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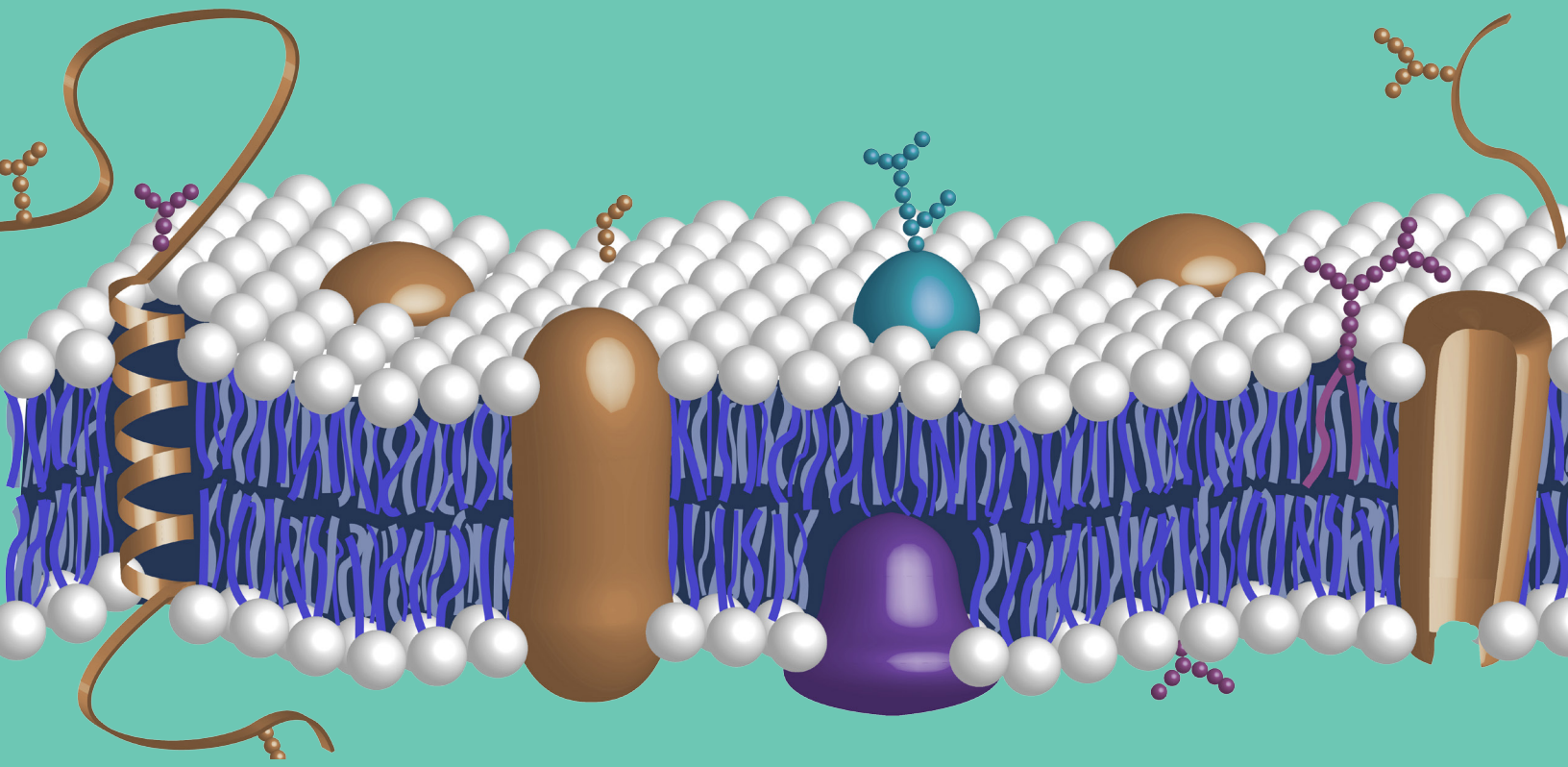


Proteoglycans & Glycosaminoglycans

Glycobiology Research

Hyaluronic Acid - Chondroitinase ABC - Assays - Enzymes - Antibodies

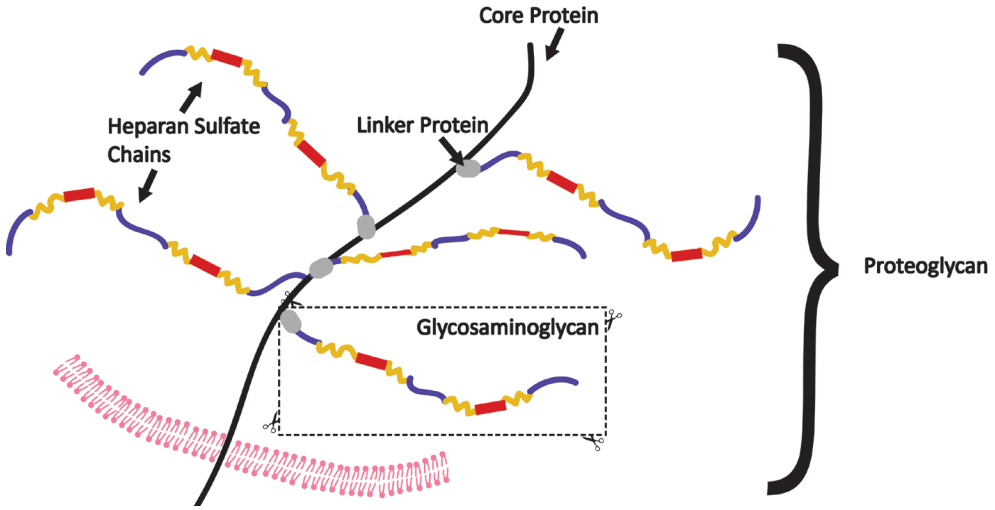
www.amsbio.com | www.proteoglycan.info

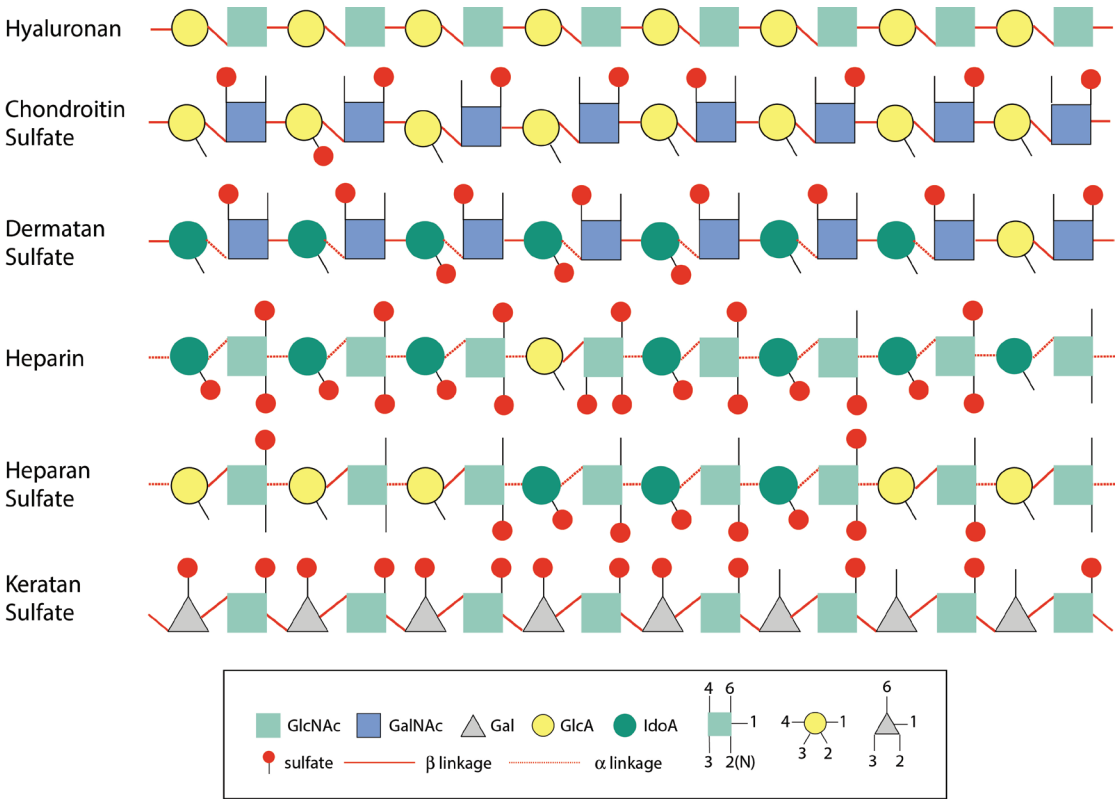
Table of Contents

Structure of Glycosaminoglycans

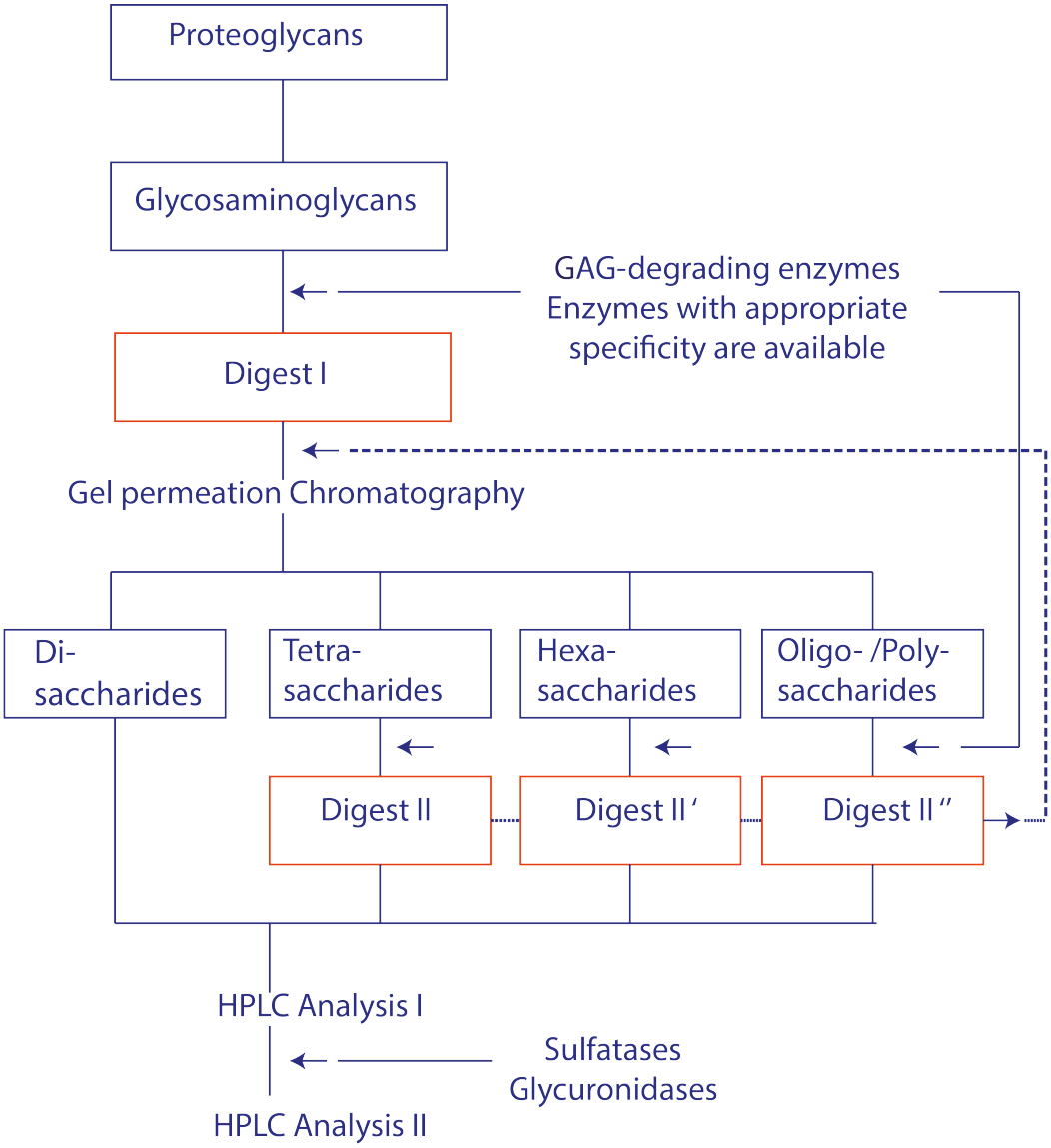
Proteoglycans are glycosylated proteins which have covalently attached highly anionic glycosaminoglycans (GAGs). Proteoglycans are present in different forms within different types of extracellular matrices and connective tissues. Heparan sulfate proteoglycans (HSPGs) are composed of a core protein with heparan sulfate (HS) GAG chains attached.

