dermatan Sulfate side chains of proteoglycans. Highly specific for the galactosaminoglycan (GAG) chains without activity on core proteins, keratan sulfate chains, and heparin/heparan sulfate chains.

![Diagram of chondroitinase action](image)

**Table: Chondroitinase Enzymes**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purified</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Chondroitinase ABC protease free- 
  *(Proteus vulgaris)* | AMS.E1028-02     | 2 U       |
|                                                  | AMS.E1028-10     | 10 U      |
| Chondroitinase AC-I- *(Flavobacterium heparinum)* | AMS.CDACI-ENZ-S  | 5 IU      |
|                                                  | AMS.CDACI-ENZ BU | 20 IU     |
|                                                  | AMS.CDACI-ENZ BU2 | 50 IU    |
|                                                  | AMS.CDACI-ENZ BU3 | 100 IU   |
|                                                  | AMS.CDACI-ENZ BU4 | 250 IU   |
| Chondroitinase B- *(Flavobacterium heparinum)*   | AMS.CDB-ENZ      | 1 IU      |
|                                                  | AMS.CDB-ENZ BU   | 2 IU      |
|                                                  | AMS.CDB-ENZ BU2  | 5 IU      |
|                                                  | AMS.CDB-ENZ BU3  | 10 IU     |
|                                                  | AMS.CDB-ENZ BU4  | 20 IU     |
|                                                  | AMS.CDB-ENZ BU5  | 50 IU     |
| **Recombinant**                                  | AMS.50-013       | 0.5 IU    |

Chondroitinases attack and cleave the following GAGs:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitinase ABC</td>
<td>Hylauronic acid, Chondroitin sulfate, Dermatan Sulfate</td>
</tr>
<tr>
<td>Chondroitinase AC-I</td>
<td>Chondroitin Sulfate A &amp; C</td>
</tr>
<tr>
<td>Chondroitinase AC-II</td>
<td>Chondroitin Sulfate A &amp; C</td>
</tr>
<tr>
<td>Chondroitinase B</td>
<td>Dermatan Sulfate</td>
</tr>
</tbody>
</table>
Perineuronal Net Removal by Chondroitinase ABC

Perineuronal nets (PNNs) are specialized extracellular matrix structures responsible for synaptic stabilization in the adult brain. They are largely composed of Chondroitin Sulfate Proteoglycans (CSPGs), which are involved in the inhibition of axon regeneration after various forms of damage to the Central Nervous System, including stroke and spinal cord injury. The enzyme Chondroitinase ABC (from Proteus vulgaris) degrades these CSPGs, and has been shown to promote functional recovery and neural regeneration in addition to its role as a tool in glycoanalysis.

Chondroitinase ABC Citations

Chondroitinase ABC (AMS.E1028)

Neural Regeneration Models

**Chondroitinase ABC (AMS.E1028)**

**Glycoanalysis**


**Chondroitinase ABC (AMS.E1028)**

**Musculoskeletal Models**


**Highly cited papers featuring Seikagaku Chondroitinase ABC (100332-1A)**


**Background / Reviews**

Heparinase Enzymes

Bacterial heparinas cleave the glycosidic linkage between amino sugars and uronic acids in heparin and heparan sulfate. Heparinase enzymes can be used in combination to achieve almost a complete depolymerisation of heparin or heparan sulfate into constituent disaccharides.

Heparinase I: Degrades heparin and the S-domains of heparan sulfate. Mainly attacks and cleaves low sulfate region.

Heparinase II: Degrades heparin and heparan sulfate. Mainly attacks and cleaves low sulfate region.

Heparinase III: Degrades heparan sulfate. Only attacks and cleaves high sulfate region.

Alternative Names for GAG-degrading enzymes:

<table>
<thead>
<tr>
<th>Alternative Classification</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparinase I - is equivalent to:</td>
<td>Heparinase</td>
</tr>
<tr>
<td>Heparinase II and III - are equivalent to:</td>
<td>Heparitinase</td>
</tr>
<tr>
<td>Heparinase III - is equivalent to:</td>
<td>Heparitinase I</td>
</tr>
<tr>
<td>Heparinase II - is equivalent to:</td>
<td>Heparitinase II</td>
</tr>
</tbody>
</table>

**Seikagaku Enzymes**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparinase</td>
<td>100700-3</td>
<td>0.1 U</td>
</tr>
<tr>
<td>Heparinase II</td>
<td>100705-1</td>
<td>0.1 U</td>
</tr>
<tr>
<td>Heparinase I</td>
<td>100704-1A</td>
<td>0.1 U</td>
</tr>
<tr>
<td>Heparitinase*</td>
<td>100703-3</td>
<td>0.1 U</td>
</tr>
</tbody>
</table>

*Seikagaku’s 100703-3 (Heparitinase from F. heparinum, 0.1U) is a mix of 100704-1A (Heparitinase I) and 100705-1 (Heparitinase II). Mixing ratio is 4(100704):1(100705).
Three structural domains can be distinguished in heparan sulfate:

**N-acetylated (NAc) domains:** Repeating sequences of non-sulfated N-acetylated disaccharides. Degraded by heparinase III.

**Transition (T) zones:** Alternating sequences of N-acetylated and N-sulfated disaccharides, variable O-sulfation at C6 of the glucosamine residues, these regions not sulfated at C2 of IdoA residue. Degraded by Heparinase III.

**Sulfated (S-) domains:** Internal repeating sequences of GlcNSO$_3$ (+/-6SO$_3$) and IdoA,2SO$_4$, variable O-sulfation at C6 of the N-sulfated glucosamine residues and occasional O-sulfation at C3 of glucosamine. Degraded by heparinase I.
K5 Heparan Lyase Enzyme

K5 Heparan Lyase (AMS.HL01) cleaves heparan sulfate in the non-sulfated regions of the polymer chain. This action may release activities that lie cryptic in the intact heparan sulphate chain. It can be used to remove heparan sulfate from proteoglycans and it is the only enzymatic method available for excising the entire sulfated regions from heparan sulfate chains. It differs from heparinase III (heparitinase I) in that it will not degrade the transition zones that have an intermediate level of sulfation. (Murphy et al. (2004), J. Biol. Chem. 279: 27239-27245). An excellent substrate for this enzyme is K5 polysaccharide (AMS.K5001) which can be used to monitor the progress of the enzyme action by measuring absorbance at 232nm.

