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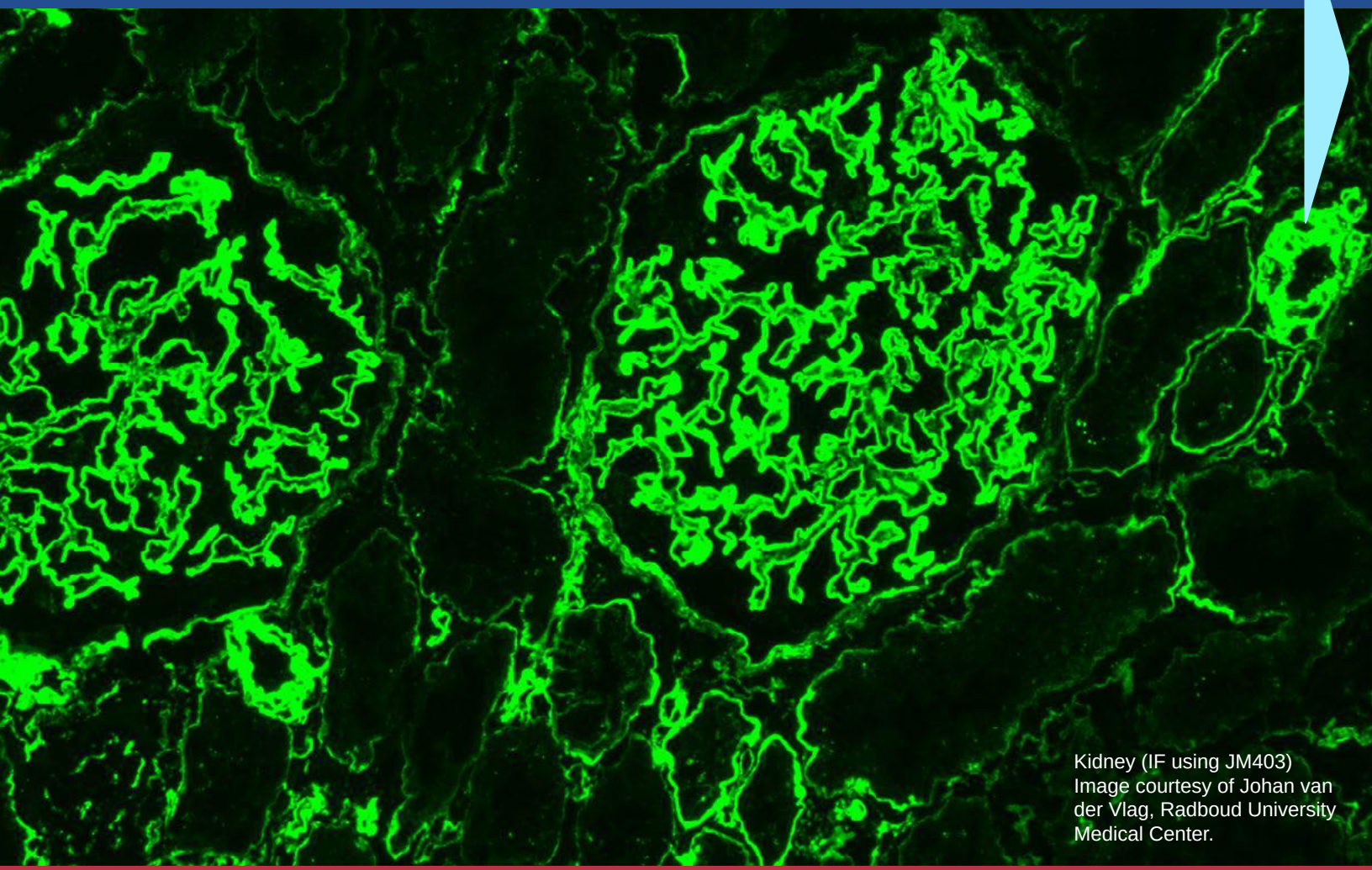
Heparan Sulfate Antibodies

Application Guide

10E4 | JM403 | 3G10 : Protocols | Citations



Kidney (IF using JM403)
Image courtesy of Johan van der Vlag, Radboud University Medical Center.

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Heparan Sulfate Antibodies

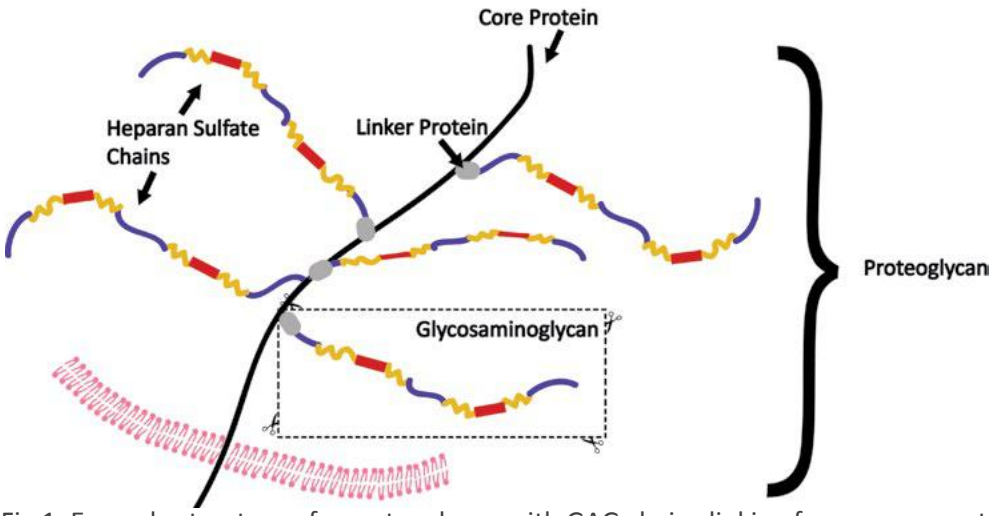


Fig 1. Example structure of a proteoglycan with GAG chains linking from a core protein.

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.

AMSBIO offers quality heparan sulfate antibodies from the important clones F69-3G10, F58-10E4 and JM403, ideal for targeted binding of HS for HSPG research. AMSBIO provides various anti-HS antibodies recognizing distinct HS substructures as research reagents, which have been well-characterized in previous studies. Heparan sulfate (HS) is synthesized as the glycosaminoglycan component of heparan sulfate proteoglycans (HSPGs). It is expressed on the cell surface of virtually all cell types and basement membranes in mammals. It displays specific interactions with many biologically active proteins and, thus, is involved in many important biological processes. It has been important to examine the dynamic distribution of HSPG in tissues and also to analyse HS structures related to its function for the elucidation of the biological functions of HSPG.

The non-immunogenic character of HS makes this type of antibody difficult to raise, therefore the few hybridoma-derived mouse anti-HS antibodies such as JM403, 10E4 and 3G10 are valuable tools for HS research. GAGs are not species-specific, the length of the heparan sulfate chain and the distribution of sulfate groups are different in different tissues due to its synthesis from multiple cell types, so our GAG antibodies will usually react across a wide range of species. 10E4 antibodies have been found to recognize heparan sulfate from hamster, mouse, human, pig, chicken, *Xenopus laevis*, zebrafish, *Hemicentrotus pulcherrimus* and *Drosophila melanogaster*. 3G10 is effective in hamster, mouse and chicken. JM403 has been found to be reactive in rat, human and bovine species. Using different antibodies with well characterized epitopes helps to track changes in heparan sulfate. Using multiple HS antibodies that recognize subtle differences in HS patterning can be useful in applications such as Flow cytometry allowing multiple cell types to be directly compared. Also fluorescent-activated cell sorting (FACS) can sort cells based on their HS-epitope expression (Holley et al. (2015) Chapter 21, DOI 10.1007/978-1-4939-1714-3).

Clones **F58-10E4** and **JM403** recognize common epitopes on native heparan sulfate (HS), which can be found on both basement membrane and cell-associated HS species.

Clone **F58-10E4** is the most widely-cited HS antibody, recognising the common 10E4 epitope (including N-sulfated glucosamine residues), which is widely found across different tissues and species.

Clone **JM403** (which recognises an epitope including N-unsubstituted glucosamine residues) is superior in obtaining high quality and convincing pictures of HS expression in different tissues using immunofluorescence microscopy. Furthermore, JM403 is a robust glycoprobe that can be applied in ELISAs that are for example aimed to quantify HS in fluids, or that are used to determine heparanase activity.

Clone **F69-3G10** recognizes the 3G10 neo-epitope (unsaturated uronic acid) exposed by digestion with heparinase III enzyme (which destroys reactivity to 10E4 and JM-403); so that the 3G10 clone can be used as control to 10E4 or JM403 (and vice versa). Only one unsaturated urinate residue per chain will remain linked to the core protein, so the extent to which 3G10 reacts with heparinase III-treated samples will therefore trace the number of HS chains carried by these cores or expressed in a particular tissue, rather than the mass of HS that was originally present.