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



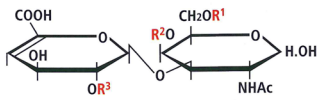


Systematic Analysis of Glycosaminoglycan (GAG) Structure



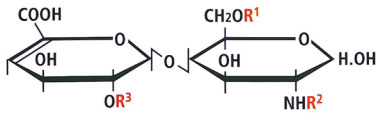
Structure of the Unsaturated Disaccharides

Chondroitin Sulfate Family



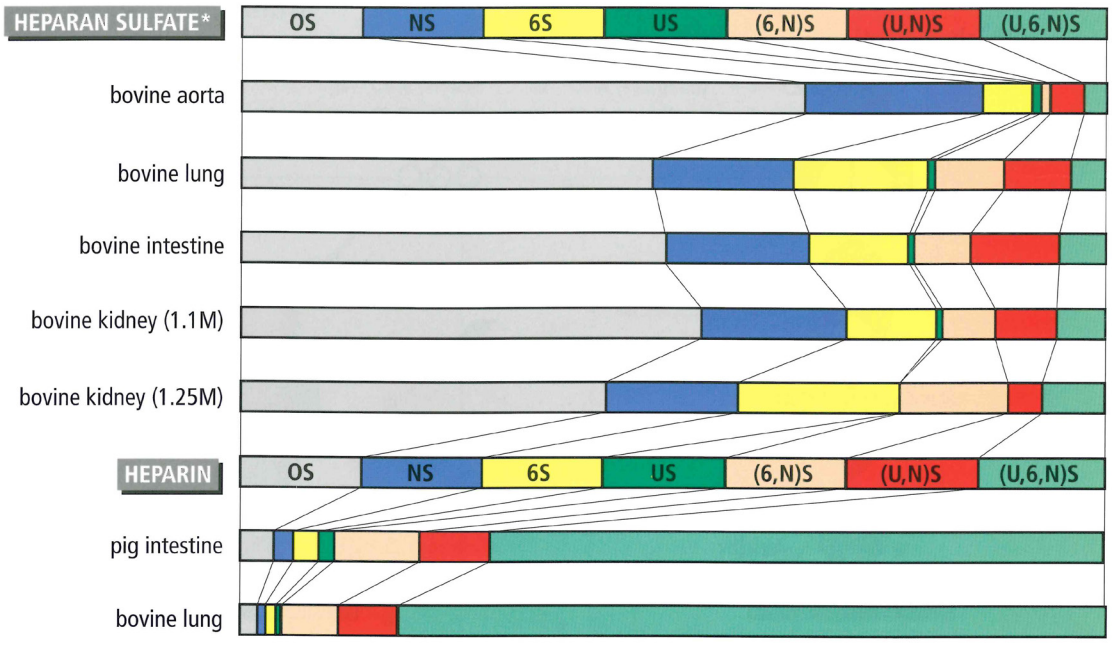
ΔDi	R^1	R^2	R^3	Abbreviation
-OS	H	H	H	
-6S	SO^{3-}	H	H	*C*
-4S	H	SO^{3-}	H	*A*
-US	H	H	SO^{3-}	
-(U,6)S	SO^{3-}	H	SO^{3-}	*D*
-(U,4)S	H	SO^{3-}	SO^{3-}	*(B)*
-(4,6)S	SO^{3-}	SO^{3-}	H	*E*
-(U,4,6)S	SO^{3-}	SO^{3-}	SO^{3-}	

Heparan Sulfate Family



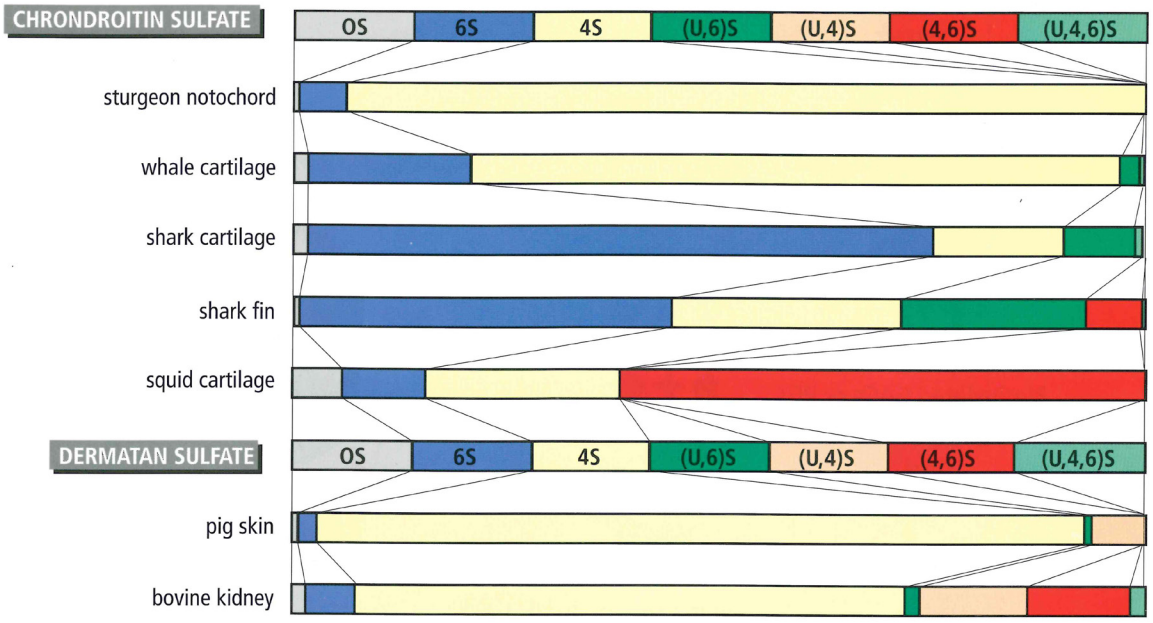
ΔDiHS	R^1	R^2	R^3
-OS	H	Ac	H
-NS	H	SO^{3-}	H
-6S	SO^{3-}	Ac	H
-US	H	Ac	SO^{3-}
-(6,N)S	SO^{3-}	SO^{3-}	H
-(U,N)S	H	SO^{3-}	SO^{3-}
-(U,N,6)S	SO^{3-}	SO^{3-}	SO^{3-}

Disaccharide Composition of Heparan Sulfate and Heparin



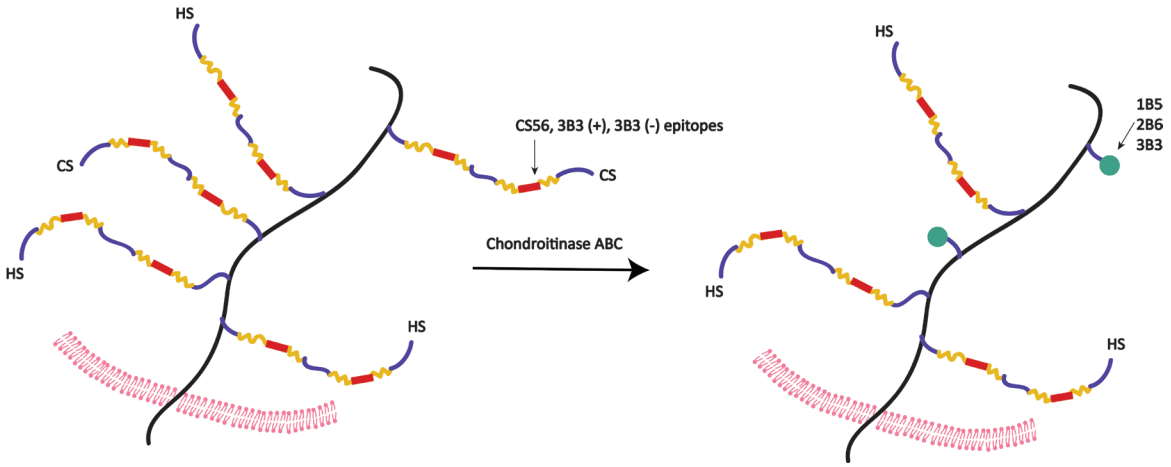
* Maccarna, M. et al., J. Biol. Chem., 271, 17804-17810 (1996)

Disaccharide Composition of Chondroitin Sulfate and Dermatan Sulfate

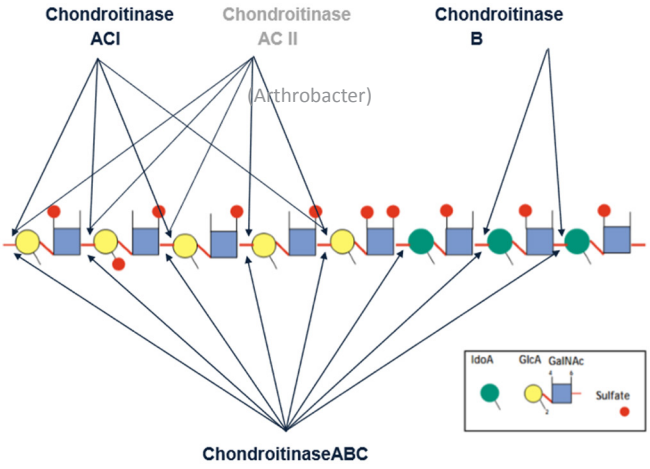


Chondroitinase Enzymes

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.



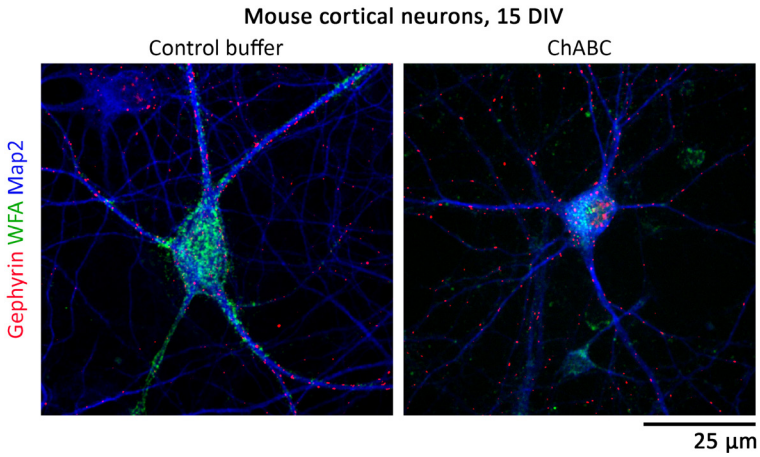
Description	Cat No.	Pack Size
Purified		
Chondroitinase ABC protease free-(*Proteus vulgaris*)	AMS.E1028-02	2 U
	AMS.E1028-10	10 U
Chondroitinase AC-I-(*Flavobacterium heparinum*)	AMS.CDACI-ENZ-S	5 IU
	AMS.CDACI-ENZ BU	20 IU
	AMS.CDACI-ENZ BU2	50 IU
	AMS.CDACI-ENZ BU3	100 IU
	AMS.CDACI-ENZ BU4	250 IU
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		
Chondroitinase AC, Research Grade-(Recombinant *Flavobacterium heparinum*)	AMS.50-013	0.5 IU



Chondroitinases attack and cleave the following GAGs:

Ezyme	Substrate
Chondroitinase ABC	Hylauronic acid, Chondroitin sulfate, Dermatan Sulfate
Chondroitinase AC-I	Chondroitin Sulfate A & C
Chondroitinase AC-II	Chondroitin Sulfate A & C
Chondroitinase B	Dermatan Sulfate

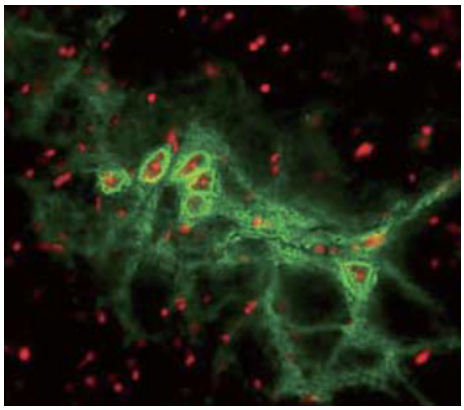
Perineuronal Net Removal by Chondroitinase ABC



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.

Shows digestion of perineuronal net by our Chondroitinase ABC AMS.E1028 (ChABC application decreased WFA reactivity, indicating the digestion of the perineuronal net, in mouse dissociated cortical cultures).

Images above courtesy of: Cell biology of the synapse laboratory, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS, INSERM U1024, Ecole Normale Supérieure, PSL Research University, F-75005 Paris, France, Corresponding author (for image): Alexandra Nothnagel, Institut de Biologie de l'ENS (IBENS), 46 rue d'Ulm, F-75005, Paris, France



Perineuronal Net in Rat Cerebellum 1B5(+): Green: 1B5(+), Orange: Nuclear Staining.

Chondroitinase ABC Citations

Chondroitinase ABC (AMS.E1028)

Neural Regeneration Models

- Abu-Rub, M. T., Newland, B., Naughton, M., Wang, W., McMahon, S., & Pandit, A. (2016). Non-Viral Xylosyltransferase-1 siRNA Delivery as an Effective Alternative to Chondroitinase in an In Vitro Model of Reactive Astrocytes. Neuroscience.
- Heidemann, M., Streit, J., & Tscherter, A. (2014). Functional regeneration of intraspinal connections in a new in vitro model. Neuroscience, 262, 40-52.
- Paveliev, M., Fenrich, K. K., Kislin, M., Kuja-Panula, J., Kulesskiy, E., Varjosalo, M., ... Kulesskaya, N. & Rauvala, H. (2016). HB-GAM (pleiotrophin) reverses inhibition of neural regeneration by the CNS extracellular matrix. Scientific Reports, 6, 33916.
- Silver, D. J., Siebzehnrubl, F. A., Schildts, M. J., Yachnis, A. T., Smith, G. M., Smith, A. A., ... & Steindler, D. A. (2013). Chondroitin sulfate proteoglycans potently inhibit invasion and serve as a central organizer of the brain tumor microenvironment. The Journal of Neuroscience, 33(39), 15603-15617.
- Wu, D., Klaw, M. C., Connors, T., Kholodilov, N., Burke, R. E., & Tom, V. J. (2015). Expressing Constitutively Active Rheb in Adult Neurons after a Complete Spinal Cord Injury Enhances Axonal Regeneration beyond a Chondroitinase-Treated Glial Scar. The Journal of Neuroscience, 35(31), 11068-11080.
- Wu, D., Klaw, M. C., Kholodilov, N., Burke, R. E., Detloff, M. R., Côté, M. P., & Tom, V. J. (2016). Expressing constitutively active Rheb in adult dorsal root ganglion neurons enhances the integration of sensory axons that regenerate across a chondroitinase-treated dorsal root entry zone following dorsal root crush. Frontiers in Molecular Neuroscience, 9. doi: 10.3389/fnmol.2016.00049
- Xu, C., Klaw, M. C., Lemay, M. A., Baas, P. W., & Tom, V. J. (2015). Pharmacologically inhibiting kinesin-5 activity with monastrol promotes axonal regeneration following spinal cord injury. Experimental neurology, 263, 172-176.

Chondroitinase ABC (AMS.E1028)

Glycoanalysis

- Dagälv, A., Lundequist, A., Filipek-Górniok, B., Dierker, T., Eriksson, I., & Kjellén, L. (2015). Heparan sulfate structure: methods to study N-sulfation and NDST action. Glycosaminoglycans: Chemistry and Biology, 189-200.
- Fang, J., Song, T., Lindahl, U., & Li, J. P. (2016). Enzyme overexpression–an exercise toward understanding regulation of heparan sulfate biosynthesis. Scientific Reports, 6, 31242.
- Nelson, A., Berkestedt, I., & Bodelsson, M. (2014). Circulating glycosaminoglycan species in septic shock. Acta Anaesthesiologica Scandinavica, 58(1), 36-43.
- Roucourt, B., Meeussen, S., Bao, J., Zimmermann, P., & David, G. (2015). Heparanase activates the syndecan-syntenin-ALIX exosome pathway. Cell research, 25(4), 412-428.
- Scavenius, C., Nikolajsen, C. L., Stenvang, M., Thøgersen, I. B., Wyrożemski, Ł., Wisniewski, H. G., ... & Enghild, J. J. (2016). The compact and biologically relevant structure of inter-α-inhibitor is maintained by the chondroitin sulfate chain and divalent cations. Journal of Biological Chemistry, 291(9), 4658-4670.