Hyaluronidase

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Description</th>
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</tr>
</thead>
<tbody>
<tr>
<td>25118.01</td>
<td>Hyaluronidase (ovine testes)</td>
<td>50mg</td>
</tr>
<tr>
<td>25118.02</td>
<td>Hyaluronidase (ovine testes)</td>
<td>500mg</td>
</tr>
</tbody>
</table>

Keratanase Enzymes

**Keratanase**: Attacks and cleaves low sulfate region.

**Keratanase II**: Attacks and cleaves high sulfate region.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratanase (Pseudomonas sp.)</td>
<td>100810-1</td>
<td>10 units</td>
</tr>
</tbody>
</table>

---

**GAG degrading enzyme**

- Hyaluronic acid
- Chondroitin sulfate
- Dermatan sulfate
- Heparan sulfate
- Heparin
- Keratan sulfate

**Glycosidase /GIU-ase Sulfatase**

- Chondro-6-sulfatase
- Chondro-4-sulfatase

**Disaccharide**

- Δι-6-ΔΩ-Δη
- Δη-Δη-Δη
- Δη-Δη
- Δη-Δη
- Δη-Δη
- Δη-Δη
- Δη-Δη
- Δη-Δη

**GAG**

- Hyaluronic acid (Pig skin)
- Hyaluronic acid (Umbralical cord)
- Chondroitin sulfate A (Sturgeon roach)
- Chondroitin sulfate C (Shark cartilage)
- Chondroitin sulfate D (Shark cartilage)
- Chondroitin sulfate E (Pig cartilage)

---

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### Proteoglycan Antibodies

<table>
<thead>
<tr>
<th>Monoclonals to Proteoglycans</th>
<th>Clone</th>
<th>Pack Size</th>
<th>Cat No.</th>
<th>IHC</th>
<th>ELISA</th>
<th>WB</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti Aggrecan</td>
<td>6F4</td>
<td>2 ML</td>
<td>AMS.PRPG-AG-M01</td>
<td>1:5 to 1:50</td>
<td>1:10 – 1:150</td>
<td>1:10 to 1:30</td>
<td>1:5 – 1:10</td>
</tr>
<tr>
<td>Anti Aggrecan</td>
<td>5D3</td>
<td>2 ML</td>
<td>AMS.PRPG-AG-M02</td>
<td>1:5 to 1:50</td>
<td>1:10 – 1:150</td>
<td>1:10 to 1:30</td>
<td>1:5 – 1:10</td>
</tr>
<tr>
<td>Anti Aggrecan</td>
<td>5G2</td>
<td>2 ML</td>
<td>AMS.PRPG-AG-M03</td>
<td>1:5 - 1:50</td>
<td>1:10 - 1:150</td>
<td>1:10 - 1:30</td>
<td>1:5 - 1:10</td>
</tr>
<tr>
<td>Anti Aggrecan</td>
<td>7B7</td>
<td>2 ML</td>
<td>AMS.PRPG-AG-M04</td>
<td>1:5 - 1:50</td>
<td>1:10 - 1:150</td>
<td>1:10 - 1:30</td>
<td>1:5 - 1:10</td>
</tr>
<tr>
<td>Anti Biglycan</td>
<td>905A7</td>
<td>2 ML</td>
<td>AMS.PRPG-BG-M01</td>
<td>1:25 to 1:100</td>
<td>1:10 - 1:150</td>
<td>✓</td>
<td>1:10 - 1:50</td>
</tr>
<tr>
<td>Anti Decorin</td>
<td>889C7</td>
<td>2 ML</td>
<td>AMS.PRPG-DC-M01</td>
<td>1:25 - 1:75</td>
<td>1:10 - 1:150</td>
<td>1:10 - 1:50</td>
<td></td>
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<tr>
<td>Anti Neuroglycan C</td>
<td></td>
<td>200 UL</td>
<td>AMS.CAC-NU-07-003</td>
<td>1:200</td>
<td>1:10,000</td>
<td>1:500</td>
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<tr>
<td>Anti Neurocan peptides</td>
<td></td>
<td>200 UL</td>
<td>AMS.CAC-NU-07-005</td>
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<tr>
<td>Anti Neurocan</td>
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<td>200 UL</td>
<td>AMS.CAC-NU-07-002</td>
<td>1:100</td>
<td>1:10,000</td>
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<tr>
<td>Anti N-syndecan</td>
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<td>100 UL</td>
<td>AMS.CAC-NU-07-004</td>
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<tr>
<td>Anti NG2/ CSPG4</td>
<td>2164H5</td>
<td>2 ML</td>
<td>AMS.PRPG-NG-M01</td>
<td>1:5 to 1:50</td>
<td>1:10 - 1:150</td>
<td>1:10 to 1:30</td>
<td>1:5 - 1:10</td>
</tr>
<tr>
<td>Anti Versican/CSPG2</td>
<td>5C12</td>
<td>2 ML</td>
<td>AMS.PRPG-VS-M01</td>
<td>1:25 to 1:75</td>
<td>1:50 - 1:150</td>
<td>1:20 to 1:60</td>
<td></td>
</tr>
<tr>
<td>Anti Versican/CSPG2</td>
<td>4C5</td>
<td>2 ML</td>
<td>AMS.PRPG-VS-M02</td>
<td>1:25 - 1:50</td>
<td>1:50 - 1:150</td>
<td>1:20 - 1:40</td>
<td></td>
</tr>
<tr>
<td>Anti Versican</td>
<td>6B10</td>
<td>2 ML</td>
<td>AMS.PRPG-VS-M04</td>
<td>1:20 - 1:40</td>
<td>1:50 - 1:150</td>
<td>1:20 - 1:60</td>
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</tr>
<tr>
<td>Anti Versican</td>
<td>2B3</td>
<td>2 ML</td>
<td>AMS.PRPG-VS-M03</td>
<td>1:20 - 1:40</td>
<td>1:50 - 1:150</td>
<td>1:20 - 1:60</td>
<td></td>
</tr>
</tbody>
</table>