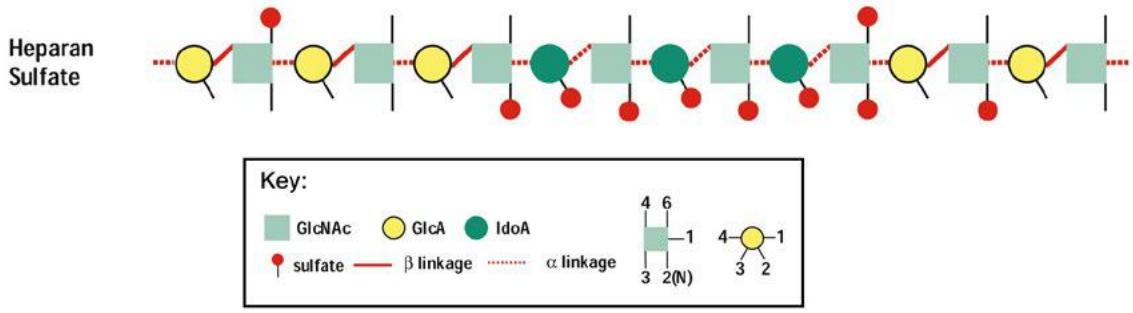


Fig 2. Structure of heparan sulfate and the unsaturated disaccharides in the heparan sulfate family.

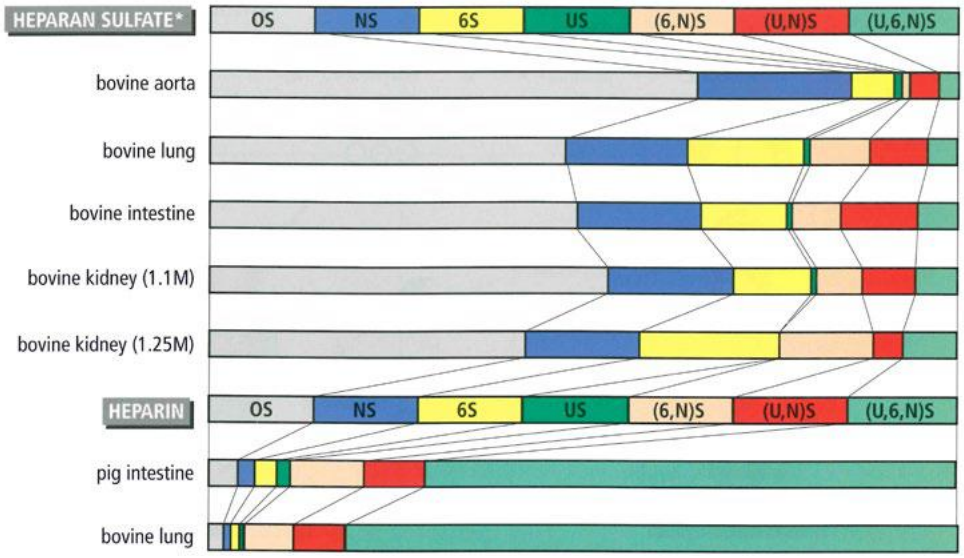


Fig 3. Disaccharide composition heparan sulfate and heparin (Maccarana, M et al. (1996), J. Biol. Chem. 271, 17804-17810). The heparan sulfate chain contains NS-domains (N-sulfated domains) with a high degree of sulfation, non-sulfated NA-domains (N-acetylated regions), and NA/NS mixed domains (Dagälv et al. (2015), Chapter 17, DOI 10.1007/978-1-4939-1714-3).

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

Antibody	Occurrance in HS	Required GlcN N-substitution	Requirement for		Epitope characteristics
			O-Sulfate	IdoUA	
JM403	Common	$-NH_3+$	No	No	GlcUA-rich sequences with Nunsubstituted GlcN units
10E4	Common	N-Sulfate & Nacetyl	No	No	Mixed N-acetylated /N-Sulfated sequence
3G10	Epitope exposed following heparinase III enzyme activity on the HS chain.	No	No	No	Artificially generated HS 'stub' (unsaturated uronic acid): only one binding site per HS chain

Table: Adapted from van den Born et al. (2005) J. Biol. Chem. 280, 20516-20523.

Species Reactivity for Heparan Sulfate Antibodies

GAGs are not species-specific, so our GAG antibodies will usually react across a wide range of species. Here are some published examples where our Heparan Sulfate antibodies have been used against a range of species:

10E4 & 3G10 reactivity in mouse and hamster samples

Bai, X. M., Van der Schueren, B., Cassiman, J. J., Van den Berghe, H., & David, G. (1994). Differential expression of multiple cell-surface heparan sulfate proteoglycans during embryonic tooth development. Journal of Histochemistry & Cytochemistry, 42(8), 1043-1054.

10E4 & 3G10 reactivity in human and hamster samples

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.

JM-403 & 10E4 reactivity in bovine, human and rat samples

Van Den Born, J., Salmivirta, K., Henttinen, T., Östman, N., Ishimaru, T., Miyaura, S., ... & Salmivirta, M. (2005). Novel heparan sulfate structures revealed by monoclonal antibodies. Journal of Biological Chemistry, 280(21), 20516-20523.
Online at http://www.jbc.org/content/280/21/20516.short

10E4 in pig

Khan, A. I., Haskard, D. O., Malhotra, R., & Landis, R. C. (2002). Identification and characterization of L-selectin ligands in porcine lymphoid tissues. Immunology, 105(4), 441-449.
Online at http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2567.2002.01393.x/full

10E4 and 3G10 in leghorn chicken (*Gallus gallus domesticus*)

Kobayashi, T., Habuchi, H., Nogami, K., Ashikari-Hada, S., Tamura, K., Ide, H., & Kimata, K. (2010). Functional analysis of chick heparan sulfate 6-O-sulfotransferases in limb bud development. Development, growth & differentiation, 52(2), 146-156.
Online at http://onlinelibrary.wiley.com/doi/10.1111/j.1440-169X.2009.01148.x/full

10E4 in *Xenopus laevis*

Walz, A., McFarlane, S., Brickman, Y. G., Nurcombe, V., Bartlett, P. F., & Holt, C. E. (1997). Essential role of heparan sulfates in axon navigation and targeting in the developing visual system. Development, 124(12), 2421-2430.
Online at http://dev.biologists.org/content/124/12/2421.short

10E4 in zebrafish (*Danio rerio*)

Liu, I., Zhang, C., Kim, M. J., & Cole, G. J. (2008). Retina development in zebrafish requires the heparan sulfate proteoglycan agrin. Developmental neurobiology, 68(7), 877-898.
Online at http://onlinelibrary.wiley.com/doi/10.1002/dneu.20625/full

10E4 in sea urchin (*Hemicentrotus pulcherrimus*)

Fujita, K., Takechi, E., Sakamoto, N., Sumiyoshi, N., Izumi, S., Miyamoto, T., ... & Yamamoto, T. (2010). HpSulf, a heparan sulfate 6-O-endosulfatase, is involved in the regulation of VEGF signaling during sea urchin development. Mechanisms of development, 127(3), 235-245.
Online at http://www.sciencedirect.com/science/article/pii/S0925477309014993

10E4 in *Drosophila melanogaster*

Park, Y., Rangel, C., Reynolds, M. M., Caldwell, M. C., Johns, M., Nayak, M., ... & Datta, S. (2003). *Drosophila perlecan* modulates FGF and hedgehog signals to activate neural stem cell division. Developmental biology, 253(2), 247-257.
Online at http://www.sciencedirect.com/science/article/pii/S0012160602000192

Heparan Sulfate Antibody Product Specifications

	JM403 (# 370730-1)	10E4 (#370255-1)	3G10 (#370260-1)
Antigen:	Heparan sulfate proteoglycan derived from rat glomerular basement membrane.	Heparan sulfate proteoglycan derived from human fetal lung fibroblast (F58).	Δ-Heparan sulfate proteoglycan derived from human fetal lung fibroblast treated with heparinase III.
Clone:	JM403	F58-10E4	F69-3G10
Reactivity:	Reacts with many types of heparan sulfate proteoglycans. The epitope includes N-unsubstituted glucosamine residues that are critical for the reactivity of the antibody. Its reactivity is almost completely eliminated by heparinase III treatment. It does not react with heparin, hyaluronan, chondroitin sulfate, dermatan sulfate, or keratan sulfate.	Reacts with many types of heparan sulfate proteoglycans. It is essential for the reactivity of 10E4 for N-sulfated glucosamine residues to be present in the structure of heparan sulfate. Its reactivity is eliminated by heparinase III treatment. It does not react with hyaluronic acid, chondroitin sulfate, dermatan sulfate, or DNA.	Reacts with neo-epitope of heparan sulfate treated with heparinase III. It does not react with chondroitin sulfate treated with chondroitinase ABC or chondroitinase AC.
Ig Subclass:	Mouse IgM κ chain.	Mouse IgM κ chain.	Mouse IgG2b κ chain.
Applications*:	-Immunohistochemistry Fozen section: 1:500-1000 -ELISA: 1:500-1000	- Flow cytometry: 1:70-140, 0.5-1 µg with label about 10^5 cells - Immunohistochemistry: 1:35-70	- Flow cytometry: 1:100-200, 0.5-1 µg with label about 10^6 cells - Immunohistochemistry: 1:100-200 -ELISA: 1:200-500
Form:	Purified monoclonal antibody, solution dissolved in PBS (-), 0.02% sodium azide at an antibody concentration of 1mg/ml.	Purified monoclonal antibody, solution dissolved in PBS (-), 0.02% sodium azide at an antibody concentration of 1mg/ml.	Purified monoclonal antibody, solution dissolved in PBS (-), 0.02% sodium azide at an antibody concentration of 1mg/ml.
Pack size:	200 µg	200 µg or 50 µg	200 µg or 50 µg

*The exact dilutions must be determined by the researcher.