Chondroitinase ABC (AMS.E1028)

Musculoskeletal Models

- Dierker, T., Bachvarova, V., Krause, Y., Li, J. P., Kjellén, L., Seidler, D. G., & Vortkamp, A. (2016). Altered heparan sulfate structure in Glce−/− mice leads to increased Hedgehog signaling in endochondral bones. Matrix Biology 49, 82–92
- Eftestøl, E., Egner, I. M., Lunde, I. G., Ellefsen, S., Andersen, T., Sjåland, C., ... & Bruusgaard, J. C. (2016). Increased hypertrophic response with increased mechanical load in skeletal muscles receiving identical activity patterns. American Journal of Physiology-Cell Physiology, ajpcell-00016.
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- Melleby, A. O., Strand, M. E., Romaine, A., Herum, K. M., Skrbic, B., Dahl, C. P., ... & Lunde, I. G. (2016). The Heparan Sulfate Proteoglycan Glypican-6 Is Upregulated in the Failing Heart, and Regulates Cardiomyocyte Growth through ERK1/2 Signaling. PloS one, 11(10), e0165079.
- Strand, M. E., Aronsen, J. M., Braathen, B., Sjaastad, I., Kvaløy, H., Tønnessen, T., ... & Lunde, I. G. (2015). Shedding of syndecan-4 promotes immune cell recruitment and mitigates cardiac dysfunction after lipopolysaccharide challenge in mice. Journal of molecular and cellular cardiology, 88, 133-144.

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

- Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., & McMahon, S. B. (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature, 416(6881), 636-640.
- Moon, L.D.F., Asher, R.A., Rhodes, K.E., & Fawcett, J.W. (2001) Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nature Neuroscience 4, 465-466.
- Thuret, S., Moon, L.D.F., & Gage, F.H. (2006) Therapeutic strategies for SCI. Nature Reviews Neuroscience 7, 628-643.

Background / Reviews:

- Burnside, E. R., & Bradbury, E. J. (2014) Review: Manipulating the extracellular matrix and its role in brain and spinal cord plasticity and repair. Neuropathology and applied neurobiology, 40(1), 26-59.
- Caterson, B. (2012) Fell-Muir Lecture: Chondroitin sulphate glycosaminoglycans: fun for some and confusion for others. International journal of experimental pathology, 93(1), 1-10.
- Fox, K., & Caterson, B. (2002) Freeing the brain from the perineuronal net. Science, 298 (5596), 1187-1189.

Heparinase Enzymes

Bacterial heparinases 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:

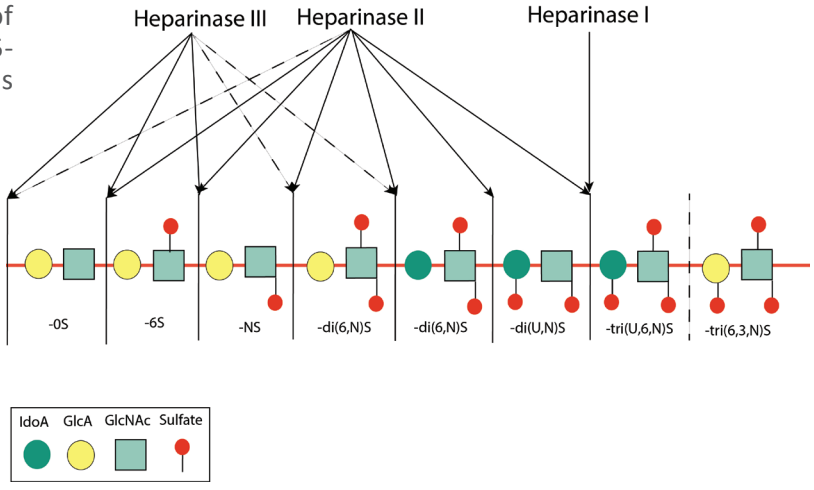
Alternative Classification	Classification
Heparinase I - is equivalent to:	Heparinase
Heparinase II and III - are equivalent to:	Heparitinase
Heparinase III - is equivalent to:	Heparitinase I
Heparinase II - is equivalent to:	Heparitinase II

Description	Cat No.	Pack Size
Seikagaku Enzymes		
Heparinase	100700-3	0.1 U
Heparinase II	100705-1	0.1 U
Heparinase I	100704-1A	0.1 U
Heparitinase*	100703-3	0.1 U

*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).

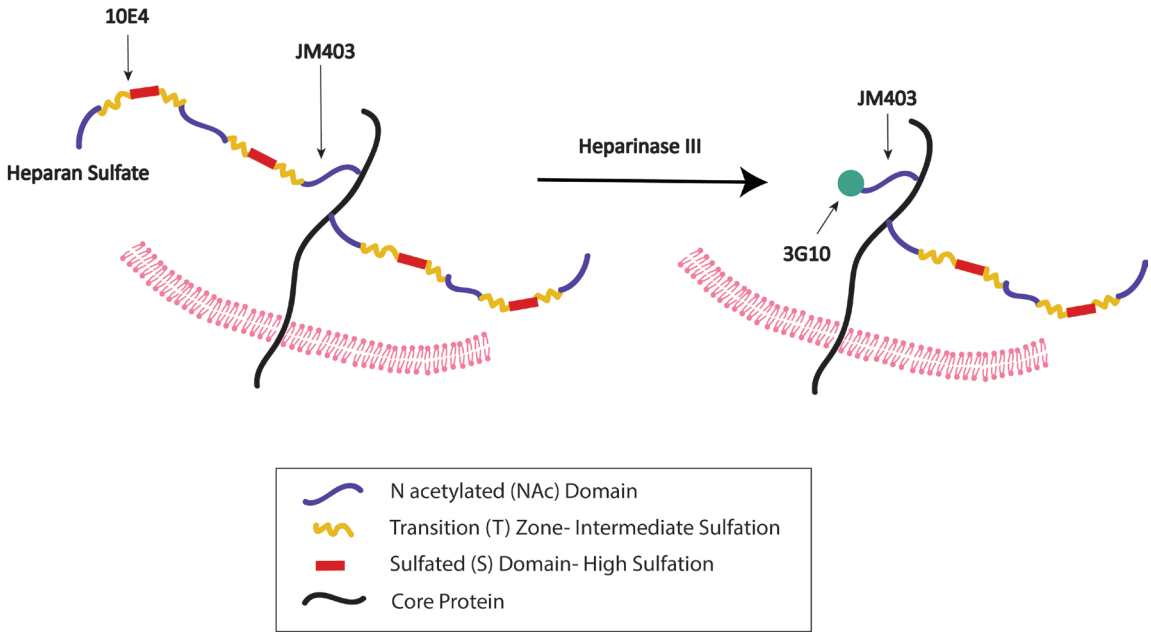
Description	Cat No.	Pack Size
Purified Bacterial Heparinases from *Flavobacterium heparinum*		
Heparinase I (EC 4.2.2.7)	AMS.HEP-ENZ I-S	0.1 IU
	AMS.HEP-ENZ I BU	2 IU
	AMS.HEP-ENZ I BU2	5 IU
	AMS.HEP-ENZ I BU3	10 IU
	AMS.HEP-ENZ I BU4	20 IU
Heparinase II	AMS.HEP-ENZ II-S	0.1 IU
	AMS.HEP-ENZ II	0.25 IU
	AMS.HEP-ENZ II BU	0.5 IU
	AMS.HEP-ENZ II BU2	1 IU
	AMS.HEP-ENZ II BU3	2 IU
	AMS.HEP-ENZ II BU4	5 IU
Heparinase III (EC 4.2.2.8)	AMS.HEP-ENZ III-S	0.1 IU
	AMS.HEP-ENZ III	0.5 IU
	AMS.HEP-ENZ III BU	1 IU
	AMS.HEP-ENZ III BU2	2 IU
	AMS.HEP-ENZ III BU3	5 IU
	AMS.HEP-ENZ III BU4	10 IU

Description	Cat No.	Pack Size
Recombinant Bacterial Heparinases from *Flavobacterium heparinum*		
Heparinase I	AMS.50-010	0.5 IU
Heparinase II	AMS.50-011	0.5 IU
Heparinase III	AMS.50-012	0.5 IU



Heparinase I-III Citations

- Douet, V., Kerever, A., Arikawa-Hirasawa, E., & Mercier, F. (2013). Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. Cell proliferation, 46(2), 137-145.Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. Cell proliferation, 46(2), 137-145. Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. Cell proliferation, 46(2), 137-145.
- Eftestøl, E., Egner, I. M., Lunde, I. G., Ellefsen, S., Andersen, T., Sjåland, C., ... & Bruusgaard, J. C. (2016). Increased hypertrophic response with increased mechanical load in skeletal muscles receiving identical activity patterns. American Journal of Physiology-Cell Physiology, ajpcell-00016.
- Keenan, T. D., Toso, M., Pappas, C., Nichols, L., Bishop, P. N., & Hageman, G. S. (2015). Assessment of Proteins Associated With Complement Activation and Inflammation in Maculae of Human Donors Homozygous Risk at Chromosome 1 CFH-to-F13BCFH-to-F13B Locus and Complement Activation in Macula. Investigative ophthalmology & visual science, 56(8), 4870-4879.
- Strand, M. E., Aronsen, J. M., Braathen, B., Sjaastad, I., Kvaløy, H., Tønnessen, T., ... & Lunde, I. G. (2015). Shedding of syndecan-4 promotes immune cell recruitment and mitigates cardiac dysfunction after lipopolysaccharide challenge in mice. Journal of molecular and cellular cardiology, 88, 133-144.
- Strand, M. E., Herum, K. M., Rana, Z. A., Skrbic, B., Askevold, E. T., Dahl, C. P., ... & Carlson, C. R. (2013). Innate immune signaling induces expression and shedding of the heparan sulfate proteoglycan syndecan-4 in cardiac fibroblasts and myocytes, affecting inflammation in the pressure-overloaded heart. FEBS Journal, 280(10), 2228-2247.



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. 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

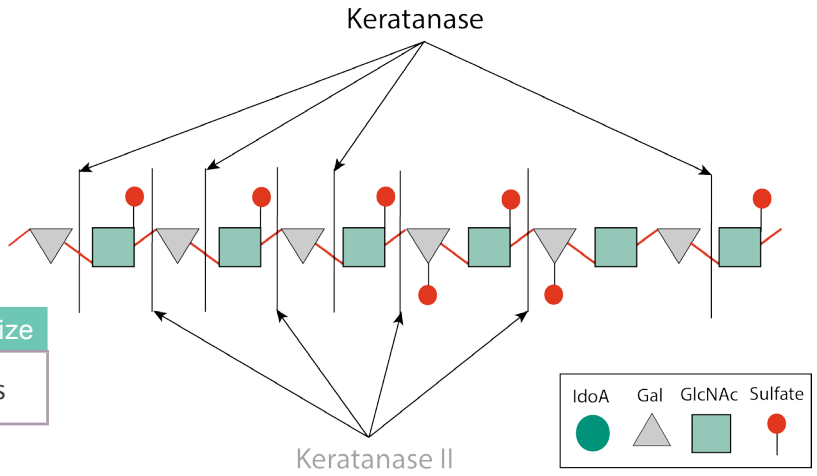
Cat No.	Description	Pack Size
25118.01	Hyaluronidase (ovine testes)	50mg
25118.02	Hyaluronidase (ovine testes)	500mg

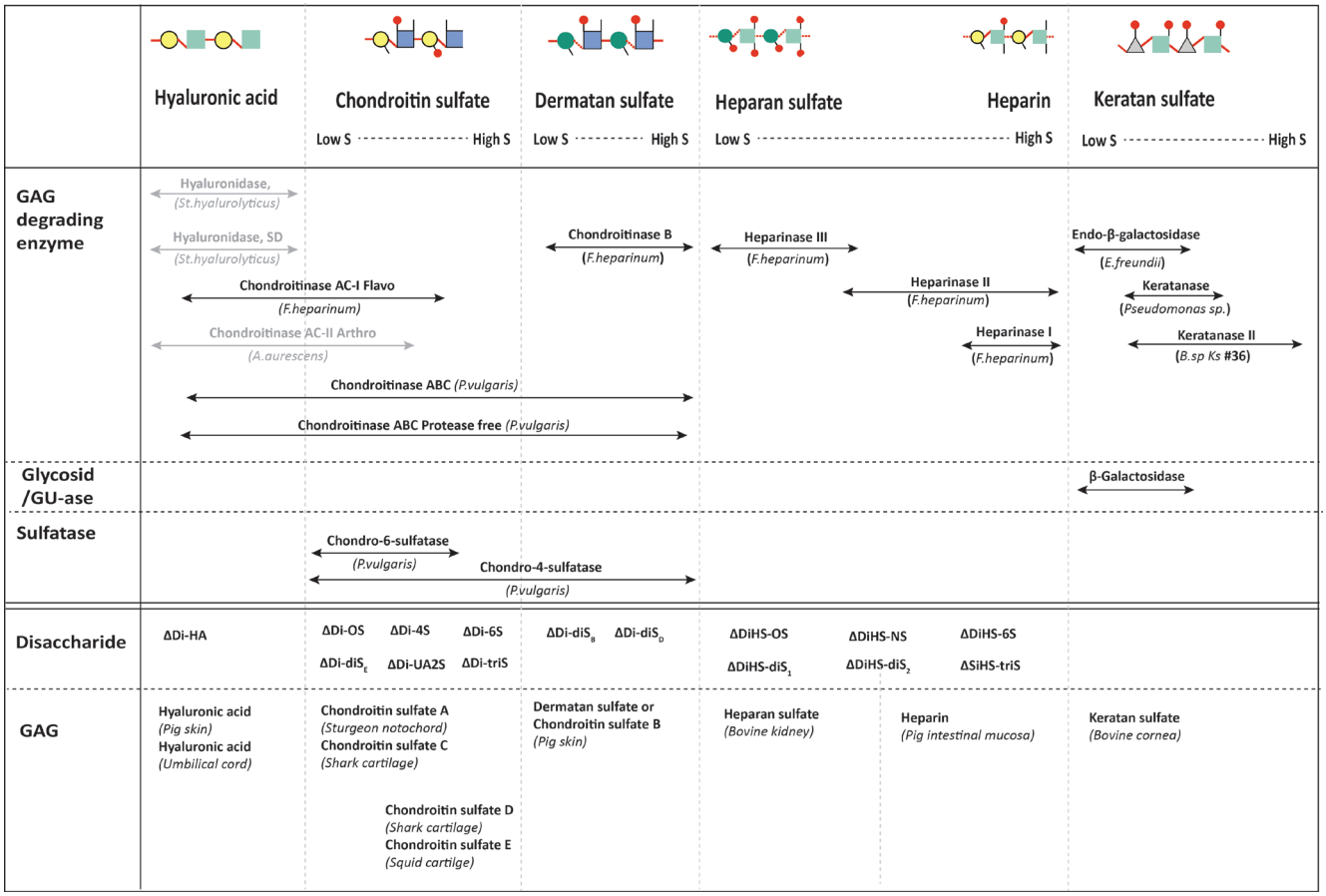
Keratanase Enzymes

Keratanase: Attacks and cleaves low sulfate region.