### Glycosaminoglycan (GAG) Antibodies

<table>
<thead>
<tr>
<th>Description</th>
<th>Clone</th>
<th>Pack Size</th>
<th>Cat No.</th>
<th>IHC</th>
<th>FC</th>
<th>ELISA</th>
<th>WB</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti Keratan Sulfate, purified; recognises KS lacking oversulfated structures</td>
<td>R-10G</td>
<td>100 UG</td>
<td>AMS.RIT-M001</td>
<td>10 µg / mL</td>
<td>3 µg / mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoclonal Anti Keratan Sulfate supernatant</td>
<td>5D4</td>
<td>1 ML</td>
<td>270427-CS</td>
<td>1:20</td>
<td></td>
<td>1:100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti Keratan Sulfate</td>
<td>373E1</td>
<td>2 ML</td>
<td>AMS.PRPG-KS-M01</td>
<td>1:50 to 1:150</td>
<td></td>
<td>✓</td>
<td>1:50 to 1:170</td>
<td>✓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Clone</th>
<th>Host</th>
<th>Cat No.</th>
<th>Pack Size</th>
<th>IHC</th>
<th>FC</th>
<th>ELISA</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ-Heparan Sulfate Antibody</td>
<td>F69-3G10</td>
<td>Mouse</td>
<td>370260-1</td>
<td>200 µg</td>
<td>1:100-200</td>
<td>1:100-200</td>
<td>1:200-500</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>370260-S</td>
<td>50 µg</td>
<td>1:100-200</td>
<td>1:100-200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparan Sulfate Monoclonal Antibody</td>
<td>F58-10E4</td>
<td>Mouse</td>
<td>370255-1</td>
<td>200 µg</td>
<td>1:50-100</td>
<td>1:100-200</td>
<td>1:100-150</td>
<td>✓</td>
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<tr>
<td></td>
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<td>370255-S</td>
<td>50 µg</td>
<td>1:50-100</td>
<td>1:100-200</td>
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<tr>
<td>Heparan Sulfate Monoclonal Antibody</td>
<td>JM403</td>
<td>Mouse</td>
<td>370730-1</td>
<td>200 µg</td>
<td>1:500-1000</td>
<td>✓</td>
<td>1:500-1000</td>
<td></td>
</tr>
</tbody>
</table>
Antibodies to Native Chondroitin Sulfate

<table>
<thead>
<tr>
<th>Antibodies to Native Chondroitin Sulfate</th>
<th>Cat No.</th>
<th>Clone</th>
<th>Format</th>
<th>Pack Size</th>
<th>WB</th>
<th>IHC</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin Sulfate A (C-4-S)</td>
<td>370710-IEC</td>
<td>2H6</td>
<td>Purified</td>
<td>200 ug</td>
<td>1:10,000</td>
<td>1:100 (P)</td>
<td>1:1,000 – 1:2,000</td>
</tr>
<tr>
<td>Chondroitin Sulfate D (C-2,6-S)</td>
<td>AMS.A2872</td>
<td>MO-225</td>
<td>Purified</td>
<td>200 ug</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Chondroitin Sulfate “stub” Monoclonal Antibodies

3 antibodies that specifically recognize unsulfated (0S), 4-sulfated (4S) & 6-sulfated (6S) Chondroitin & Dermatan Sulfate, following Chondroitinase ABC digestion of various proteoglycans.

<table>
<thead>
<tr>
<th>Chondroitin Sulfate Stub Antibodies</th>
<th>Cat No.</th>
<th>Clone</th>
<th>Format</th>
<th>Pack Size</th>
<th>WB</th>
<th>IHC</th>
<th>ELISA</th>
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<tr>
<td>ΔDi-0S</td>
<td>270431-CS</td>
<td>1B5</td>
<td>Supernatant</td>
<td>1 ml</td>
<td>1:100</td>
<td>1:20</td>
<td>✓</td>
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<tr>
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<td>2B6</td>
<td>Supernatant</td>
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<td>1:100</td>
<td>1:20</td>
<td>✓</td>
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<tr>
<td>ΔDi-6S</td>
<td>270433-CS</td>
<td>3B3</td>
<td>Supernatant</td>
<td>1 ml</td>
<td>1:100</td>
<td>1:20</td>
<td>✓</td>
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</table>

1B5: Recognises unsulfated unsaturated disaccharide neoepitopes (i.e. C-0-S “stubs”) generated at the non-reducing terminal of Chondroitin Sulfate GAG chains that have been pre-digested with either Chondroitinase ABC or Chondroitinase ACI.

2B6: Recognises 4-sulfated unsaturated disaccharide neoepitopes (i.e. C-4-S “stubs”) generated at the non-reducing terminal of Chondroitin Sulfate or Dermatan Sulfate GAG chains that have been pre-digested with Chondroitinase ABC but only Chondroitin Sulfate GAG chains pre-digested with Chondroitinase ACII or only Dermatan Sulfate GAG chains pre-digested with Chondroitinase B.

3B3: Recognises 6-sulfated unsaturated disaccharide neoepitopes (i.e. C-6-S “stubs”) generated at the non-reducing terminal of Chondroitin Sulfate GAG chains that have been pre-digested with either Chondroitinase ABC or Chondroitinase ACII. 3B3 also recognises a non-reducing end saturated disaccharide epitope in ‘native’ Chondroitin Sulfate GAG chains consisting of a terminal glucuronic acid adjacent to 6-sulfated N-acetyl-galactosamine. The chondroitinase-generated neoepitope is often denoted as 3B3(+) and the ‘native’ terminal epitope as 3B3(-) in publications.

Reactivity of antibodies to Glycosaminoglycans

Assay Method for GAG Antibodies

Assay Method for ΔGAG Antibodies

www.amsbio.com | info@amsbio.com
Hyaluronic Acid Binding Protein (HABP)

Purified cartilage proteoglycans bind specifically to hyaluronate to form high-MW aggregates in which many proteoglycans are bound to each hyaluronate chain. The proteoglycans bind by a specific site at one end of the protein backbone that is largely devoid of glycosaminoglycan chains (HA-binding region) and has a high affinity for a decasaccharide unit of hyaluronate. The link protein is an integral part of the aggregate structure and has been proposed to form additional bonds, by bridging the proteoglycan molecule and the hyaluronate chain, thereby increasing the strength of binding and giving a more stable aggregate structure (ternary complex).

HABP is available from AMSBIO as purified or botinylated:

- Recombinant human Hyaluronic Acid Binding Protein (rHABP): produced by expression induced culturing in the presence of IPTG using *E. coli* BL21(DE3) RIL transfected with human versican G1-domain expression vector pRK172VG1.