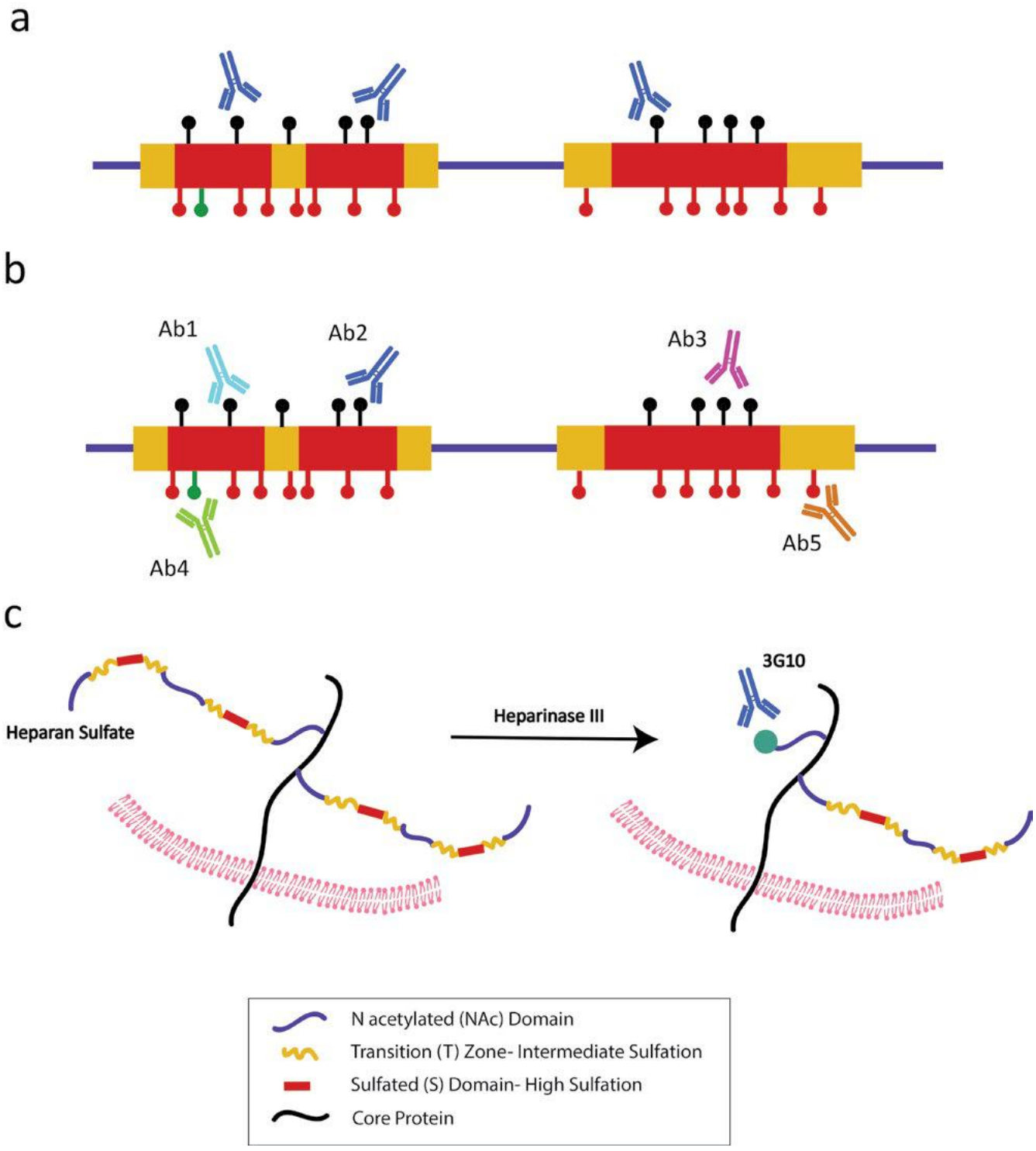


Fig 4. Schematic representation of a. Heparan sulfate antibodies with preference for specific sulfate motifs at different locations along the HS chain, b. multiple HS antibodies recognizing different patterns within the HS chain allowing them to be used as a panel and c. HS domain structures showing the 3G10 stub created after degradation with Heparinase III.

JM403 Antibody

The antibody (clone JM403) reacts with an epitope present in many types of heparan sulfate. The JM403 epitope includes (an) N-unsubstituted glucosamine residue(s) that are critical for the reactivity of the antibody. The reactivity of the JM403 antibody with most heparan sulfates is nearly completely abolished after treatment of the glycosaminoglycan with bacterial Heparitinase I (Heparinase III) from Flavobacterium heparinum; EC 4.2.2.8). The JM403 antibody does not react with heparin, hyaluronan, chondroitin sulfate, dermatan sulfate and keratan sulfate. Certain HS-epitope-specific antibodies such as JM403 have a preference for specific sulfate motifs which can occur at multiple sites along the HS chain. Therefore one antibody may potentially bind a single HS chain multiple times. Multiple epitope specific HS antibodies can be used togeher as panels (Holley et al. (2015) Chapter 21, DOI 10.1007/978-1-4939-1714-3).

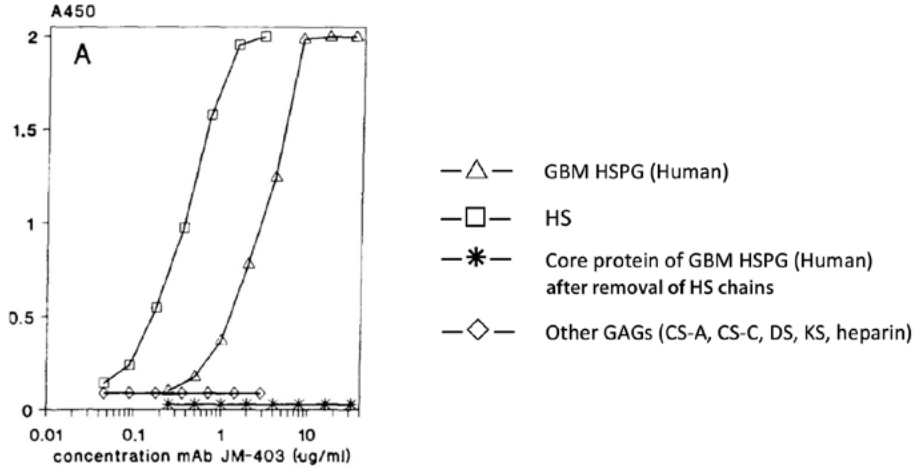


Fig 5. ELISA reactivity of heparan sulfate JM403 antibody to GBM HSPG (human), core protein of GBM HSPG (human), HS, and other GAGs (CS-A, CS-A, DS, KS and heparin). Source: van den Born et al. 1994.