Keratanase II: Attacks and cleaves high sulfate region.

Description	Cat No.	Pack Size
Keratanase (Pseudomonas sp.)	100810-1	10 units





✓ = Indicates that clone has been used for this application, but no suggested dilutions available.
* Optimal dilutions/concentrations should be determined by end-user.

Proteoglycan Antibodies

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

Glycosaminoglycan (GAG) Antibodies

Description	Clone	Pack Size	Cat No.	IHC	FC	ELISA	WB	IP
Anti Keratan Sulfate, purified; recognises KS lacking oversulfated structures	R-10G	100 UG	AMS.RIT-M001	10 µg / mL			3 µg / mL	
Monoclonal Anti Keratan Sulfate supernatant	5D4	1 ML	270427-CS	1:20			1:100	
Anti Keratan Sulfate	373E1	2 ML	AMS.PRPG-KS-M01	1:50 to 1:150	✓	1:100 – 1:500	1:50 to 1:170	✓

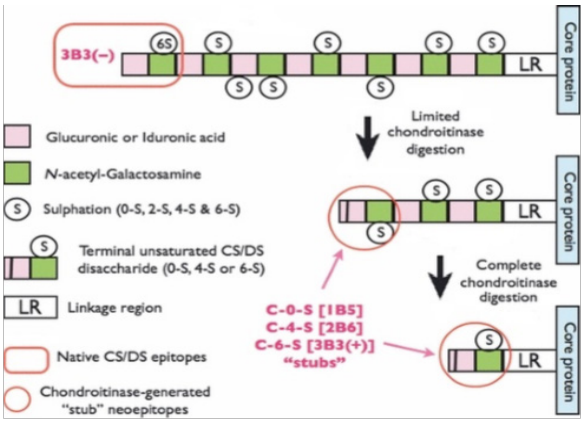
Description	Clone	Host	Cat No.	Pack Size	IHC	FC	ELISA	WB
Δ-Heparan Sulfate Antibody	F69-3G10	Mouse	370260-1	200 µg	1:100-200	1:100-200	1:200-500	✓
			370260-S	50 µg				
Heparan Sulfate Monoclonal Antibody	F58-10E4	Mouse	370255-1	200 µg	1:50-100	1:100-200	1:100-1:500	✓
			370255-S	50 µg				
Heparan Sulfate Monoclonal Antibody	JM403	Mouse	370730-1	200 µg	1:500-1000	✓	1:500-1000	

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

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.

Chondroitin Sulfate Stub Antibodies	Cat No.	Clone	Format	Pack Size	WB	IHC	ELISA
ΔDi-OS	270431-CS	1B5	Supernatant	1 ml	1:100	1:20	✓
ΔDi-4S	270432-CS	2B6	Supernatant	1 ml	1:100	1:20	✓
ΔDi-6S	270433-CS	3B3	Supernatant	1 ml	1:100	1:20	✓



Epitope mapping scheme of chondroitin sulfate-detecting antibodies. (Anti CS, clone 1B5, 2B6, 3B3).

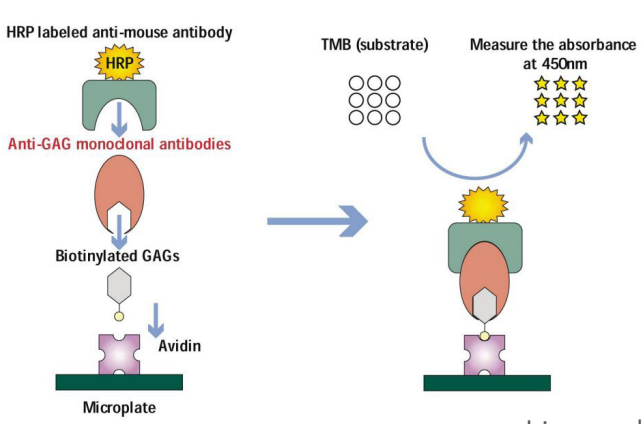
1B5: Recognises unsulfated unsaturated disaccharide neoepitopes (i.e. **C-0-S "stubs"**) generated at the non-reducing terminal of **Chondroitin Sulfate** GAG chains that have been pre-digested with either **Chondroitinase ABC** or **Chondroitinase ACI**.

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.

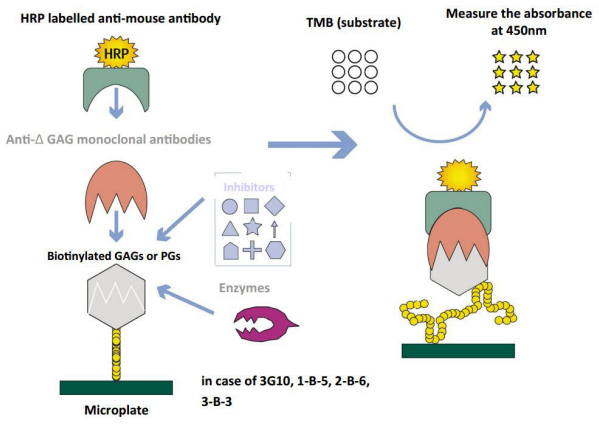
3B3: Recognises 6-sulfated unsaturated disaccharide neoepitopes (i.e. **C-6-S "stubs"**) generated at the non-reducing terminal of Chondroitin Sulfate GAG chains that have been pre-digested with either Chondroitinase ABC or Chondroitinase ACII. 3B3 also recognises a non-reducing end saturated disaccharide epitope in 'native' Chondroitin Sulfate GAG chains consisting of a terminal glucuronic acid adjacent to 6-sulfatedN-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



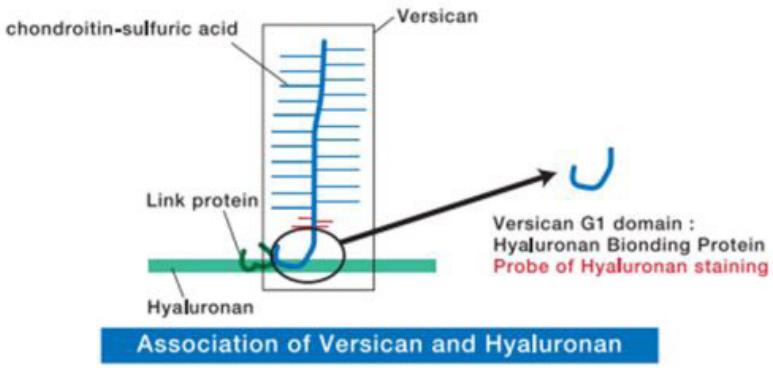
Hyaluronic Acid Binding Protein (HABP)

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 ex-pression vector pRK172VG1.

Applications:
✓ ELISA
✓ IHC



Description	Cat No.	Pack Size
Hyaluronan Binding Protein [HABP], Recombinant, Human, Purified	AMS.HKD-BC40	50ug
Hyaluronan Binding Protein [HABP], Recombinant, Human, Biotin	AMS.HKD-BC41	50ug

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

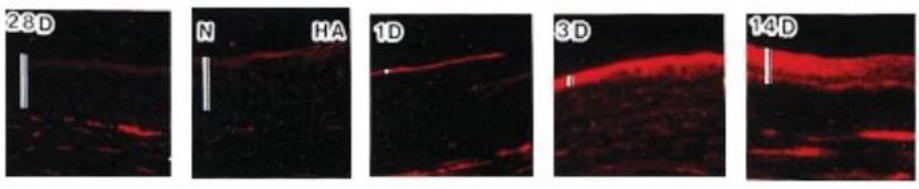
Reagents:

Biotinylated Hyaluronic Acid Binding Protein
Hyaluronidase (Streptmyces 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.
Treat with Hyaluronidase (200TRU/mL; 100mM Sodium acetate buffer, pH6.0) for 2hrs at 60°C. Wash with PBS.
2. Blocking endogeneous avidin biotin activity. Treat with avidin solution for 20min at RT. Wash. Treat with biotin solution for 20min at RT. Wash.
3. Treat with 0.1% BSA solution for 1hr at RT. Wash.
4. Treat with Biotinylated HABP (2µg/mL) for 1-2hrs at RT. Wash.
5. Treat with Fluorophore conjugated Streptavidin for 15min at RT.

Example: Histochemical staining of hyaluronan (Texas red) in rabbit cornea.
N: Normal cornea; 1, 3, 14 and 28 days: corneas 1, 3, 14 and 28 days after wounding

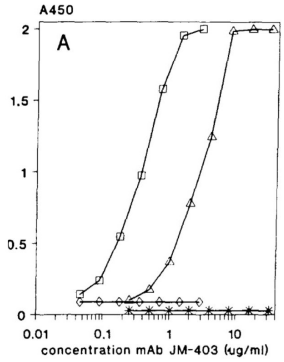


ELISA Procedure for Hyaluronic Acid using B-HABP

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

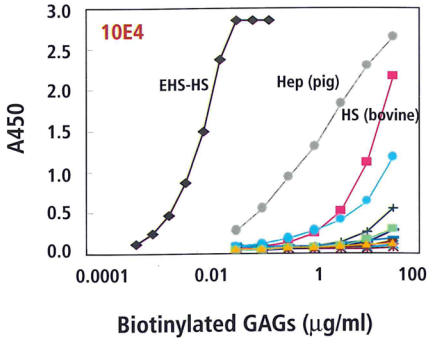
Glycobiology Antibodies: Reactivity

Reactivity of HS Antibodies to Glycosaminoglycans



JM403

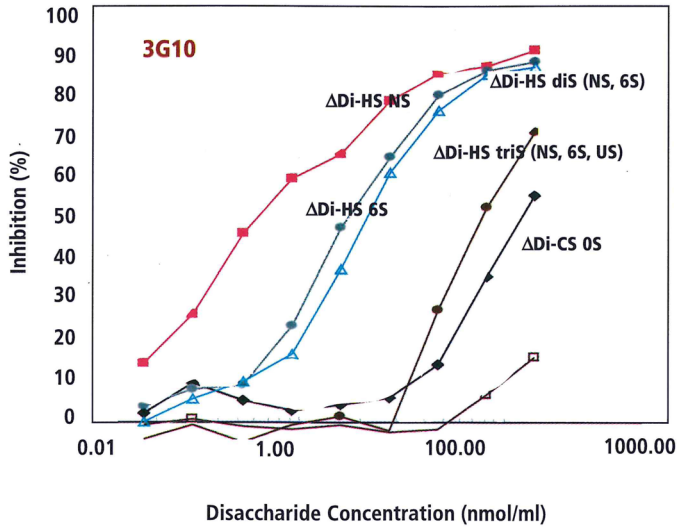
—△— GBM HSPG (Human)
—□— HS
—✱— Core protein of GBM HSPG (Human) after removal of HS chains
—◇— Other GAGs (CS-A, CS-C, DS, KS, heparin)



EHS-HS
CS-A (whale)
CS-C (shark)
HS (bovine)
CS-A (sturgeon)
CS-E (squid)
Hep (pig)
CS-B (pig)
KS (bovine)
HA
CS-D (shark)

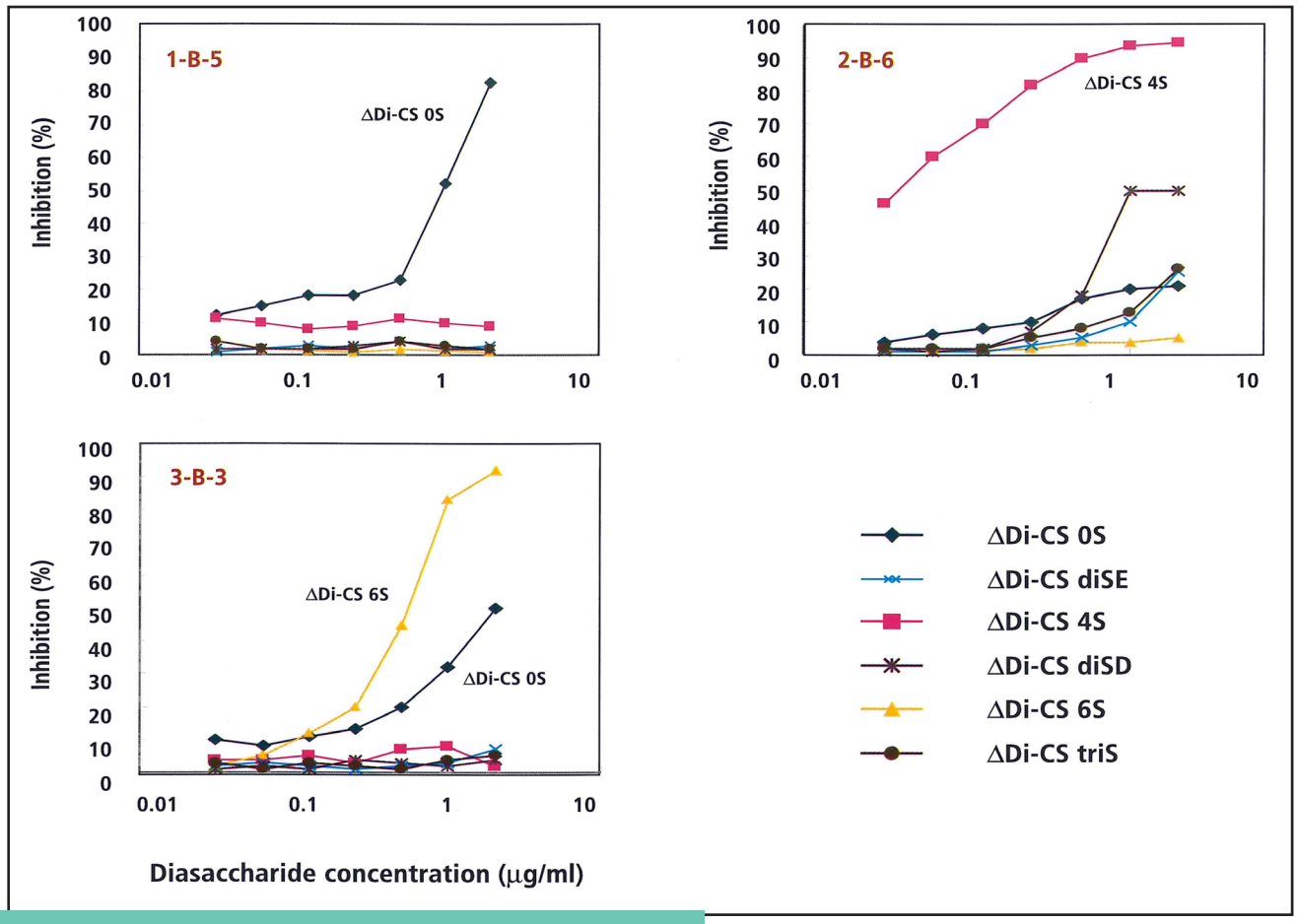
Reactivity of Antibodies to Unsaturated Glycosaminoglycans