Applications:
- ELISA
- IHC

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<td>Hyaluronan Binding Protein [HABP], Recombinant, Human, Biotin</td>
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<td>50ug</td>
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</table>

Immunohistochemistry for Hyaluronic acid using B-HABP (Bovine Nasal Cartilage)

Reagents:
- Biotinylated Hyaluronic Acid Binding Protein
- Hyaluronidase (Streptomyces hyalurolyticus)
- Chondroitinase ABC Protease Free (Proteus vulgaris)*
- Trypsin *
- Avidin solution/ Biotin solution
- Fluorophore conjugated Streptavidin
  * For proteoglycan digestion when HA is masked.

Procedure

1. Pretreatment with Hyaluronidase (Negative control). Treat with reaction buffer of Hyaluronidase (100mM Sodium acetate buffer, pH6.0) for 15min at 37°C. No wash.
2. Treat with Hyaluronidase (200TRU/mL; 100mM Sodium acetate buffer, pH6.0) for 2hrs at 60°C. Wash with PBS.
4. Treat with 0.1% BSA solution for 1hr at RT. Wash.
5. Treat with Biotinylated HABP (2μg/mL) for 1-2hrs at RT. Wash.
6. Treatment with Fluorophore conjugated Streptavidin for 15min at RT.
ELISA Procedure for Hyaluronic Acid using B-HABP

1. Coat Costar plate with HA (1 ug) or use BSA-HA plates
2. Wash with T-PBS buffer (200ul) 3 times
3. Add bHABP (e.g. 50 ng/mL) [T-PBS/1% BSA as a negative control]
4. Incubate covered by Parafilm at 37 C for 1 hour
5. Discard excess bHABP
6. Wash with T-PBS buffer (200ul) 3 times
7. Add 100ul HRP-sAv solution (ImmunoPure® Streptavidin, Horseradish Peroxidase Conjugated, 1mg)
8. Incubate covered by Parafilm at 37 C for 1 hour
9. Discard excess HRP-SAV
10. Wash with T-PBS buffer (200ul) 3 times
11. Add 100 ul TMB substrate
12. Incubate covered by Parafilm at 37 C for 15 min (prevent light exposure)
13. Add 100ul 1N HCl
14. Measure the endpoint at A450 minus A630nm
Immunohistochemistry for Heparan Sulfate and Chondroitin Sulfate

Reagents

Antibodies to Heparan Sulfate:
10E4,3G10

Antibodies to Chondroitin Sulfate:
1B5,2B6,3B3

GAGases:
Heparinase III (20 mU/ml of sodium acetate buffer-3.3 mM calcium chloride, pH 7.0)
Chondroitinase ABC Protease free (1-5 U/ml of 20 mM Tris-HCl buffer, pH 8.0)

References

Antibodies to Heparan Sulfate

Antibodies to Chondroitin Sulfate

Procedure

1. Prepare slides and controls.
2. Preincubate sections with reaction buffer of GAGase for 15 minutes at 37°C
3. Incubate with GAGase for 1-2 hours at 37°C. Wash.
4. Block endogenous peroxidase with 0.3% H$_2$O$_2$ methanol.
5. Incubate with 1% BSA in PBS for 1 hour at room temperature.
6. Incubate with anti-HS or anti-CS antibody for 1-2 hours at room temperature. Wash.
7. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hour at room temperature. Wash.
8. Incubate with HRP substrate. Wash.
Immunohistochemistry of Hamster's Embryo Tissues Using MAb (10E4,3G10) to Heparan Sulfate
Hamster Fetus (14 days)
Immunostaining using MAb (10E4) to Heparan Sulfate

Immunofluorescence Staining on Normal Human Kidney Sections using MAb (JM403) to Heparan Sulfate

Immunofluorescence staining on normal human kidney sections with JM403 antibody (magnification 20x).

Immunofluorescence Staining on Normal Rat Kidney Sections using MAb (JM403) to Heparan Sulfate

Immunofluorescence staining on normal rat kidney sections with JM403 antibody (magnification 40x).
IHC using CS antibodies

Immuno electron microscopy showing specific staining of the cytolytic granules with anti CS-4 (clone 2B6, 270432-CS). Sections were treated with 1U Chondroitinase ABC (AMS. E1028) for 2H at 37C, followed by staining with anti-CS4. Staining was detected by gold labelled Protein A. Specific staining allowed for the quantitation of granularity in primary NK cells drawn from the blood. Sectioning and staining performed for Malmberg lab, Oslo University Hospital by Andreas Brech at the Norwegian Radium Hospital Institute for Cancer Research.

Immunohistochemistry of Hamster Embryo Vertebrae, using monoclonal antibodies (1B5, 2B6, 3B3) to Chondroitin Sulfate. Showing results with & without treatment with Chondroitinase ABC (Chase ABC).

Immunohistochemistry of Articular Cartilage using MAb to Proteoglycan ΔDi-0S, ΔDi-4S & ΔDi-6S

ΔDi-0S (Clone 1-B-5)

**Figure 1:** Chase ABC-

**Figure 2:** Chase ABC+

ΔDi-4S (Clone 2-B-6)

**Figure 1:** Chase ABC-

**Figure 2:** Chase ABC+
Immunohistochemistry of Western Blot using MAb (5D3) to Aggrecan (ACAN/ Chondroitin sulfate proteoglycan)

(A) Immunostaining of human articular cartilage.
(B) Double immunostaining for aggrecan and neurofilaments in human adult cerebral cortex. Cell nuclei are counterstained with TO-PRO-1. Right lower panel: SDS-PAGE on 3-8% linear gradient gels under reducing conditions of purified human articular cartilage aggrecan prior to (intact) / after combined keratanase I, endo-beta-galactosidase and chondroitinase ABC-digestion

Immunohistochemistry using MAb (373E1) to Keratan Sulfate

(Left) Immunohistochemical staining (FITC-conjugated secondary antibodies) with mAb 373E1 of keratan sulfates of the ECM deposited with a glomerule of human kidney (PFA-OCT embedding and cryosectioning).

(Right) Immunohistochemical staining of keratan sulfates deposited within Langerhans islands of human adult pancreas (Formalin-paraffin embedding)
Immunohistochemistry using MAb (905A7) to Biglycan

(A) Human intestine and (B) human skeletal muscle.

Immunohistochemistry using MAb (889C7) to Decorin

(A) Human prostate and (B) human breast.

Immunohistochemistry and Western Blot using MAb (5C12) to Versican (CSPG2)

(A) Immunoblotting of intact versican (mixture of V1 & V2 isoforms) in untreated and Chase ABC-digested form, or after combined digestion with Chase ABC and a number of exo- and endoglycosydases.