Analysis of Reactivity of JM403 with Heparan Sulfate in ELISA

Protocol

1. Coat Heparan sulfate(Sigma H7640) 20 µg/ml (in 90% saturated ammonium sulfate) in Maxisorb 96-wells plates (Nunc439454) in 100 µl/well overnight at 4 °C
2. Wash plate 3 times with PBS-0.05% Tween (PBS-T)
3. Block for 1 h at room temperature in 200 µl/well with 1% gelatin (Difco, Bacto 014301) in phosphate buffered saline(PBS)
4. Wash plate 5 times with PBS-T
5. Incubate in 100 µl/well with JM403 serial dilutions Start concentration of JM403 5 ug/ml in PBS for 1 h at room temperature
6. Wash plate 5 times with PBS-T
7. Incubate in 100 µl/well with detecting antibody (secondary antibody) Goat anti mouse-IgM(u -spec)-HRP (Southern Biotechnology, 1021-05) at the appropriate dilution
8. Wash plate 5 times with PBS-T
9. Develop by addition of 100 µl/well TMB solution for 15 minutes at room temperature
10. Stop peroxidase reaction by adding 100 µl/well 2M H2SO4
11. Measure absorbance at 450 nm

Results

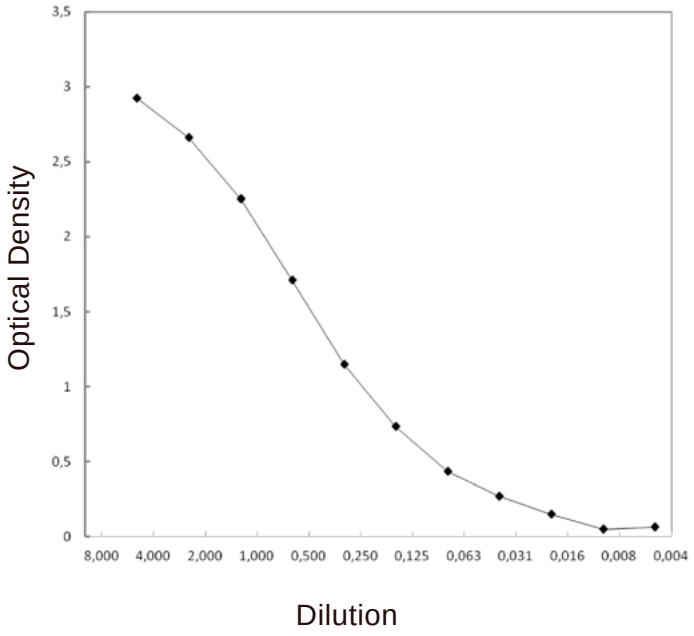


Fig 6. JM403 reactivity with heparan sulfate (bovine kidney) coated in ELISA.

JM403 Antibody Immunofluorescence Staining of Normal Frozen Kidney (2 µm) Sections

Protocol

1. Fix tissue sections for 10 minutes in aceton at 4 °C
2. Dry in the air
3. Dilute JM403 in PBS containing 1% BSA (1:100 starting dilution for JM403) and ,incubate for 45 min on sections at room temperature
4. Wash three times in PBS
5. Dilute detecting antibodies (Goat anti Mouse IgG H&L Alexa488 (Cat #A11001) of Goat anti Mouse IgM Alexa 488 (Cat #A21042) in PBS containing 1% BSA at the appropriate dilution and incubate for 30-45 min at room temperature
6. Wash three times in PBS
7. Fix with 1% paraformaldehyde in PBS for 15 min
8. Wash three times in PBS
9. Embed in Vectashield mounting medium

Results

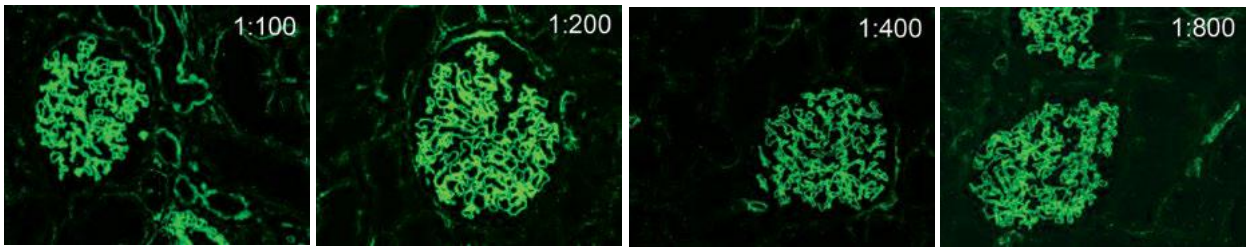


Fig 7.Immunofluorescence staining on normal human kidney sections with JM403 antibody (magnification 20x).

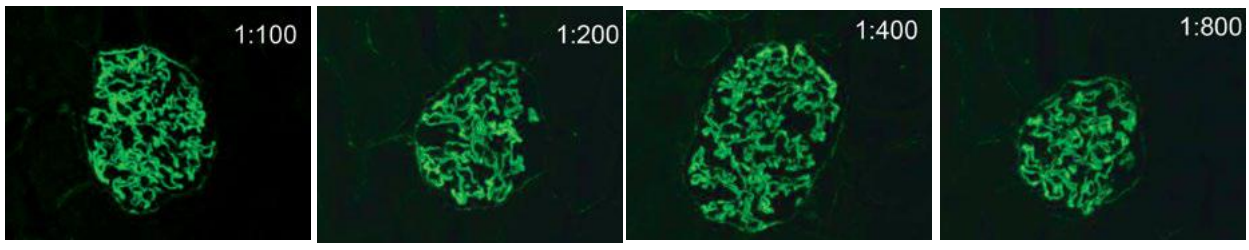


Fig 8.Immunofluorescence staining on normal rat kidney sections with JM403 antibody (magnification 40x).

10E4 Antibody

The anti-heparan sulfate monoclonal 10E4 antibody (F58-10E4 clone) reacts with the 10E4 epitope, which is present in many types of heparan sulfate proteoglycans. The structure of heparan sulfate includes N-sulfated glucosamine residues that are critical for the reactivity of the F58-10E4 antibody. The reactivity of the antibody with most heparan sulfates is nearly completely abolished after treatment of the glycosaminoglycan with bacterial Heparinase III (heparatinase I) from Flavobacterium heparinum (EC 4.2.2.8). The 10E4 antibody does not react with hyaluronan, chondroitin sulfate, dermatan sulfate, keratan sulfate or DNA.

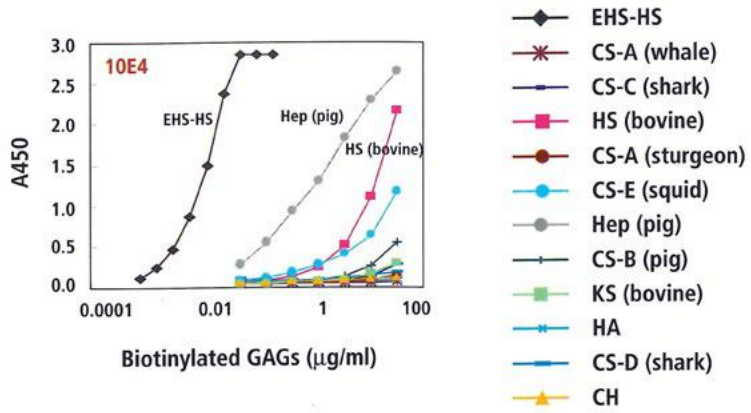
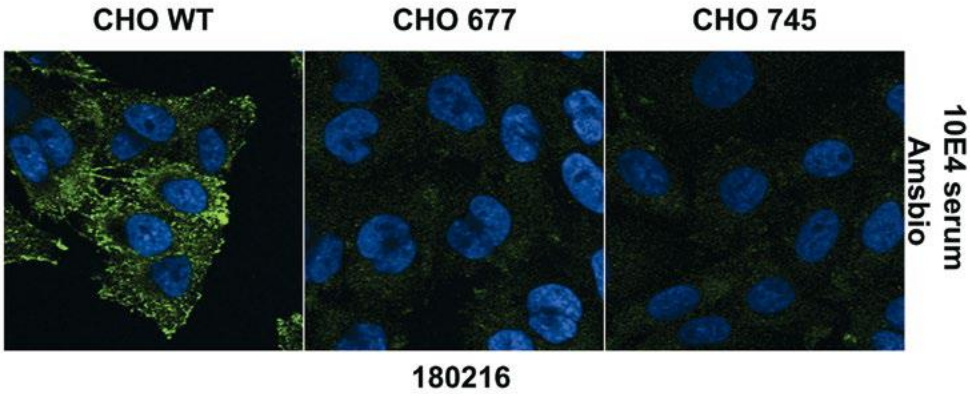


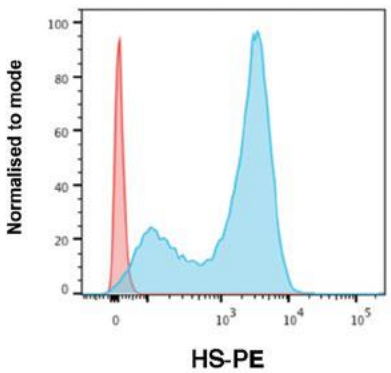
Fig 9. Reactivity of heparan sulfate 10E4 antibody to biotinylated glycosaminoglycans.

10E4 antibody staining on wild type CHO cells, alongside CHO cell mutants deficient in heparan sulfate

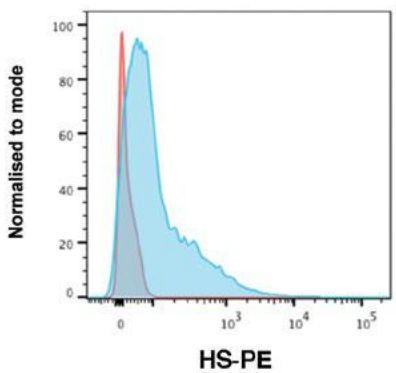
Fig 10. 10E4 antibody (from AMSBIO) staining (green) and Hoechst (blue) on wild type CHO cells, alongside CHO cell mutants deficient in heparan sulfate (CHO 677 and CHO 745). Image courtesy of R. Ghossoub, P. Zimmermann Lab - KU Leuven and CRCM.