Δ Heparan Sulfate



ΔDi-CS 0S
ΔDi-HS NS
ΔDi-HS 6S
ΔDi-HS diS(NS, 6S)
ΔDi-HS diS(US, NS)
ΔDi-HS triS(NS, 6S, US)

Δ Chondroitin Sulfate



Glycobiology Antibodies: IHC and IF

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 calciumchloride,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
1.David,G.et al.J.Cell Biol.,119,961-975 (1992)

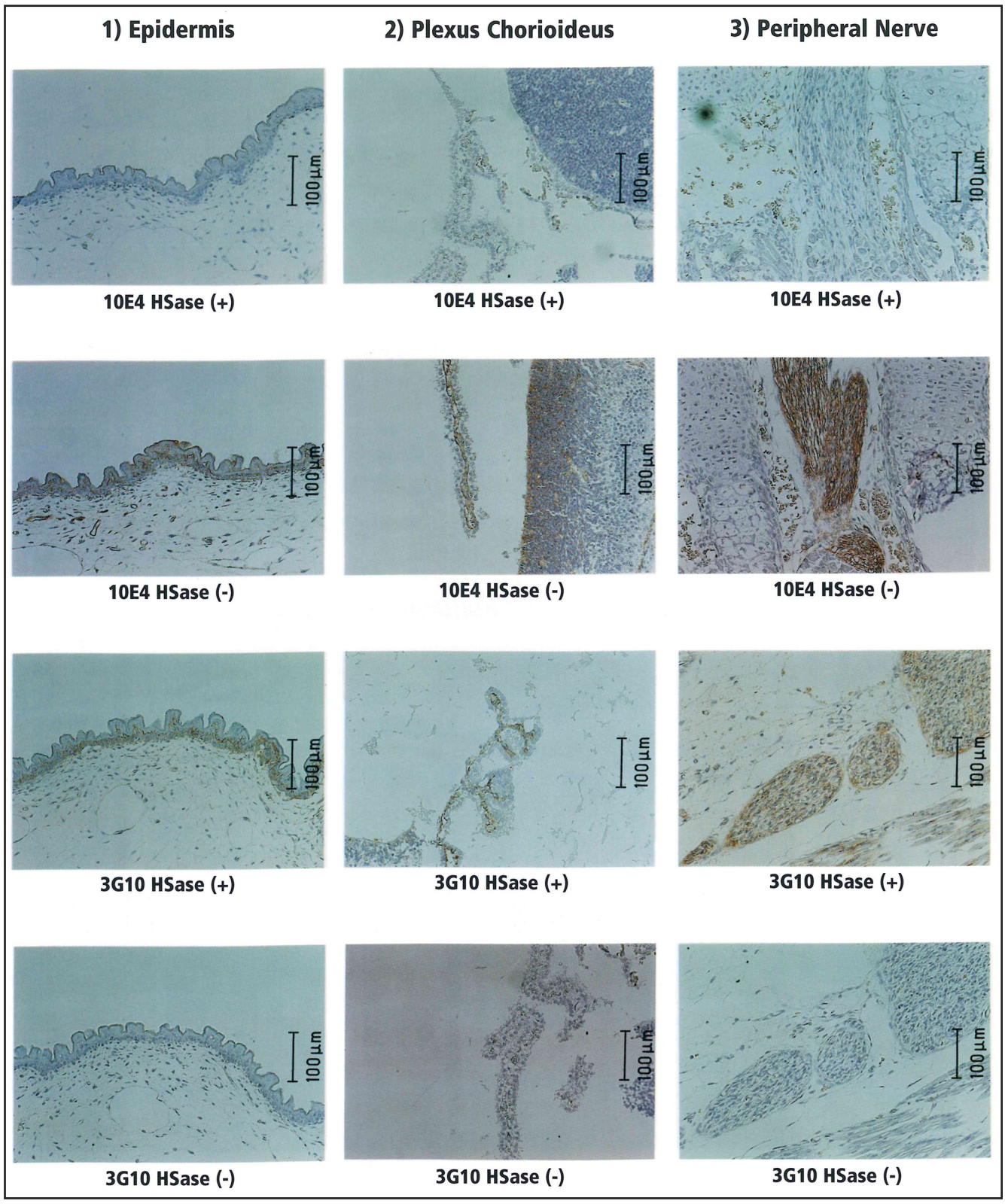
Antibodies to Chondroitin Sulfate
1. Mark,M.P.et al.Develop.Biol.,133,475-488 (1989)
2. Fukatsu,T.et al.Br.J.Cancer,57,74-78 (1987)
3. Sorrell,J.M.etal.J.Immunol.,140,4263-4270 (1988)

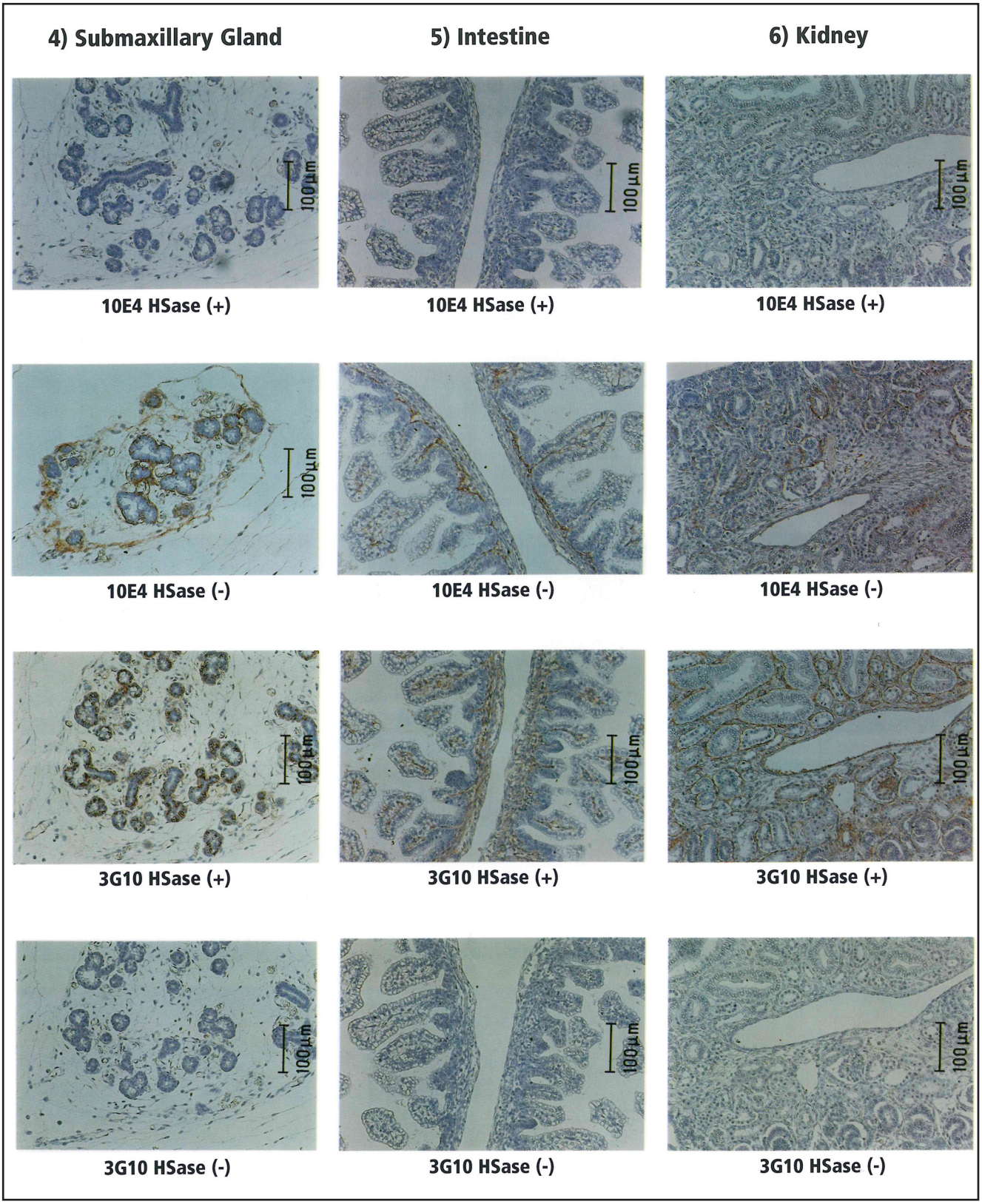
Procedure

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_2O_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.
9. Observe by microscopy.

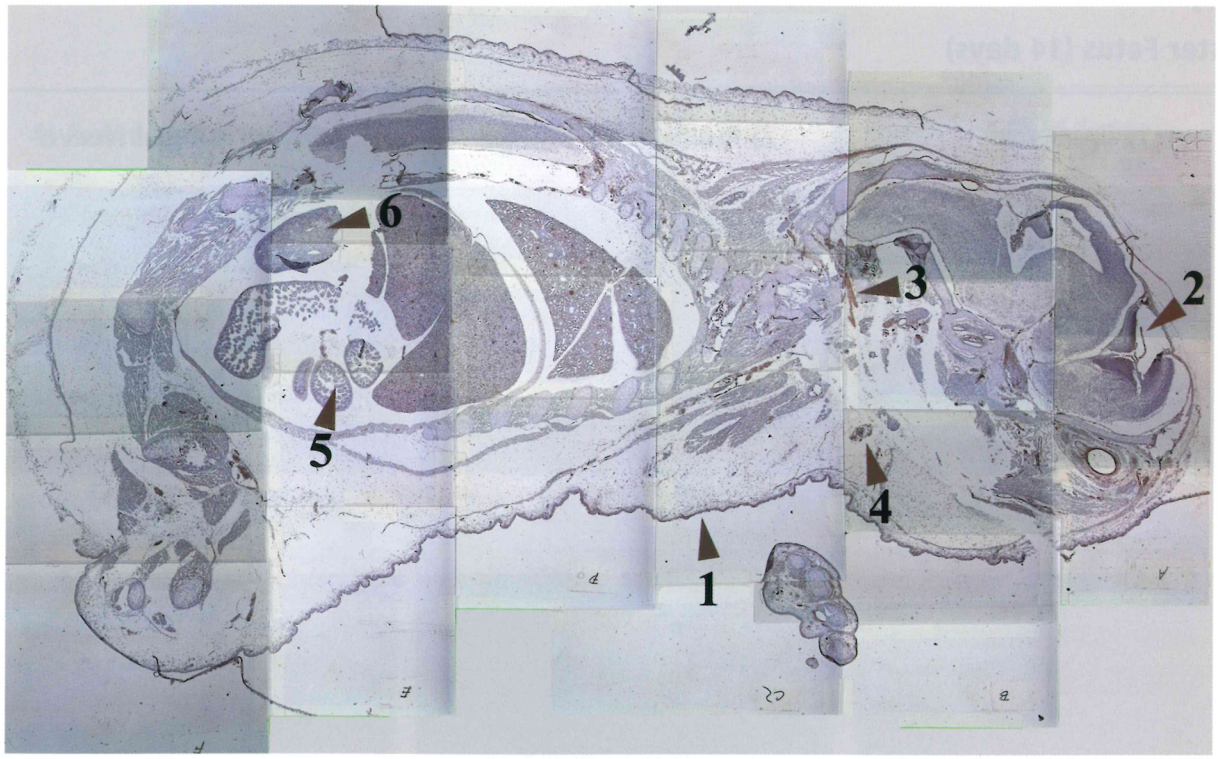
Immunohistochemistry of Hamster's Embryo Tissues Using MAb (10E4,3G10) to Heparan Sulfate

Hamster Fetus (14 days)



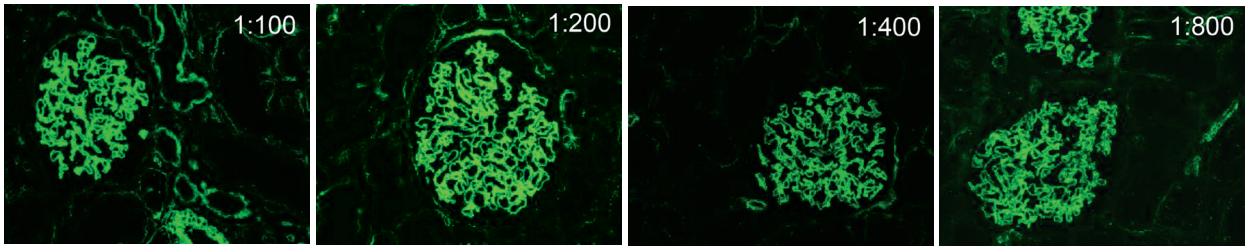

4) Submaxillary Gland
5) Intestine
6) Kidney
100 µm
10E4 HSase (+)
10E4 HSase (+)
10E4 HSase (+)
10E4 HSase (-)
10E4 HSase (-)
10E4 HSase (-)
3G10 HSase (+)
3G10 HSase (+)
3G10 HSase (+)
3G10 HSase (-)
3G10 HSase (-)
3G10 HSase (-)

Immunostaining using MAb (10E4) to Heparan Sulfate



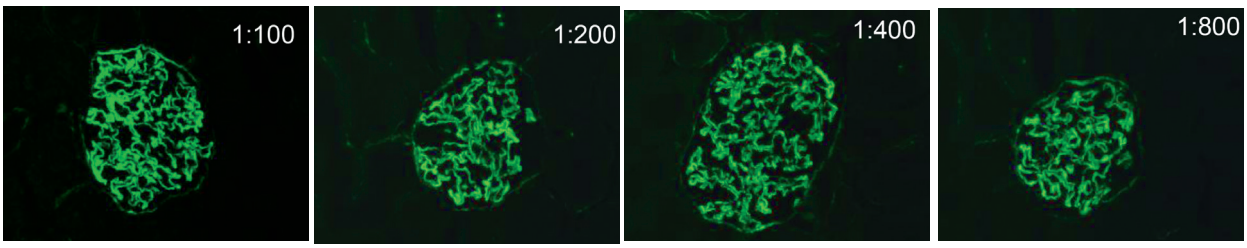
1) Epidermis 2) Plexus Chorioideus 3) Peripheral Nerve 4) Submaxillary Gland 5) Intestine 6) Kidney

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).