(B) Immunohistochemistry on human normal urinary bladder.

(C) Immunostaining of versican in the matrix deposited in vitro of human microvascular endothelial cells after TNF stimulation.

(D) Immunostaining of versican lining the wall of a larger vein in human kidney (PFA-fixed frozen section).
Flow Cytometry using MAbs (10E4 and 3G10) to Heparan Sulfate

Procedure
1. Incubate $1 \times 10^6$ cells with 100 μl of Heparinase III or PBS for 20 minutes at 37°C. Wash.
2. Incubate cells with anti-HS for 30 minutes at 4°C. Wash.
3. Incubate cells with FITC conjugated F(ab')2 fragment anti-mouse IgG or IgM for 30 minutes at 4°C. Wash.
4. Analyze using manufacturer's instructions.

Reagents
Antibody to Heparan Sulfate: 10E4, 3G10
Cells: K-562, PLC/PRF/5
GAGases: Heparitinase I (50 mU/ml of phosphate buffer saline, pH 7.4 (PBS))

Results

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<tr>
<th>Cell Line</th>
<th>10E4</th>
<th>3G10</th>
</tr>
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<tbody>
<tr>
<td>KM3</td>
<td>HSase I (-)</td>
<td>-</td>
</tr>
<tr>
<td>Daudi</td>
<td>Weakly +</td>
<td>+</td>
</tr>
<tr>
<td>EB2</td>
<td>+ (&gt;90%)</td>
<td>↓ (50%)</td>
</tr>
<tr>
<td>CCRF-SB</td>
<td>Weakly +</td>
<td>↓ (-)</td>
</tr>
<tr>
<td>Molt 4</td>
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<td>+ (60%)</td>
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<tr>
<td>HPBALL</td>
<td></td>
<td>↓ (10%)</td>
</tr>
<tr>
<td>K-562</td>
<td>+ (80%)</td>
<td>↓ (10%)</td>
</tr>
<tr>
<td>PB (M)</td>
<td></td>
<td>↓ (-)</td>
</tr>
<tr>
<td>PB (L)</td>
<td></td>
<td>↓ (+)</td>
</tr>
<tr>
<td>PB (G)</td>
<td></td>
<td>↓ (10%)</td>
</tr>
<tr>
<td>MKN 74</td>
<td>+ (100%)</td>
<td>↓ (90%)</td>
</tr>
<tr>
<td>COLO 201</td>
<td>+ (80%)</td>
<td>↓ (-)</td>
</tr>
<tr>
<td>PLC/PRF/5</td>
<td>+ (80%)</td>
<td>~ +</td>
</tr>
<tr>
<td>Hep G2</td>
<td>+ (100%)</td>
<td>↓ (80%)</td>
</tr>
<tr>
<td>G32TG</td>
<td>+ (90%)</td>
<td>↓ (+)</td>
</tr>
</tbody>
</table>

+: positive, -: negative, ↑: increase, ↓: decrease, →: no change, (%) : positive rate
Western Blot for Heparan Sulfate & Chondroitin Sulfate Proteoglycan

Reagents
Antibody to Heparan Sulfate:
3G10
Antibodies to Chondroitin Sulfate:
1-B-5, 2-B-6, 3-B-3

GAGases:
Heparinase III (200 mU/ml of sodium acetate buffer-3.3 mM calcium chloride, pH 7.0). Chondroitinase ABC Protease free (1-5 U/ml of 20 mM Tris-HCL buffer, pH 8.0)

Membranes:
PVDF membrane or nitrocellulose membrane

References
Antibodies to Heparan Sulfate

Antibodies to Chondroitin Sulfate

Western Blot Examples

Western blot analysis of rat liver proteoglycans using MAb to \( \Delta \) heparan sulfate (3G10)

Western blot analysis of rat liver proteoglycans using MAb to \( \Delta \) Di-0S (1-B-5), \( \Delta \) Di-4S (2-B-6), \( \Delta \) Di-6S (3-B-3)

1. Incubate partially purified proteoglycan fractions with GAGase for 1 hour at 37°C.
   a) Treat 1.5 \( \mu \)g of sample with 2 mU of Heparitinase I for heparan sulfate proteoglycan
   b) Treat 1 \( \mu \)g of sample with 100 mU of Chondroitinase ABC for chondroitin sulfate proteoglycan.

2. Run samples on SDS-PAGE under reducing conditions.

3. Transfer it to membrane.

4. Blocking with 10% skim milk in PBS for 30 minutes at 37°C.

5. Incubate with anti-HS or anti-CS antibody for 1 hour at room temperature. Wash.

6. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hour at room temperature. Wash.


Western Blot using MAb (373E1) to Keratan Sulfate

Western blotting of purified human articular cartilage aggrecan using keratan sulfate (373E1), resolved prior to and after keratanase II, endo-B-galactosidase or chondroitinase ABC-digestion on SDS-Agarose electrophoresis (left gel) or 3-8% gradient gels.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>HUVECs</td>
<td>human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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</table>
Immunoprecipitation for Chondroitin Sulfate Proteoglycan

Reagents

Antibodies to Chondroitin Sulfate: 1B5, 2B6

GAGases:
Chondroitinase ABC Protease free (1-5 U/ml of 100 mM sodium acetate buffer, pH 8.0 or 20 mM Tris-HCl buffer, pH 8.0)

Procedure
1. Prepare 125I proteoglycan fractions by Chloramin T method.
2. Incubate 125I labeled proteoglycan fractions with GAGase for 1 hour at 37°C.
   (Treat 3 μg of sample with 100 mU of Chondroitinase ABC)
3. Incubate sample with normal mouse IgG and Protein G Sepharose for 1 hour at 4°C.
4. Remove Protein G Sepharose binding non-specific immune complexes and save supernatant.
5. Incubate supernatant with anti-CS antibody and new Protein G Sepharose for 3 hours at 4°C. Wash.
6. Boil Protein G Sepharose binding specific immune complexes with SDS-PAGE sample buffer for 5 minutes.
7. Save supernatant after centrifugation.
8. Run supernatant on SDS-PAGE under reducing conditions.
9. Place gel in direct contact with X-ray film and develop using manufacturers instructions.