FACS analysis of isolated primary mouse beta cells following staining with 10E4 mAb



Intracellular HS in isolated mouse beta cells



Cell surface HS on isolated mouse beta cells

Fig 11.Flow cytometry shows that isolated primary mouse beta cells stain more strongly for intracellular heparan sulfate (HS; blue histogram, GMFI=1458, left panel) than cell surface HS (blue histogram,GMFI=138, right panel) using 10E4 mAb and a PE-conjugated second antibody. Red histogram shows background autofluorescence (GMFI=15, left panel; GMFI=21, right panel). Image courtesy of C. Simeonovic, Australia.

Intra-islet Immunostaining of Heparan Sulfate (post-antigen retrieval) using 10E4 Monoclonal Antibody

The intracellular localisation of HS in islet beta cells is unique, because HS is conventionally localised on the surface of cells, in basement membranes and in extracelllular matrix.

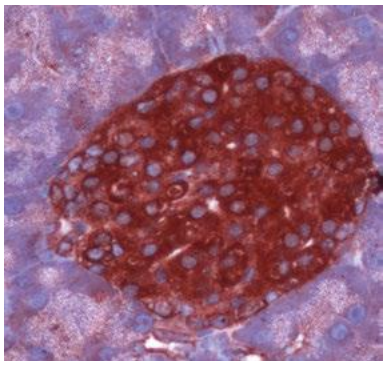


Fig 12.Immunostaining of mouse islet beta cells showing intracellular localization of heparan sulfate (post-antigen retreival) using 10E4 monoclonal antibody. Image courtesy of C. Simeonovic, Australia.

3G10 Antibody

The anti-Δ-heparan sulfate monoclonal antibody (F69-3G10 clone) reacts with heparan sulfate neo-epitope (unsaturated uronic acid) 3G10, generated by digesting heparan sulfate with heparitinase I (Heparinase III) from Flavobacterium heparinum (EC 4.2.2.8). The desaturated hexuronate (glucuronate) that is present at the non-reducing end of the heparan sulfate fragments created by the enzyme is critical for the reactivity of the antibody. The 3G10 antibody does not react with intact heparan sulfate, oligosaccharides generated from chondroitin sulfates with bacterial chondroitinase ABC or AC, or generated from heparan sulfate with heparinase (Heparinase I): EC 4.2.2.7). There is only one 3G10 epitope on the HS stub which can only be recognized by one antibody at a time which allows these to be utilized as a tool for measuring how many chains are present per cell.

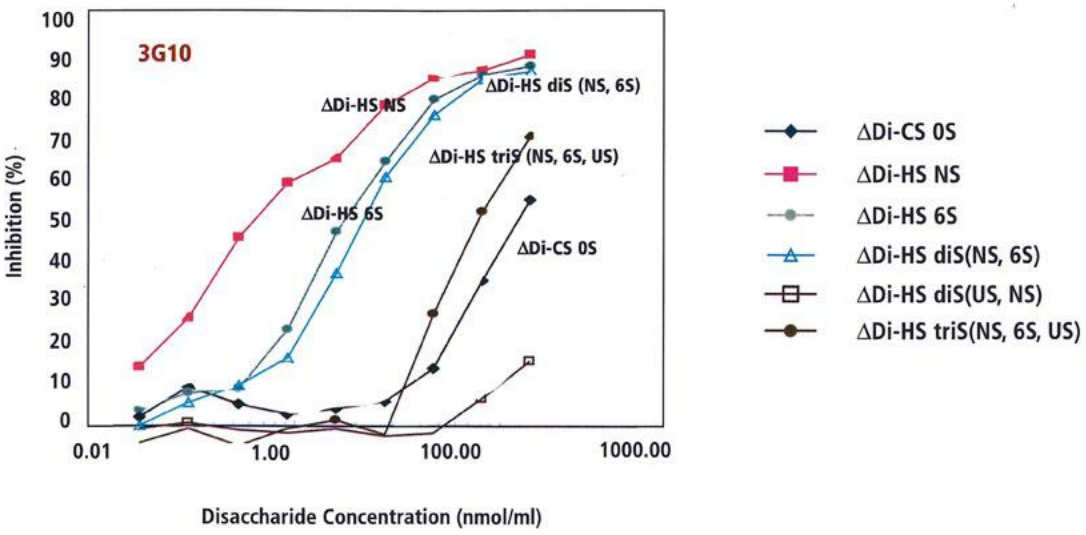


Fig 13. Reactivity of heparan sulfate 10E4 antibody to biotinylated glycosaminoglycans.

Western Blot using Monoclonal Antibody 3G10 to Heparan Sulfate

Procedure

1. Incubate partially purified proteoglycan fractions with GAGase for 1 hour at 37°C.
   (Treat 1.5 µg of sample with 2 mU of Heparinase III (200 mU/ml of sodium acetate buffer-3.3 mM calcium chloride, pH 7.0): digestion degrades 10E4 epitope but exposes 3G10 epitope.)
2. Run samples on SDS-PAGE under reducing conditions.
3. Transfer it to membrane (PVDF membrane or nitrocellulose membrane).
4. Blocking with 10% skim milk in PBS for 30 minutes at 37oC.
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.

References

Bai,X.M.et al.J.Histochem.Cytochem.,42,1043-1054 (1994)

Results

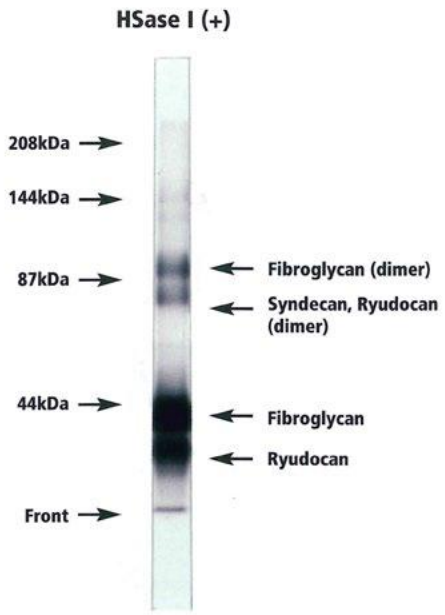


Fig 14. Western blot analysis of rat liver proteoglycans using monoclonal antibody 3G10 to Δ-Heparan sulfate.

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

Combined use of Heparan sulfate Antibodies

The anti-heparan sulfate monoclonal antibodies 10E4 and 3G10 can be used for immunohistostaining, immunoblotting, flow cytometric analysis, and ELISA, they are also useful in clarifying the relationship between the structure and function of the proteoglycan. Heparan sulfate antibodies can be used together with the heparinase III enzyme. Clones 10E4 and JM403 recognize common epitopes on heparan sulfate (HS), which can be found on both basement membrane and cell-associated HS species. The 3G10 clone recognizes a neo-epitope exposed by digestion with heparinase III (HSase I) enzyme (which destroys reactivity to 10E4 and JM-403); so that the 3G10 clone can be used as control to 10E4 or JM-403(and vice cersa). Clones 10E4 and JM-403 were used together as capture antibodies in Seikagaku's Heparan Sulfate ELISA Kit.

Reactivity of 10E4 and 3G10 to BA-HSPG in ELISA

Procedure:

1. Coat bovine aorta-derived heparan sulfate proteoglycan (BA-HSPG) to a microplate at 4°C overnight (0.1mg/well).
2. React with Heparinase III* at 37°C for 90 minutes (optional step: digestion degrades 10E4 epitope but e poses 3G10 epitope - see results below).
3. Wash with 0.5% Tween 20-PBS (-).
4. Block with 3% BSA-PBS (-) at room temperature for 30 minutes.
5. Wash with T-PBS.
6. React with primary antibody (10E4 or 3G10) at room temperature for 20 minutes.
7. Wash with T-PBS.
8. React with secondary antibody (peroxidase-labelled rabbit anti-mouse IgG + IgM)†.
9. Wash with T-PBS.
10. Develop color with o-phenylene diamine /H2O .

* Use Heparinase III (eg. Seikagaku #. 100704 or AMS.HEP-ENZ III-S).
† Use HRP-rabbit-anti-mouse IgG + IgM (Jackson # 286255).