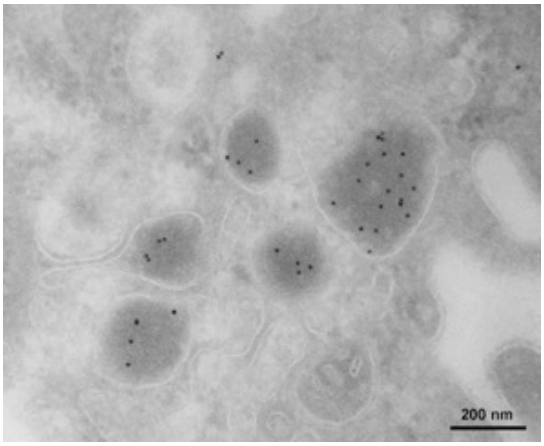
Immunofluorescence Staining on Normal Rat Kidney Sections using MAb (JM403) to Heparan Sulfate

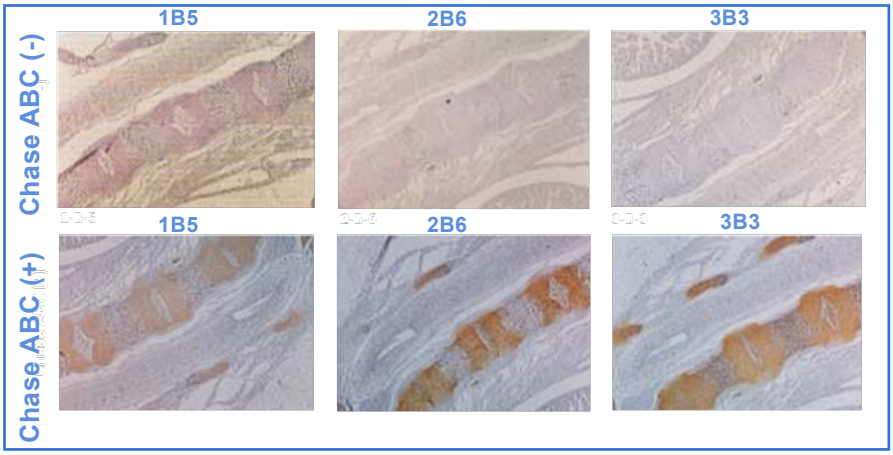


Immunofluorescence staining on normal rat kidney sections with JM403 antibody (magnification 40x).

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.*

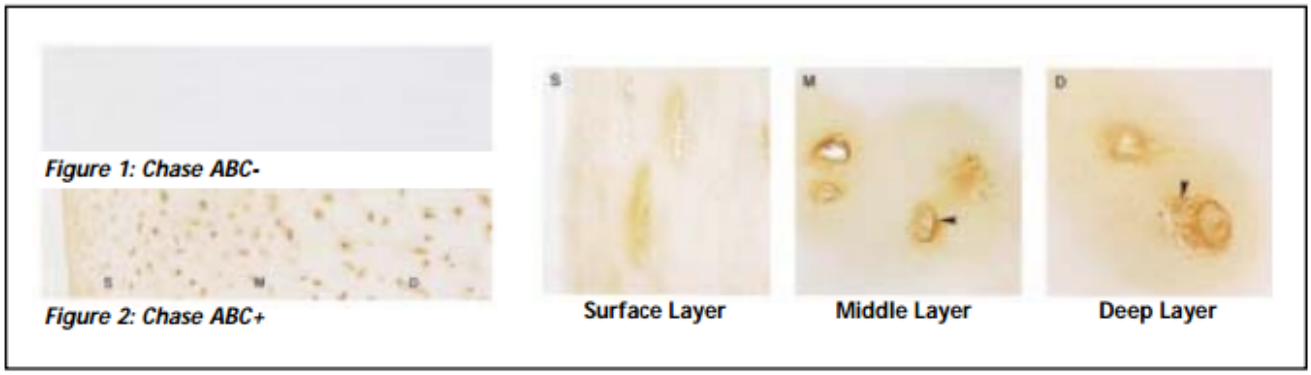




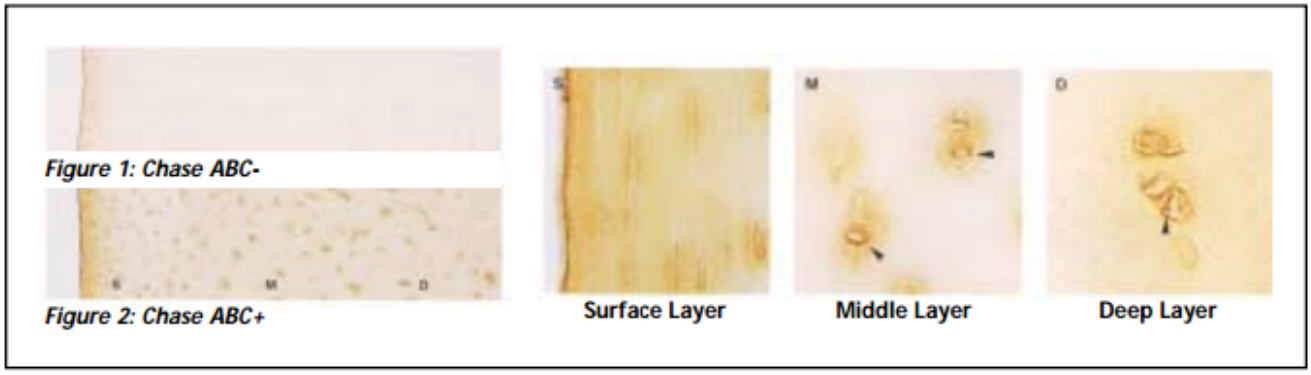
Immunohistochemistry of Hamster Embryo Vertebrae, using monoclonal antibodies (1B5, 2B6, 3B3) to Chondroitin Sulfate. Showing results with & without treatment with Chondroitinase ABC (Chase ABC).

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

ΔDi-0S(Clone 1-B-5)



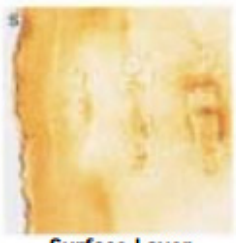
ΔDi-4S(Clone 2-B-6)



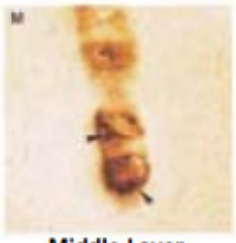
ΔDi-6S(Clone 3-B-3)

Figure 1: Chase ABC-

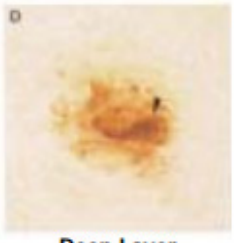
Figure 2: Chase ABC+



Surface Layer

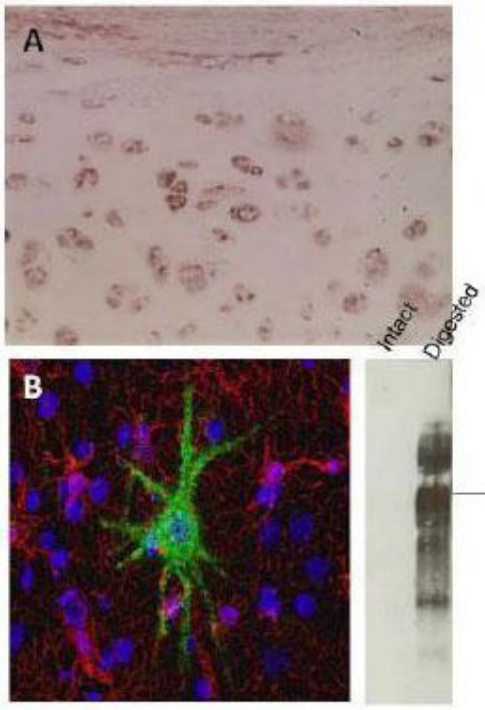


Middle Layer



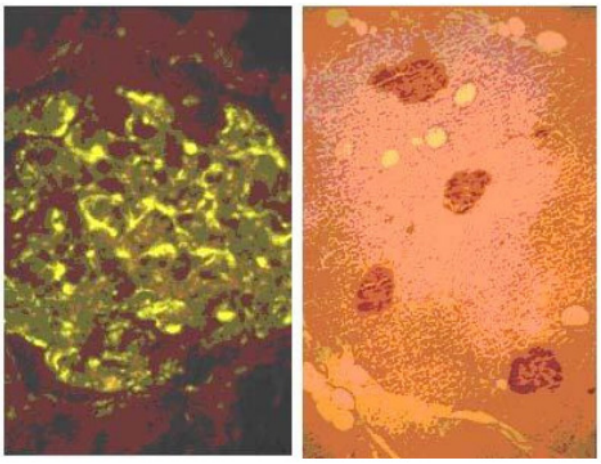
Deep Layer

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

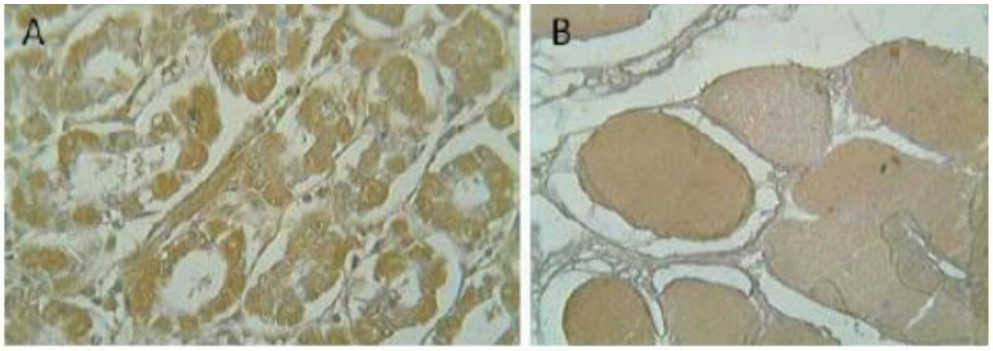
Immunohistochemistry using MAb (373E1) to Keratan Sulfate



(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).

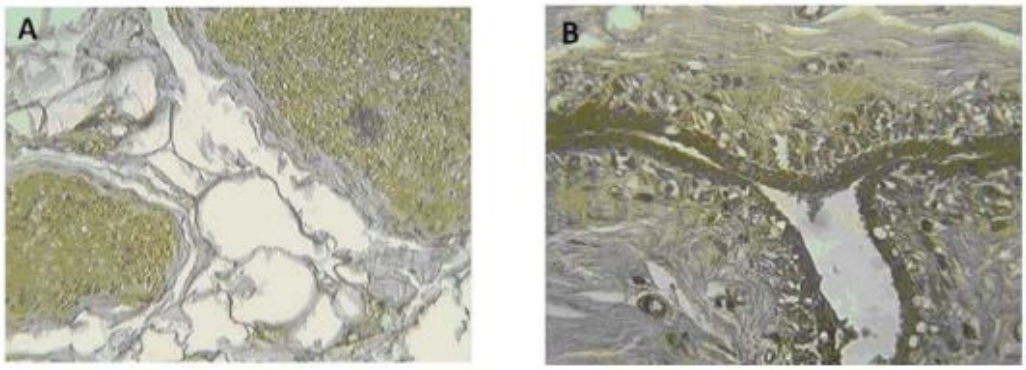
(Right) Immunohistochemical staining of keratan sulfates deposited within Langerhans islands of human adult pancreas (Formalin-paraffin embedding)

Immunohistochemistry using MAb (905A7) to Biglycan



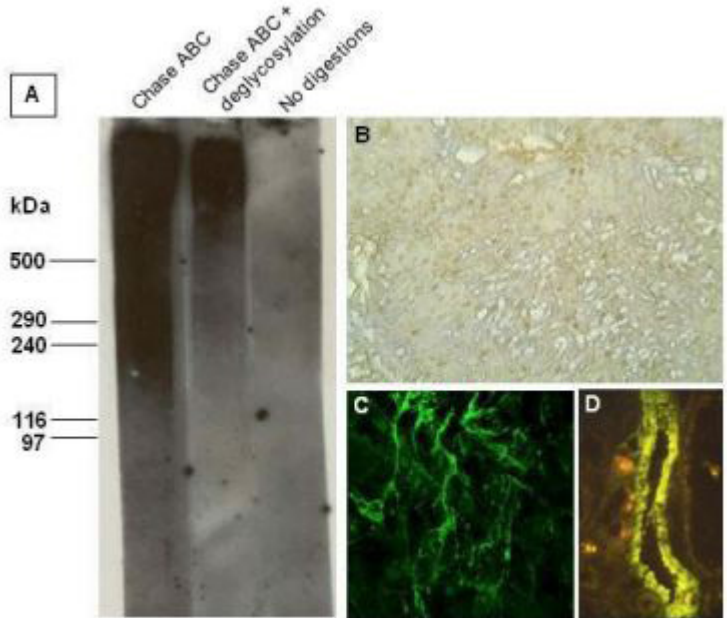
(A) Human intestine and (B) human skeletal muscle.