Glycobiology Antibodies: Citations

10E4 and 3G10 Antibodies

10E4, 3G10 and JM-403 Antibodies
**10E4 and JM-403 Antibodies**

**10E4, 3G10 and 3B3 Antibodies**

**JM403 Citations**

**Stub Antibodies**

---

**3B3 (270433-1)**

**2B6 (270432-1)**

**1B5, 2B6, 3B3, 10E4 (Seikagaku)**
1B5, 2B6, 3B3, 3G10


2B6 (270432-CS)


Native CS antibodies


MO-225, 2H6, and LY111


MO225, CS56, LY111, and 2H6 (Seikagaku)


473HD, CS-56, and MO-225


2H6 and MO-225


Biotinylated Hyaluronan Binding Protein [B-HABP] Citations for AMS.HKD-BC41


Proteoglycans Detection Kit

Sulfated GAGs can be measured directly by use of a metachromatic dye, 1, 9 Dimethylmethylene blue (DMMB). The GAG-dye complex results in an absorption spectrum shift which can be measured at between 515 and 530 nm.

The Proteoglycan Assay uses the metachromatic dye 1, 9-dimethylmethylene blue to quantify the amount of sulfated glycosaminoglycans in the standard and test samples. The binding of the sulfated glycosaminoglycans to the dye induces a shift in the absorption spectrum which is directly proportional to the amount of sulfated glycosaminoglycans. The sample values, μg/ml of sulfated glycosaminoglycans, are determined by the standard curve. The assay detects chondroitin 4 and 6 sulfates, and heparan, keratan, and dermatan sulfates. Hyaluronic acid will not be detected and also will not interfere with the assay.

Various biologic fluids can be tested following papain digestion such as synovial fluid, serum, amniotic fluid and urine. Tissue culture medium can be tested directly. Tissue samples such as cartilage, skin, and other organs must first be digested with papain or extracted with 3M guanidinium HCl before testing (see Sample Preparation).

### Proteoglycan Detection Kit

<table>
<thead>
<tr>
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<tr>
<td>Proteoglycan Detection Kit</td>
<td>280560-N</td>
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</table>

**Proteoglycan Detection Kit Citation:**

**Razie Enzyme Activity Assays**

AMSBIO supply Razie assay kits for quantitative detection of Heparanase and Hyaluronidase in cell culture supernatants, human plasma, biological fluids and tissue samples.

**Kit features**
- Suitable for inhibitor screening
- Non-radioactive
- Fast and easy to use
- Sensitive and specific
- Uses a universal 96-well plate format ideal for inhibitor studies Heparanase Assay
Heparanase Assay Kit

A handicap in Heparan Sulfate research has been a lack of a sensitive and more importantly specific test for human eparanase activity. Furthermore unavailability of a purified enzyme or instability of the cloned enzyme limits assay design. To date the available tests have the above shortcomings and are time consuming, not applicable for inhibitor screening or lack an appropriate positive control.

Heparanase Assay Citations

- Masola, V., Gambaro, G., Tibaldi, E., Onisto, M., Abaterusso, C., & Lupo, A. (2011). Regulation of heparanase by albumin and advanced glycation end products in proximal tubular cells Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1813(8), 1475-1482

Hyaluronidase Assay Kit

For screening of Hyaluronidase inhibitors, and quantification of Hyaluronidase activity

PRINCIPLES OF THE TEST (HEPARANASE ASSAY)

GAG-coated 96-well plates available separately

Hyaluronidase Assay Citation

### Glycosaminoglycans

High grade purified Heparan Sulfate, Chondroitin Sulfate, Dermatan Sulfate, Keratan Sulfate and Hyaluronic Acid standards from a variety of sources. Also available: Dermatan Sulfate and Over Sulfated Chondroitin Sulfate Standards, highly purified from contaminated Heparin Sodium.

<table>
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<td>Whale Cartilage</td>
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<td>400676-1A</td>
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<td>Squid Cartilage</td>
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<td>AMS.CSR-NACS-E2.SQC-10</td>
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<td>Squid Cartilage</td>
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<td>31254.01</td>
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<td>2 mg</td>
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<td>25 mg</td>
<td>Crude heparin</td>
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<tr>
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<td>20 mg</td>
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<td>Pig skin</td>
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<td>AMS.CSR-NADS-B2-PGS-3</td>
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<td>3 MG</td>
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<tr>
<td>AMS.GAG-DS01</td>
<td>Dermatan Sulfate (from pig mucosa)</td>
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<td>pig mucosa</td>
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<td><strong>Heparan Sulfate</strong></td>
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<td>400700</td>
<td>Heparan Sulfate, Na Salt</td>
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<td>Bovine Kidney</td>
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<td>AMS.GAG-HS01</td>
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<td>Pig Mucosa</td>
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<td><strong>Hyaluronic Acid</strong></td>
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<td>400720</td>
<td>Hyaluronic Acid, Na Salt</td>
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<td><strong>Keratan Sulfate</strong></td>
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<td>AMS.CSR-NAKPS2-SHC-1</td>
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<tr>
<td>AMS.CSR-NAKPS2-SHC-3</td>
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<td>AMS.CSR-NAKS2-PNC-1</td>
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<td>Sodium Keratan Sulfate (Nasal Cartilage)</td>
<td>3 MG</td>
<td>Pig Cartilage Nasal Cartilage</td>
</tr>
</tbody>
</table>
### Heparin

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin Sodium, research grade</td>
<td>24590.01</td>
<td>500 mg</td>
</tr>
<tr>
<td>Heparin Sodium, research grade</td>
<td>24590.02</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Heparin Sodium, research grade</td>
<td>24590.03</td>
<td>10 g</td>
</tr>
<tr>
<td>Heparin - high grade</td>
<td>AMS.HEP001-100</td>
<td>10 mg</td>
</tr>
<tr>
<td>Low-In-Calcium Heparin</td>
<td>AMS.LCaHEP002-100</td>
<td>10 mg</td>
</tr>
<tr>
<td>Low Molecular weight heparin</td>
<td>AMS.LMW Heparin</td>
<td>10 mg</td>
</tr>
<tr>
<td>Heparin Saccharide Mw. 7400</td>
<td>AMS.HO22</td>
<td>2 mg</td>
</tr>
<tr>
<td>Heparin Saccharide Mw. 8000</td>
<td>AMS.HO24</td>
<td>2 mg</td>
</tr>
<tr>
<td>Heparin Saccharide Mw. 8700</td>
<td>AMS.HO26</td>
<td>2 mg</td>
</tr>
<tr>
<td>Heparin Polymer (&gt;9000)</td>
<td>AMS.HO30</td>
<td>2 mg</td>
</tr>
</tbody>
</table>