Results:

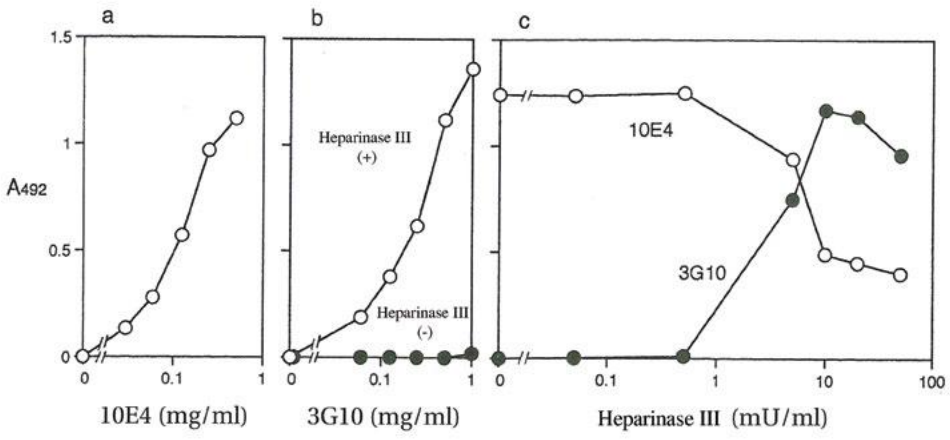


Fig 15. a) Reactivity of 10E4: Binding of 10E4 to BA-HSPG was observed in a concentration-dependent manner b) Reactivity of 3G10: 3G10 was not bound to intact BA-HSPG (●) but bound to BA-HSPG treated with Heparinase III ((1mU/100ml/well) (○), c) Sensitivity of 10E4 and 3G10 to heparinase III (antibody concentration: 1 µg/ml): Treatment with HSase I decreased the reactivity of 10E4 (○) but increased the reactivity of 3G10 (●) to BA-HSPG.

Description	Cat No.	Pack size
Heparinase III (Heparitinase I) Flavobacterium heparinum (EC 4.2.2.8)	AMS.HEP-ENZ III-S	0.1 IU
	AMS.HEP-ENZ III	0.5 IU

Flow Cytometry using Monoclonal Antibody 10E4 and 3G10 to Heparan Sulfate

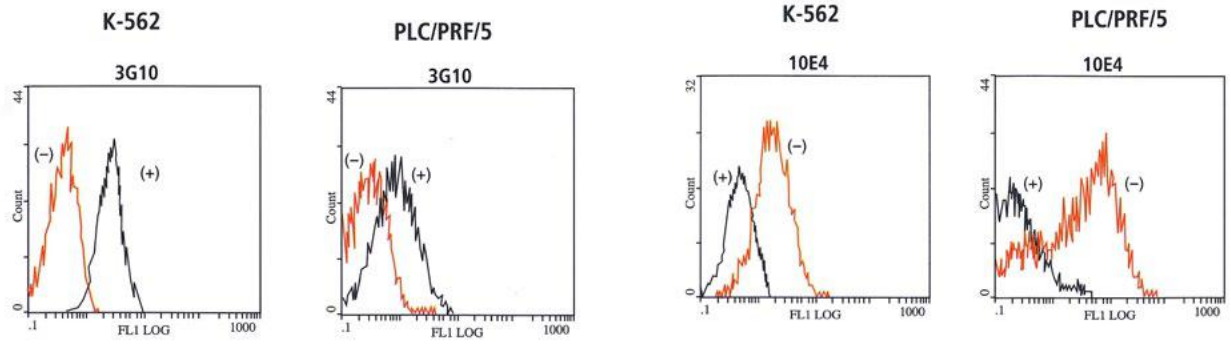
Procedure:

1. Incubate 1 x 10^6 cells with 100µl of Heparinase III (50mU/ml phosphate buffer saline, pH 7.4 PBS) or PBS for 20 minutes at 37°C. Wash. (Optional step: digestion degrades 10E4 epitope but exposes 3G10 epitope - see results below).
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



Immunostaining using Monoclonal Antibodies 10E4 and 3G10 to Heparan Sulfate

Procedure

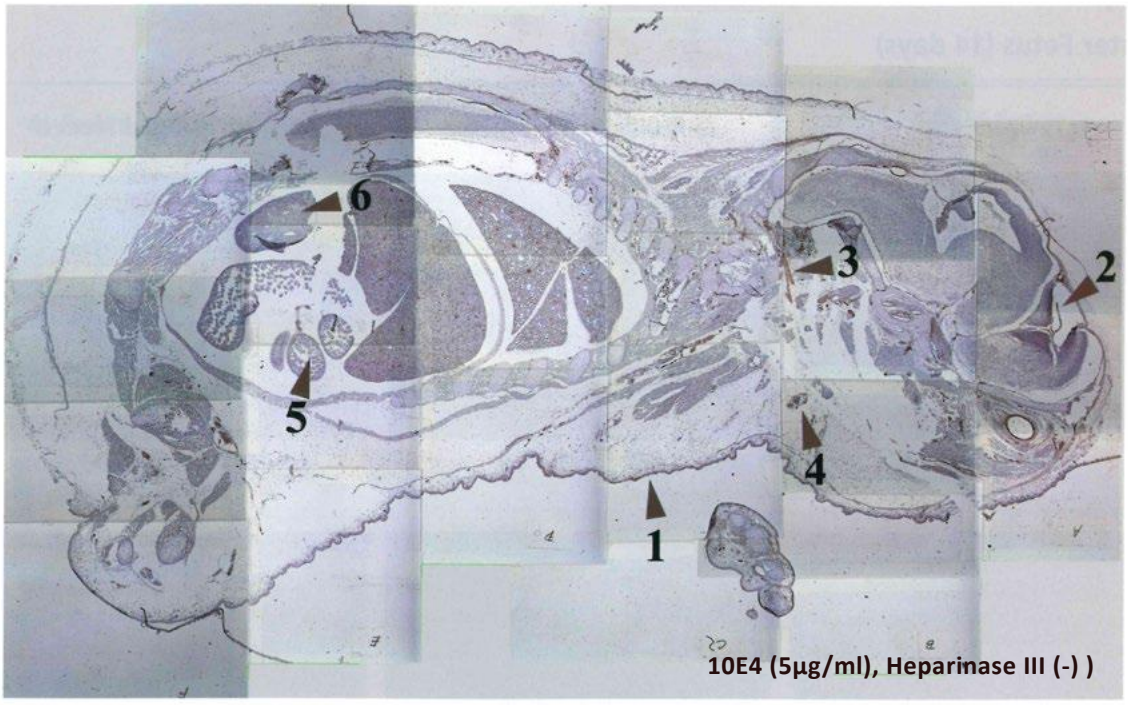
1. Prepare a paraffinized section fixed with formalin.
2. Wash with water after deparaffinization.
3. Wash with PBS (-) twice.
4. React with 25 mU/100µl/slide (10E4) or 2.5mU/100µl/slide (3G10) of Heparinase III* at 37°C for 2 hours. (optional step: digestion degrades 10E4 epitope but exposes 3G10 epitope - see results below).
5. Wash with PBS (-) twice.
6. Block with 100-150µl of 0.1% casein/PBS (-) at room temperature for 1 hour.
7. React with 5µg/ml of primary antibody (10E4 or 3G10) at room temperature for 2 hours.
8. Wash with PBS (-) three times.
9. React with a few drops of secondary antibody (biotinylated rabbit anti-mouse IgG + IgA + IgM) at room temperature for 15 minutes.
10. Wash with PBS (-) three times.
11. React with a few drops of an enzyme reagent (HRP-streptavidin† at room temperature) for 5 minutes.
12. Wash with PBS (-) three times.
13. Develop color with DAB (3-3' Diaminobenzidine).
14. Perform nuclear staining.
15. Perform dehydration and then coating.

* Use Heparinase III (eg. Seikagaku #. 100704 or AMS.HEP-ENZ III-S).
† Use HRP-rabbit-anti-mouse IgG + IgM (Jackson # 286255)

Results

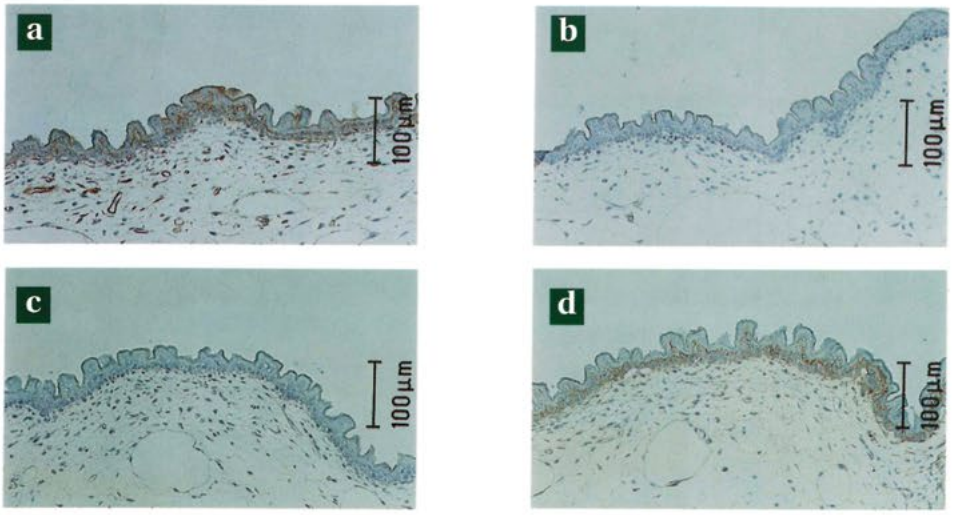
Hamster Fetus (14 days)

In the fetus, 10E4 epitope is frequently found in part of the brain and the mesenchymal tissue of the head, limbs, lungs, digestive tract, and kidneys. Regardless of the region, the reactivity of 10E4 is reduced by heparinase III treatment and 3G10 epitope appears. The following figure shows the results of a study on the reactivity of 10E4 in a 14 day old hamster fetus. Positive results for the 10E4 were obtained in the submaxillary gland, choroid plexus, peripheral nerve, intestines, kidneys, epidermis, etc. Magnified pictures of these tissues are shown in figures 1 through 6.