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

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)

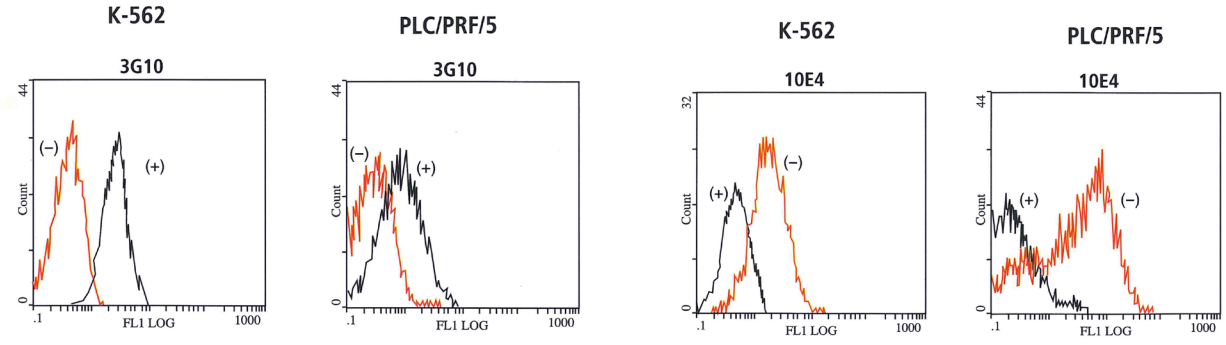
Procedure

1. Incubate 1 x 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 manufacturers instructions.

Results

		10E4		3G10	
Cell Line		HSase I (-)	HSase I (+)	HSase I (-)	HSase I (+)
KM3	Common ALL	Weakly + (10%)	→	- ~ ±	→
Daudi	Burkitt Lymphoma	- ~ ±	→	-	→
EB2	Burkitt Lymphoma (ovary)	+ (>90%)	↓ (50%)	+ (60%)	↑ (>90%)
CCRF-SB	ALL (B)	Weakly + (50%)	↓ (-)	-	↑ (>70%)
Molt 4	ALL (T)	-	→	-	→
HPBALL	ALL (T)	Weakly + (20%)	↓ (10%)	-	↑ (>90%)
K-562	Erythroleukemia	+ (80%)	↓ (10%)	-	↑ (>90%)
PB (M)	Normal Human Monocyte	-	→	-	→
PB (L)	Normal Human Lymphocyte	-	→	-	→
PB (G)	Normal Human Granulocyte	~ ±	→	~ ±	→
MKN 74	Stomach Cancer	+ (100%)	↓ (90%)	-	↑ (>90%)
COLO 201	Colon Cancer	+ (80%)	↓ (-)	-	↑ (100%)
PLC/ PRF/5	Hepatoma	+ (80%)	↓ (-)	~ ±	↑ (50~60%)
Hep G2	Hepatocellular Carcinoma	+ (100%)	↓ (80%)	-	↑ (100%)
G32TG	Hepatocellular Carcinoma	+ (90%)	↓ (±)	-	→

+: 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

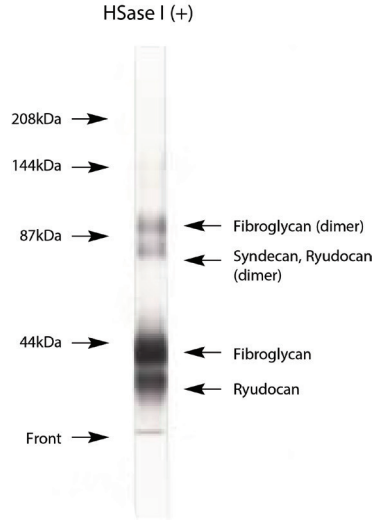
References
Antibodies to Heparan Sulfate
1.Bai,X.M.et al.J.Histochem.Cytochem.,42,1043-1054 (1994)

Antibodies to Chondroitin Sulfate
1.Yada,T.et al.J.Histochem.Cytochem.,44,969-980 (1996)

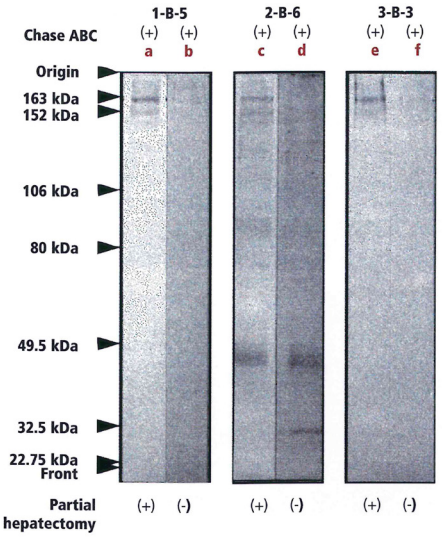
Procedure
1.Incubate partially purified proteoglycan fractions with GAGase for 1 hour at 37°C.
a) Treat 1.5 µg of sample with 2 mU of Heparitinase I for heparan sulfate proteoglycan
b) Treat 1 µg of sample with 100 mU of Chondroitinase ABC for chondroitin sulfate proteoglycan.
2.Run samples on SDS-PAGE 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.
7.Incubate with HRP substrate. Wash.

Western Blot Examples

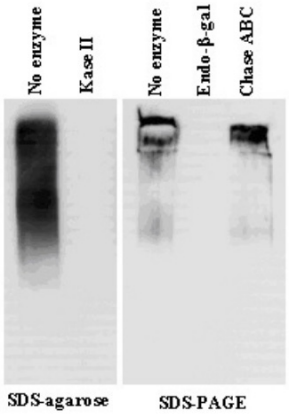
Western blot analysis of rat liver proteoglycans using MAb to Δ-heparan sulfate (3G10)



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



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-galactosididase or chondroitinase ABC-digestion on SDS-Agarose electrophoresis (left gel) or 3-8% gradient gels.

Abbreviations

BSA - Bovine serum albumin
FITC - Fluorescein isothiocyanate
SDS - Sodium dodecyl sulphate
PBS - Phosphate buffered saline
HUVECs - human umbilical vein endothelial cells
PAGE- polyacrylamide gel electrophoresis

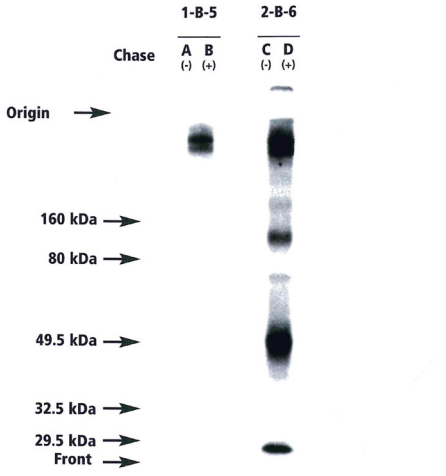
Immunoprecipitation for Chondroitin Sulfate Proteoglycan

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)

Immunoprecipitation analysis of rat liver proteoglycans using MAb to ΔDi-0S (1-B-5), ΔDi-4S (2-B-6)



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 GSepharose 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

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- David, G., Bai, X. M., Van Der Schueren, B., Cassiman, J. J., & Van Den Berghe, H. (1992). Developmental changes in heparan sulfate expression: in situ detection with mAbs. The Journal of cell biology, 119(4), 961-975.
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- Sugiura, N., Shioiri, T., Chiba, M., Sato, T., Narimatsu, H., Kimata, K., & Watanabe, H. (2012). Construction of a chondroitin sulfate library with defined structures and analysis of molecular interactions. Journal of Biological Chemistry, 287(52), 43390-43400.

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- Miller, G. M., & Hsieh-Wilson, L. C. (2015). Sugar-dependent modulation of neuronal development, regeneration, and plasticity by chondroitin sulfate proteoglycans. Experimental neurology, 274, 115-125.

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

- Alves, J. N., Muir, E. M., Andrews, M. R., Ward, A., Michelmore, N., Dasgupta, D., ... & Rogers, J. H. (2014). AAV vector-mediated secretion of chondroitinase provides a sensitive tracer for axonal arborisations. Journal of neuroscience methods, 227, 107-120.
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Glycobiology ELISA Kits

Targets:

GLYCOSAMINOGLYCANS

Chondroitin Sulfate (CS)
Chondroitin Sulfate Epitope 3B3 (CS3B3)
Heparan Sulfate (HS)
Hyaluronic Acid (HA)
Keratan sulfate (KS)

HYALURONIC ACID BINDING PROTEIN (HABP)

ENZYMES

Heparanase (HPA)
Hyaluronidase
Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 2
Hyaluronan synthase 2

PROTEOGLYCANS

Aggrecan
Bone marrow proteoglycan
Decorin (DCN)
Heparan Sulfate Proteoglycan (HSPG)
Heparan Sulfate Proteoglycan 2 (HSPG2)
Proteoglycan 4 (lubricin)
Syndecan
Versican

Species reactivities:

General (non-species specific), Human, Mouse, Rat, Rabbit, Guinea Pig, Goat, Porcine, Bovine, Canine, Monkey, Chicken,

Proteoglycan 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).

Description	Cat No.
Proteoglycan Detection Kit	280560-N

Proteoglycan Detection Kit Citation:

- Wang, Z., Winsor, K., Neinhaus, C., Hess, E., & Blackmore, M. G. (2017). Combined chondroitinase and KLF7 expression reduce net retraction of sensory and CST axons from sites of spinal injury. Neurobiology of Disease 99: 24-35.

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 creening or lack an appropriate positive control.

Description	Cat No.	Pack Size
Heparanase Kit with Bacterial Enzyme as control	Ra001- BE-K	96 rxns
Heparanase Kit without enzyme	Ra001-02-K	96 rxns

Heparanase Assay Citations

- Garsen, M., Benner, M., Dijkman, H., van Kuppevelt, T. H., Li, J. P., Rabelink, T. J., ... & van der Vlag, J. (2016). Heparanase Is Essential for the Development of Acute Experimental Glomerulonephritis. The American Journal of Pathology
- Garsen, M., Sonneveld, R., Rops, A. L., Huntink, S., van Kuppevelt, T. H., Rabelink, T. J., ... & van der Vlag, J. (2015). Vitamin D attenuates proteinuria by inhibition of heparanase expression in the podocyte The Journal of pathology, 237(4), 472-481
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- Masola, V., Maran, C., Tassone, E., Zin, A., Rosolen, A., & Onisto, M. (2009). Heparanase activity in alveolar and embryonal rhabdomyosarcoma: implications for tumor invasion BMC cancer, 9(1), 1
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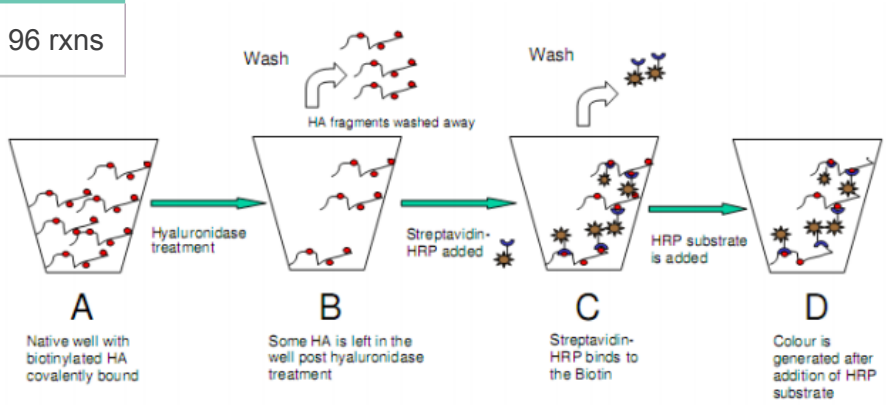
Hyaluronidase Assay Kit

Description	Cat No.	Pack Size
Hyaluronidase Kit	Ra003-01-HAK	96 rxns

For screening of Hyaluronidase inhibitors, and quantification of Hyaluronidase activity

PRINCIPLES OF THE TEST (HEPARANASE ASSAY)

Decrease in the OD of well D compared to A is directly proportional to heparanase activity.



GAG-coated 96-well plates available separately

Description	Cat No.
HS Plate (Higher concentration of bounded Biotynylated HS)	Ra1001-03-HSP
HS Plate (Higher concentration of bounded HS Non biotinylated)	Ra1001-04-NBP
Heparin Plate (heparin bounded plate, not biotynylated)	Ra1002-01-HEP
Biotinylated hyaluronic acid (hyaluronan) plate	Ra1003-01-HAP

Hyaluronidase Assay Citation

- Morris, B., & Behzad, F. (2014). The Effects of Gold and Silver Nanoparticles on an Enzymatic Reaction Between Horseradish Peroxidase and 3, 3', 5, 5'-Tetramethylbenzidine. Biochemistry & Pharmacology: Open Access, 2014.

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.

Cat No.	Description	Pack Size	Source
Chondroitin Sulfate			
AMS.CSR-NACS-A2-WHC-10	Sodium Chondroitin Sulfate A (Cartilage)	10 MG	Whale Cartilage
AMS.CSR-NACS-A2-WHC-3	Sodium Chondroitin Sulfate A (Cartilage)	3 MG	Whale Cartilage
AMS.CSR-NACS-C2-SHC-10	Sodium Chondroitin Sulfate C (Shark Cartilage)	10 MG	Shark Cartilage
AMS.CSR-NACS-C2-SHC-3	Sodium Chondroitin Sulfate C (Shark Cartilage)	3 MG	Shark Cartilage
400676-1A	Chondroitin Sulfate D, Na Salt, Super Special Grade	20 mg	Shark Cartilage
AMS.CSR-NACS-E2.SQC-3	Sodium Chondroitin Sulfate E (Squid Cartilage)	3 MG	Squid Cartilage
AMS.CSR-NACS-E2.SQC-10	Sodium Chondroitin Sulfate E (Squid Cartilage)	10 MG	Squid Cartilage
AMS.CSR-NACS-E2.SQC-100	Sodium Chondroitin Sulfate E (Squid Cartilage)	100 MG	Squid Cartilage
31254.01	Over Sulfated Chondroitin Sulfate (OSCS) Standard pure	2 mg	Contaminated heparin sodium
31254.02	Over Sulfated Chondroitin Sulfate (OSCS) Standard pure	10 mg	Contaminated heparin sodium
Dermatan Sulfate (formerly known as Chondroitin Sulfate B			
31255.01	Dermatan Sulfate Standard pure	25 mg	Crude heparin
31255.02	Dermatan Sulfate Standard pure	100 mg	Crude heparin
31255.03	Dermatan Sulfate Standard pure	250 mg	Crude heparin
400660	Chondroitin sulfate B, Na Salt (Pig skin)	20mg	Pig skin
AMS.CSR-NADS-B2-PGS-10	Sodium Dermatan Sulfate (Skin)	10 MG	Pig skin
AMS.CSR-NADS-B2-PGS-3	Sodium Dermatan Sulfate (Skin)	3 MG	Pig skin
AMS.GAG-DS01	Dermatan Sulfate (from pig mucosa)	2mg	pig mucosa
Heparan Sulfate			
400700	Heparan Sulfate, Na Salt	2mg	Bovine Kidney
AMS.GAG-HS01	Heparan Sulfate	2mg	Pig Mucosa
Hyaluronic Acid			
400720	Hyaluronic Acid, Na Salt	5mg	Pig Skin
Keratan Sulfate			
AMS.CSR-NAKPS2-SHC-1	Sodium Keratan Sulfate (Shark Cartilage)	1 MG	Shark Cartilage
AMS.CSR-NAKPS2-SHC-3	Sodium Keratan Sulfate (Shark Cartilage)	3 MG	Shark Cartilage
AMS.CSR-NAKS2-PNC-1	Sodium Keratan Sulfate (Nasal Cartilage)	1 MG	Pig Nasal Cartilage
AMS.CSR-NAKS2-PNC-3	Sodium Keratan Sulfate (Nasal Cartilage)	3 MG	Pig Nasal Cartilage

Heparin

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

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 K5 polysaccharides in which the internal uronate is glucuronic acid (GlcUA) in the native unmodified structure.