Selectively DeSulfated Heparins

These heparin products have been made from high quality heparin modified by standard chemical methods to selectively remove sulfate groups from C2 of Iduronate, (De2S Hep), C6 of glucosamine (De6SHep) or the Nsulfate of Glucosamine (DeNS Hep). The DeNS heparin contains the free amino group (NH+3); in DeNS/Ac Hep the free amino group has been modified by acetylation. This range of desulfated heparins is complemented by our series of sulfated KS polysaccharides in which the internal uronate is glucuronic acid (GlcUA) in the native unmodified structure.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-K5/NS: N-Sulfated SO3-/COO- : 1.00</td>
<td>AMS.E-K008</td>
<td>1mg</td>
</tr>
<tr>
<td>E-K5/OS(L): O-Sulfated, low SO3-/COO- : 1.16</td>
<td>AMS.E-K009</td>
<td>1mg</td>
</tr>
<tr>
<td>E-K5/OS(H): O-Sulfated, high SO3-/COO- : 3.44</td>
<td>AMS.E-K010</td>
<td>1mg</td>
</tr>
<tr>
<td>E-K5/NS, OS(L): N, O-Sulfated, low SO3-/COO- : 1.95</td>
<td>AMS.E-K011</td>
<td>1mg</td>
</tr>
<tr>
<td>E-K5/NS, OS(H): N, O-Sulfated, high SO3-/COO- : 4.03</td>
<td>AMS.E-K012</td>
<td>1mg</td>
</tr>
</tbody>
</table>

### Substrates & Standards: Oligosaccharides

#### Dermatan Sulfate Oligosaccharides

Prepared from high quality Dermatan Sulfate (porcine origin) by partial GAG-endolyase scission and isolated by high resolution gel filtration.

**General formula:**

\[
\Delta UA-GallNAc,4S – (IdoA – GalNAc,4S)n-IdoA-GalNAc, 4S
\]

where \( n \) is number of disaccharide units

- \( n = 0 \) in a tetrasaccharide (dp4)
- \( n = 1 \) in a hexasaccharide (dp6)
- \( n = 2 \) in an octasaccharide (dp8) etc..

Uronic acid (\( \Delta UA \)) at the non-reducing ends of the oligosaccharides has a C4-C5 double bond as a result of endolytic scission. The main disaccharide in the original dermatan sulfate was IdoA – GalNAc,4S (88%) with minor quantities of 6-sulfated and 2,4 disulfated units (5% and 7% respectively) also present.

#### Hyaluronic Acid Oligosaccharides

Hyaluronic Acid (HA) is a glycosaminoglycan composed of an alternating sequence of "1,3 glucuronic acid (GlcA) and "1,4 N-acetylg glucosamine (GlcNAc). In its native state HA is normally present in the extracellular matrix as a high molecular weight, high viscosity polymer essential for maintenance tissue architecture, elasticity and hydration. However it also has other key functions including the regulation of cell behaviour through specific interactions with cell surface receptors and extracellular proteins. HA binding to individual proteins commonly involves relatively short sequences in the HA polymer and there is considerable evidence that HA fragments generated in vivo have distinctive properties from the intact polymer.

AMSBIO offers a range of oligosaccharides produced by controlled endolyase scission of purified, low endotoxin Hyaluronic Acid (Streptococcal species). The oligosaccharides are separated by high resolution gel filtration and purity assessed on an analytical Superdex S75 HPLC column (see Data Sheets for profiles). Size range of oligosaccharides dp2 to dp 20 dp is degree of monosaccharide polymerisation: dp2 is a disaccharide, dp4 is a tetrasaccharide etc.
General formula:
\[ \Delta \text{HexA}^{1,3} \ (\text{GlcNAc}^{1,4} \ \text{GlcA}^{1,3})^n \ \text{GlcNAc} \quad n = \text{number of disaccharide units} \]

\( \Delta \text{HexA} \) is the C4-C5 unsaturated hexuronic acid at the non-reducing end of the oligosaccharides produced by endolyase scission of the HA polymer. The C4-C5 double bond absorbs strongly at 232nm and can be used for monitoring the oligosaccharides in various separation systems.

**Heparin Oligosaccharides**

Prepared from high grade porcine heparin using bacterial Heparinase and isolated by high resolution gel filtration.

General formula:
\[ \Delta \text{UA,2S-GlcNS,6S} - (\text{IdoA,2S} - \text{GlcNS,6S})^n - \text{IdoA,2S-GlcNS,6S} \text{ where 'n' is the number of disaccharide units} \]

\( n = 0 \) in the dp4 (HO04) tetrasaccharide
\( n = 1 \) in the dp6 (HO06) hexasaccharide
\( n = 2 \) in the dp8 (HO08) octasaccharide etc.

Uronic acid (\( \Delta \text{UA} \)) at the non-reducing end of the oligosaccharides has a C4-C5 double bond as a result of the endolytic action of bacterial heparinase.

Although the main disaccharide unit in these products is IdoA,2S – GlcNS,6S (approx 75%) saccharides in each size class show some variation in sulfation.

**Heparin Disaccharides**

Produced by the action of bacterial heparinase on high grade porcine heparin. Isolated by high resolution gel filtration and ion exchange chromatography. The uronate (\( \Delta \text{UA} \)) contains a C4-C5 double bond due to the action of the heparinas used to depolymerise heparin. Our range includes N-unsubstituted disaccharides.
Chondroitin/Dermatan Sulfate Disaccharides

Produced by bacterial chondroitinase digestion of Chondroitin and Dermatan sulfate. Isolated by high resolution gel filtration and ion exchange chromatography. Our product range includes the rare, but functionally important di and trisulfated Chondroitin/Dermatan sulfate disaccharides.