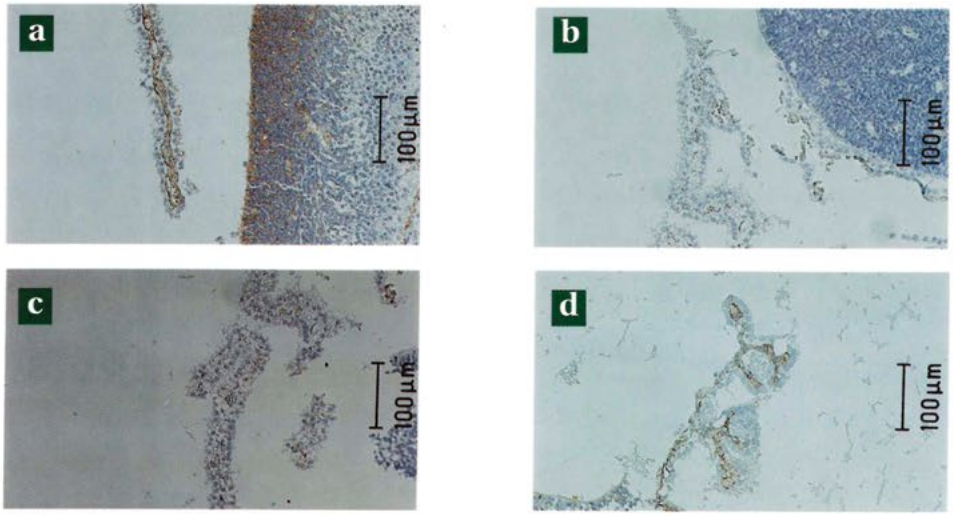


1) Epidermis 2) Plexus Chorioideus 3) Peripheral Nerve 4) Submaxillary Gland 5) Intestine 6) Kidney

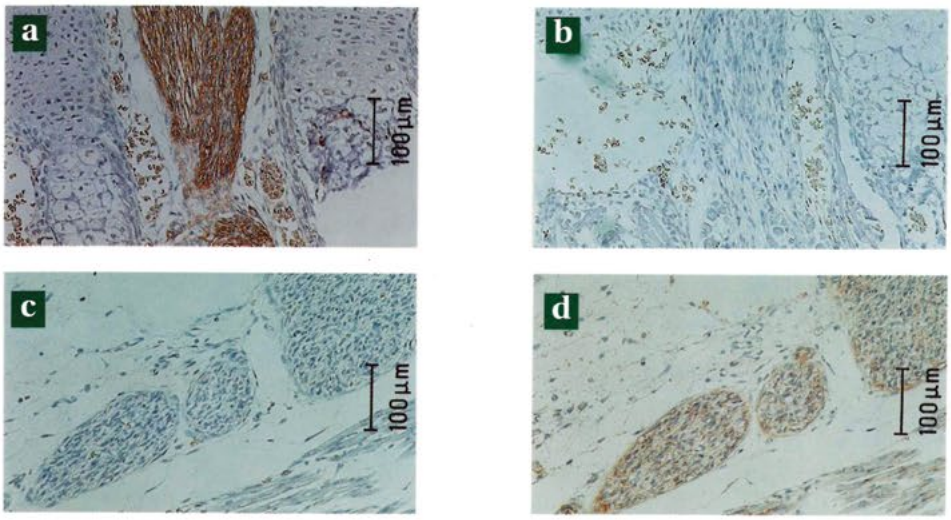
(1) Epidermis



(2) Choroid plexus

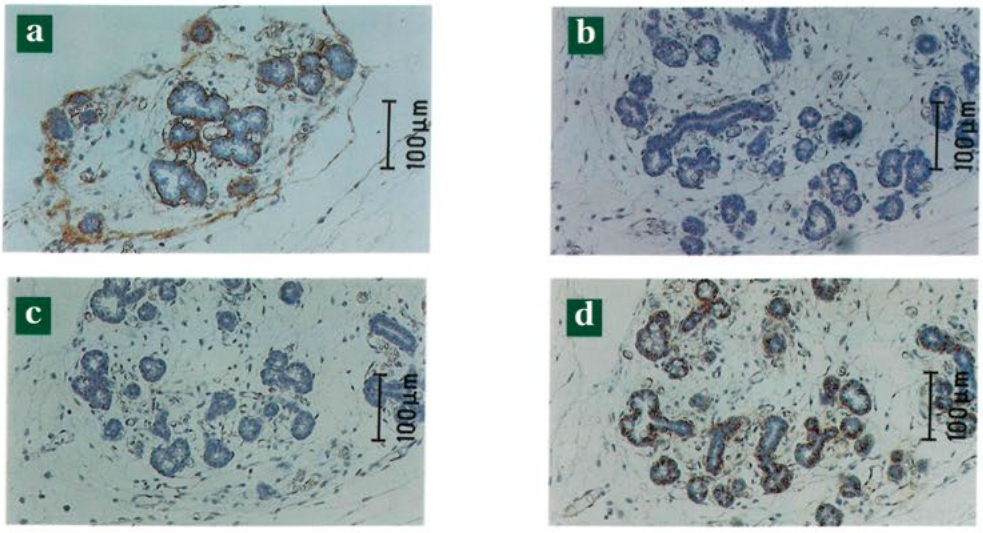


(3) Peripheral Nerve

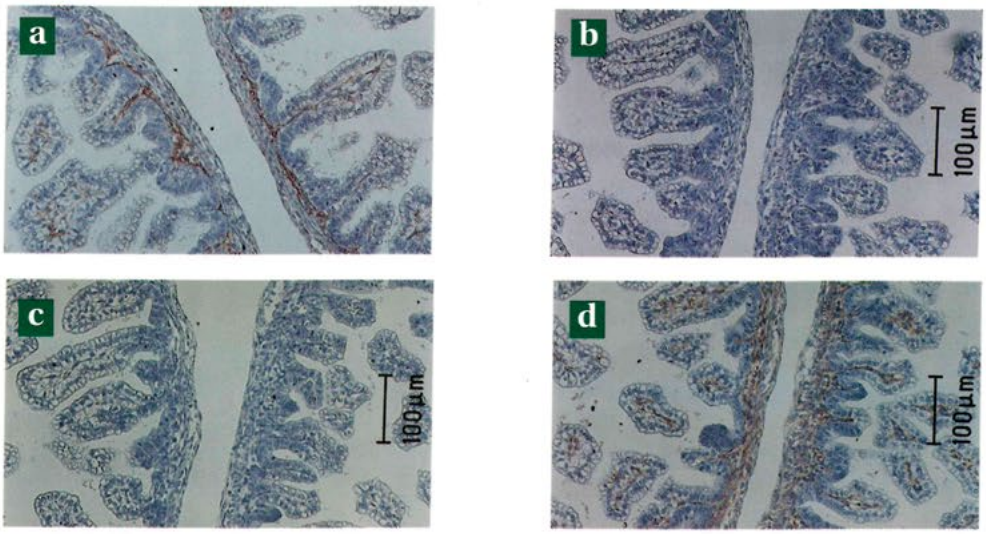


a: 10E4,Heparinase III (-)
b: 10E4, Heparinase III (+)
c: 3G10, Heparinase III (-)
d: 3G10, Heparinase III (+)