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

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:

ΔUA-GalINAc,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..

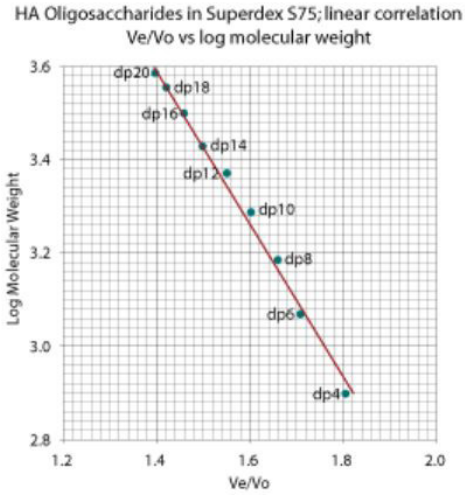
Description	Cat No.	Pack Size
Dermatan Sulfate dp4	AMS.DSO04	1mg
Dermatan Sulfate dp6	AMS.DSO06	1mg
Dermatan Sulfate dp8	AMS.DSO08	1mg
Dermatan Sulfate dp10	AMS.DSO10	1mg
Dermatan Sulfate dp12	AMS.DSO12	1mg
Dermatan Sulfate dp14	AMS.DSO14	1mg
Dermatan Sulfate dp16	AMS.DSO16	1mg
Dermatan Sulfate dp18	AMS.DSO18	1mg
Dermatan Sulfate dp20	AMS.DSO20	1mg

Uronic acid (ΔUA) 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-acetylglucosamine (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 polyimerisation: dp2 is a disaccharide, dp4 is a tetrasaccharide etc.



Description	Cat No.	Pack Size
Hyaluronic Acid dp2	AMS.HA02	1mg
Hyaluronic Acid dp4	AMS.HA04	1mg
Hyaluronic Acid dp6	AMS.HA06	1mg
Hyaluronic Acid dp8	AMS.HA08	1mg
Hyaluronic Acid dp10	AMS.HA10	1mg
Hyaluronic Acid dp12	AMS.HA12	1mg
Hyaluronic Acid dp14	AMS.HA14	1mg
Hyaluronic Acid dp16	AMS.HA16	1mg
Hyaluronic Acid dp18	AMS.HA18	1mg

General formula:
ΔHexA"1,3 [GlcNAc"1,4 GlcA"1,3]n GlcNAc n = number of disaccharide units
ΔHexA 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:

ΔUA,2S-GlcNS,6S – (IdoA,2S – GlcNS,6S)n- IdoA,2S-GlcNS,6S 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 (ΔUA) 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.

Description	Cat No.	Pack Size
Heparin dp4	AMS.HO04	2 mg
Heparin dp6	AMS.HO06	2 mg
Heparin dp8	AMS.HO08	2 mg
Heparin dp10	AMS.HO10	2 mg
Heparin dp12	AMS.HO12	2 mg
Heparin dp14	AMS.HO14	2 mg
Heparin dp16	AMS.HO16	2 mg
Heparin dp18	AMS.HO18	2 mg
Heparin dp20	AMS.HO20	2 mg

Substrates & Standards : Disaccharides

Heparin Disaccharides

Produced by the action of bacterial heparinase on high grade porcine heparin. Isolated by high resolution gel filtration and ion exchange chromotography The uronate (ΔUA) contains a C4-C5 double bond due to the action of the heparinases used to depolymerise heparin Our range includes N-unsubstituted disaccharides.

Description	Cat No.	Pack Size
ΔUA,2S – GlcNS,6S	AMS.HD001	1mg
ΔUA,2S – GlcNS	AMS.HD002	1mg
ΔUA,2S – GlcNAc,6S	AMS.HD003	1mg
ΔUA – GlcNS,6S	AMS.HD004	1mg
ΔUA – GlcNS	AMS.HD005	1mg
ΔUA – GlcNAc	AMS.HD006	1mg
ΔUA,2S – GlcNAc	AMS.HD007	1mg
ΔUA – GlcNAc,6S	AMS.HD008	1mg
ΔUA-2S (R) GlcNCOEt-6S (internal standard Disaccharide)	AMS.HD009	1mg

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

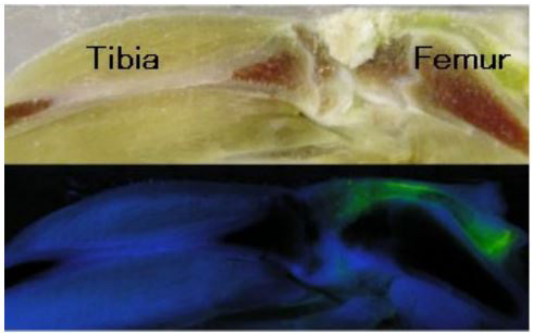
Description	Cat No.	Pack Size
ΔUA,2S – GlcN	AMS.HD010	1mg
ΔUA,2S – GlcN,6S	AMS.HD011	1mg
ΔUA – GlcN,6S	AMS.HD012	1mg
ΔUA – GlcN	AMS.HD013	1mg

Description	Cat No.	Pack Size
ΔUA – GalNAc	AMS.CD001	1mg
ΔUA – GalNAc,4S	AMS.CD002	1mg
ΔUA – GalNAc,6S	AMS.CD003	1mg
ΔUA – GalNAc,4S,6S (diE)	AMS.CD004	500ug
ΔUA,2S – GalNAc,4S (diB)	AMS.CD005	500ug
ΔUA,2S – GalNAc,6S (diD)	AMS.CD006	500ug
ΔUA,2S – GalNAc,4S,6S (triS)	AMS.CD007	500ug
ΔUA,2S – GalNAc	AMS.CD008	500ug

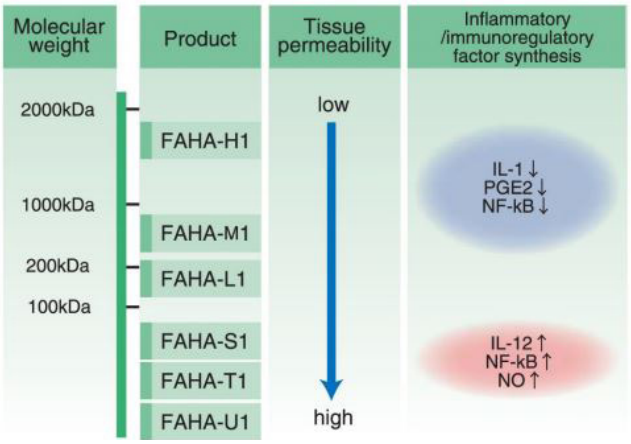
Fluorescence labeled GAGs

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

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



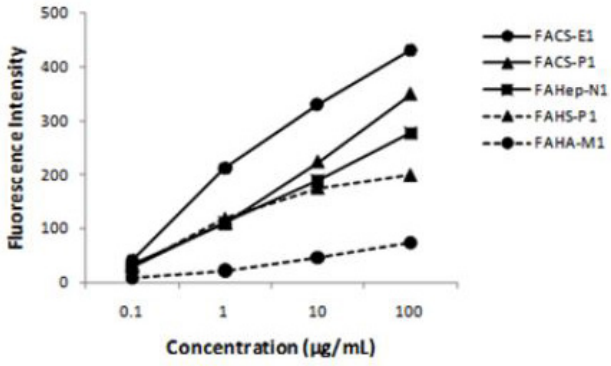
Fluorescence photograph of rat knee joint after intra-articular injection of FAHA-M1.



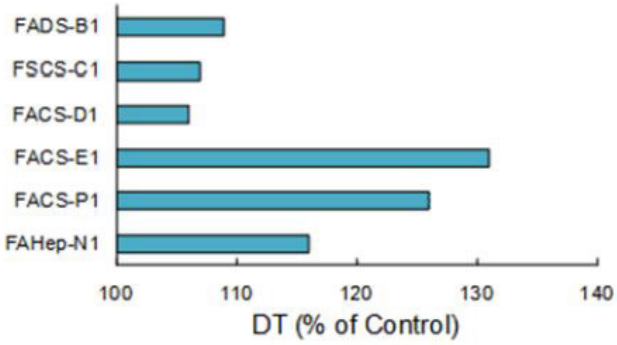
Effects of different molecular weights of sodium hyaluronate on tissue permeability and inflammatory/ immunoregulatory factor synthesis.

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.

Description	Cat No.	Pack Size
Fluoresceinamine Labeled Sodium Hyaluronate (H1)	AMS.CSR-FAHA-H1	3 ML
Fluoresceinamine Labeled Sodium Hyaluronate (H2)	AMS.CSR-FAHA-H2	3 MG
Fluoresceinamine Labeled Sodium Hyaluronate (L1)	AMS.CSR-FAHA-L1	3 ML
Fluoresceinamine Labeled Sodium Hyaluronate (L2)	AMS.CSR-FAHA-L2	3 MG
Fluoresceinamine Labeled Sodium Hyaluronate (M1)	AMS.CSR-FAHA-M1	3 ML
Fluoresceinamine Labeled Sodium Hyaluronate (M2)	AMS.CSR-FAHA-M2	3 MG
Fluoresceinamine Labeled Sodium Hyaluronate (S1)	AMS.CSR-FAHA-S1	3 ML
Fluoresceinamine Labeled Sodium Hyaluronate (S1)	AMS.CSR-FAHA-T1	3 ML



Analysis of Binding Activities with bFGF.



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

Hyalose: Size Standards For Glycobiology Research

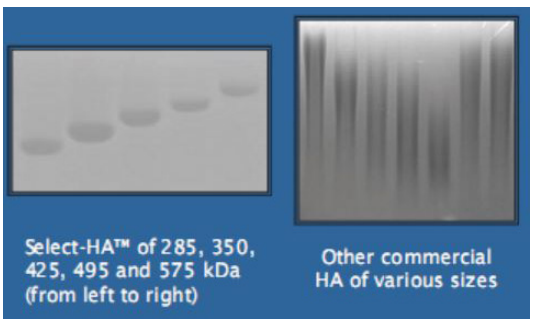
Select-HA™ - Select-HA is hyaluronic acid produced through enzymatic synthesis achieving a high level of size control. Polydispersity of the products in any size from 50kDa to 1000kDa is as low as 1.02 and averages 1.1 (a value of 1 is associated with a 'perfect' polymer). Hyalose offers one grade of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

- ✓ Various sizes (25 kDa–1000 kDa)
- ✓ Low endotoxin, certified to be less than 0.1 EU/mg of HA
- ✓ Available biotinylated

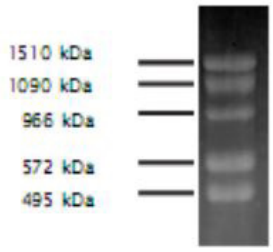
Q: What is the difference between Select-HA™and other commercial HAs?
A: The unique property of Select-HA™is that it has very narrow size distributionwhile all other commercial HA polymers are mixtures of HA with a much broader size range. Please see the pictures (agarose gels stained with Stains -All) 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 isnot a mixture of HA ranging from 25 kDato 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

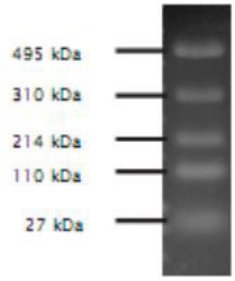


Select-HA HiLadder™ - The Select-HA HiLadder contains five Select-HA molecular mass markers in the range of ~500 kDa to ~1500kDa. The masses are 495 kDa, 572 kDa, 966 kDa, 1090 kDa and 1510 kDa. Recommended usage: agarose gel stained with StainsAll.



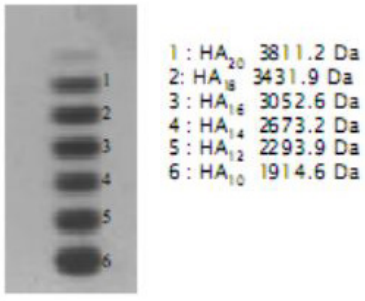
Electrophoretic Separation of Select-HA™ HiLadder Standards. Five µl of reconstituted HiLadder (Lot HL0503) was separated on a 1.0% (w/v) agarose gel by electrophoresis and stained with Stains-All.

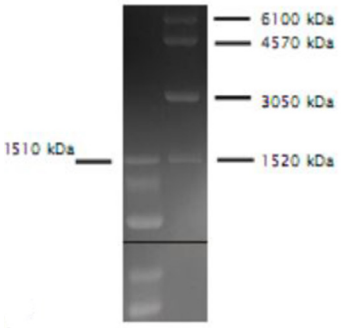
Select-HA LoLadder™ - The Select-HA LoLadder contains five Select-HA molecular mass markers in the range of ~25 kDa to ~500 kDa. The masses are 27 kDa, 110 kDa, 214 kDa, 310 kDa and 495 kDa. Recommended usage: agarose gel stained with StainsAll.



Electrophoretic Separation of Select-HA™ LoLadder Standards. Five µl of reconstituted LoLadder (Lot LL0401) was separated on a 1.0% (w/v) aga-rose gel by electrophoresis and stained with Stains-All.

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.





Electrophoretic Separation of Mega-HA™ 0.25 • gof Mega-HATM Ladder (Lot ML200505, **right**) was separated on a 1% (w/v) agarose gel by electrophoresis and stained with Stains-All. **Left**, Select-HA™HiLadder Lot: HL0503.

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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