Disaccharides with N-unsubstituted Amine

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔUA,2S – GlcN</td>
<td>AMS.HD010</td>
<td>1mg</td>
</tr>
<tr>
<td>ΔUA,2S – GlcN,6S</td>
<td>AMS.HD011</td>
<td>1mg</td>
</tr>
<tr>
<td>ΔUA – GlcN,6S</td>
<td>AMS.HD012</td>
<td>1mg</td>
</tr>
<tr>
<td>ΔUA – GlcN</td>
<td>AMS.HD013</td>
<td>1mg</td>
</tr>
</tbody>
</table>

Fluorescence labeled GAGs

Prepared by the fluorescent labeling of HA. Fluoresceinamine molecules are chemically attached to carboxyl groups of the GlcUA of HA. This solution is dissolved in PBS (−) and sterilized by filtration. The excitation wavelength is 490~500 nm and the emission wavelength is 515~525 nm.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoresceinamine Labeled Sodium Chondroitin Sulfate A (A1)</td>
<td>AMS.CSR-FACS-A1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Chondroitin Sulfate (C1)</td>
<td>AMS.CSR-FACS-C1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Chondroitin Sulfate D (D1)</td>
<td>AMS.CSR-FACS-D1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Chondroitin Sulfate E (E1)</td>
<td>AMS.CSR-FACS-E1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Chondroitin Poly-Sulfate (P1)</td>
<td>AMS.CSR-FACS-P1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Dermatan Sulfate (B1)</td>
<td>AMS.CSR-FADS-B1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Heparin (N1)</td>
<td>AMS.CSR-FAHEP-N1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Heparan Sulfate (P1)</td>
<td>AMS.CSR-FAHS-P1</td>
<td>1 ML</td>
</tr>
</tbody>
</table>


Effects of different molecular weights of sodium hyaluronate on tissue permeability and inflammatory/immunoregulatory factor synthesis.
Prepared by the fluorescent labeling of GAGs. Fluoresceinamine molecules are chemically attached to carboxyl groups of glucuronic acid of CS; GlcUA or IdoUA of DS; GlcUA or IdoUA of Hep or GlcUA or IdoUA of HS. This solution is dissolved in PBS (-) and sterilized by filtration. The excitation wavelength is 490~500 nm and the emission wavelength is 515~525 nm.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat No.</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (H1)</td>
<td>AMS.CSR-FAHA-H1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (H2)</td>
<td>AMS.CSR-FAHA-H2</td>
<td>3 MG</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (L1)</td>
<td>AMS.CSR-FAHA-L1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (L2)</td>
<td>AMS.CSR-FAHA-L2</td>
<td>3 MG</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (M1)</td>
<td>AMS.CSR-FAHA-M1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (M2)</td>
<td>AMS.CSR-FAHA-M2</td>
<td>3 MG</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (S1)</td>
<td>AMS.CSR-FAHA-S1</td>
<td>3 ML</td>
</tr>
<tr>
<td>Fluoresceinamine Labeled Sodium Hyaluronate (S1)</td>
<td>AMS.CSR-FAHA-T1</td>
<td>3 ML</td>
</tr>
</tbody>
</table>

Analysis of Binding Activities with bFGF.

Analysis of binding activities with BMP-4 by fluorescence correlation spectroscopy (FCS).

Hyalose: Size Standards For Glycobiology Research

**Select-HA™** - Select-HA is hyaluronic acid produced through enzymatic synthesis achieving a high level of size control. Polydispersity of the products in any size from 50kDa to 1000kDa is as low as 1.02 and averages 1.1 (a value of 1 is associated with a ‘perfect’ polymer). Hyalose offers one grade of Select-HA products and certifies its products as having endotoxin levels of less than 0.1 EU/mg of HA polymer.

Q: **What is the difference between Select-HA™ and other commercial HAs?**
A: The unique property of Select-HA™ is that it has very narrow size distribution while all other commercial HA polymers are mixtures of HA with a much broader size range. Please see the pictures (agarose gels stained with Stains -All) for a better understanding.

Q: **How are the sizes of Select-HA™ defined?**
A: Due to the nature of the production process, there is lot-to-lot variation. If the indicated molecular mass (determined by MALLS-SEC and reported on the Certificate of Analysis) falls within 25-75 kDa, it is called Select-HA™ 50. However, the Select-HA™ 50 is not a mixture of HA ranging from 25 kDa to 75 kDa. Remember, for any given lot, the polydispersity is close to 1 (i.e. close to monodispersity).

- Select-HA™ 50 will be within the range of 25-75 kDa
- Select-HA™ 150 will be within the range of 125-175 kDa
- Select-HA™ 500 will be within the range of 400-600 kDa
- Select-HA™ 1000 will be within the range of 800-1200 kDa

- Various sizes (25 kDa–1000 kDa)
- Low endotoxin, certified to be less than 0.1 EU/mg of HA
- Available biotinylated
**Select-HA HiLadder™** - The Select-HA HiLadder contains five Select-HA molecular mass markers in the range of ~500 kDa to ~1500 kDa. The masses are 495 kDa, 572 kDa, 966 kDa, 1090 kDa and 1510 kDa. Recommended usage: agarose gel stained with StainsAll.

**Select-HA LoLadder™** - The Select-HA LoLadder contains five Select-HA molecular mass markers in the range of ~25 kDa to ~500 kDa. The masses are 27 kDa, 110 kDa, 214 kDa, 310 kDa and 495 kDa. Recommended usage: agarose gel stained with StainsAll.

**Select-HA MegaLadder™** - The Select-HA MegaLadder is a mixture of streptavidin complexes containing one, two, three or four end-labeled biotin-Select-HA molecules of very defined sizes for use as size standards in gel electrophoresis or other separation methods. This ladder covers a range from 2 MegaDalton to 8 MegaDalton. Recommended usage: agarose gel stained with StainsAll.

**Description** | **Cat No.** | **Mol. W** | **Pack Size**  
--- | --- | --- | ---  
**Hyalose Ladders™**  
Select-HA™ LoLadder | HYA-LOLAD-20 | ~25 kDa to ~500 kDa. | 20 lanes  
Select-HA™ HiLadder | HYA-HILAD-20 | ~500 kDa to ~1500kDa. | 20 lanes  
Select-HA™ MegaLadder | HYA-MGLAD-20 | 2 to 8 MegaDalton | 20 lanes  
Super Mega-HATM Ladder | HYA-MGLAD-20_SML200903 | 2 to 8 MegaDalton | 20 lanes  
**Select-HA™**  
Select-HA™ 50K Low Endotoxin | HYA-50KEF-1 | 25-75 kDa | 1 mg  
Select-HA™ 150K Low Endotoxin | HYA-150KEF-1 | 125-175 kDa | 1 mg  
Select-HA™ 500K Low Endotoxin | HYA-500KEF-1 | 400-600 kDa | 1 mg  
Select-HA™ 1000K Low Endotoxin | HYA-1000KEF-1 | 800-1200 kDa | 1 mg
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