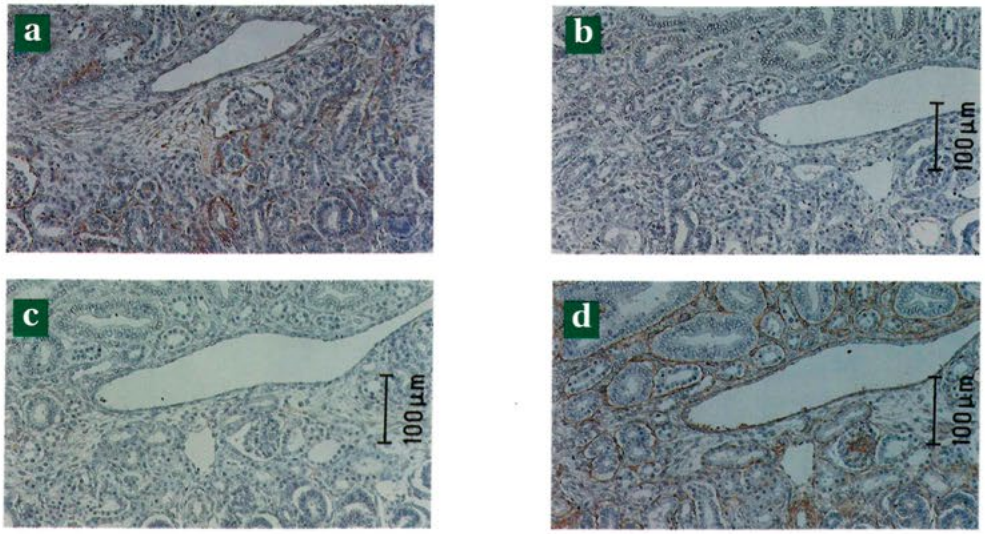
(4) Submaxillary Gland



(5) Intestine



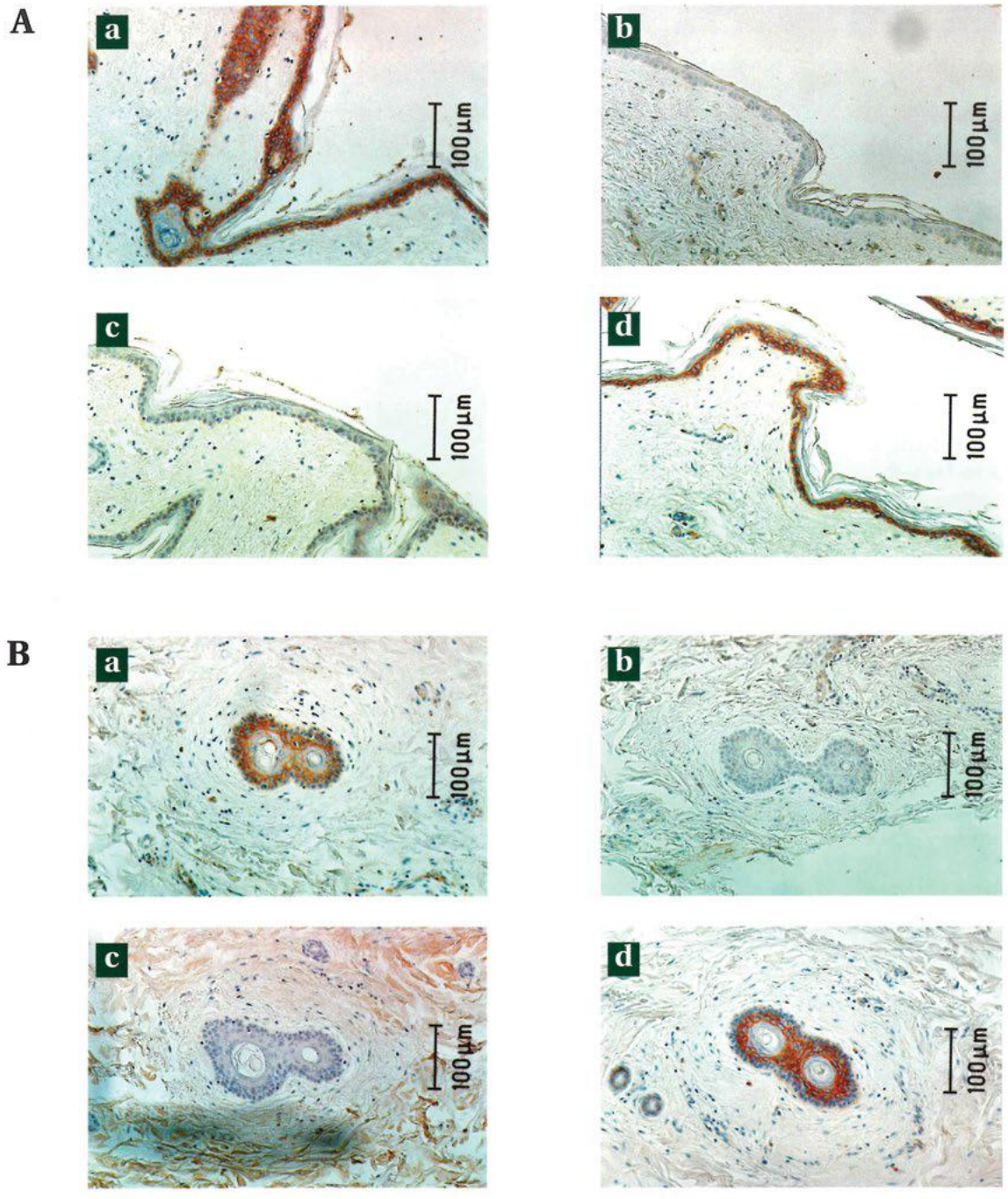
(6) Kidney



a: 10E4,Heparinase III (-)
b: 10E4, Heparinase III (+)
c: 3G10, Heparinase III (-)
d: 3G10, Heparinase III (+)

Human Epidermis

10E4 strongly stained the junction of the skin and epidermis (A-a) and the hair follicle (B-a) but 3G10 did not (A-c, B-c). After using Heparinase III treatment, 10E4 epitope disappeared (A-b, B-b) whereas 3G10 epitope appeared (A-d, B-d).



a: 10E4,Heparinase III (-)
c: 3G10, Heparinase III (-)

b: 10E4, Heparinase III (+)
d: 3G10, Heparinase III (+)

Use of 10E4 and JM403 in Heparan Sulfate ELISA

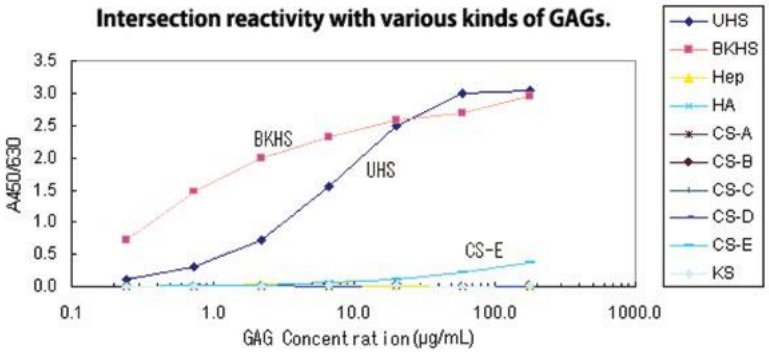
Clones 10E4 and JM-403 were used together as capture antibodies in Seikagaku's discontinued Heparan Sulfate ELISA Kit. Although these kits are no longer available for sale, the results from these ELISA kits show the reactivity of these antibodies to various GAGs and species.

Procedure

Reactivity of various kinds GAGs was evaluated with the Heparan Sulfate ELISA Kit. Three-fold dilution series at 7 concentrations [180.0, 60.0, 20.0, 6.67, 2.22, 0.74 or 0.25 µg/mL] were prepared using the GAGs solution (180µg/mL) shown in the following table and they were measured according to the protocol. OD data are shown in the graph. For Hyaluronic acid, Chondroitin sulfate A, B, C, D, E, and Keratan sulfate, the influence of endogenous Heparan sulfate was minimized by Heparitinase digestion, prior to preparation of the GAG solutions.

GAGs	Origin	Abbreviation
Heparan Sulfate	Human Urine	UHS
Heparan Sulfate	Bovine Kidney	BKHS
Heparin	Pig Intestine	Hep
Hyalronic acid	Pig Skin	HA
Chondroitin sulfate A	Whale Cartilage	CS-A
Chondroitin sulfate B	Pig Skin	CS-B
Chondroitin sulfate C	Shark Cartilage	CS-C
Chondroitin sulfate D	Shark Cartilage	CS-D
Chondroitin sulfate E	Squid Cartilage	CS-E
Keratan sulfate	Bovine Cornea	KS

Results



The Heparan Sulfate ELISA Kit which reacted with human urine origin Heparan sulfate (UHS) and Bovine kidney origin Heparan sulfate (BKHS) was evaluated with solutions of Heparin, Hyaluronic acid, Chondroitin Sulfate A, B, C, D, and Keratan sulfate in the range 1800.25µg/mL. Although some reactivity was found in the high concentration solutions (more than 20µg /mL) of Chondroitin sulfate E, it was about 1/100 UHS and about 1/1,000 BKHS and therefore a very weak reaction.

Heparinase III Digestive Examination

Using Heparinase III digestive examination, the specificity of the Heparan sulfate in the various samples was checked with the Heparan Sulfate ELISA Kit.

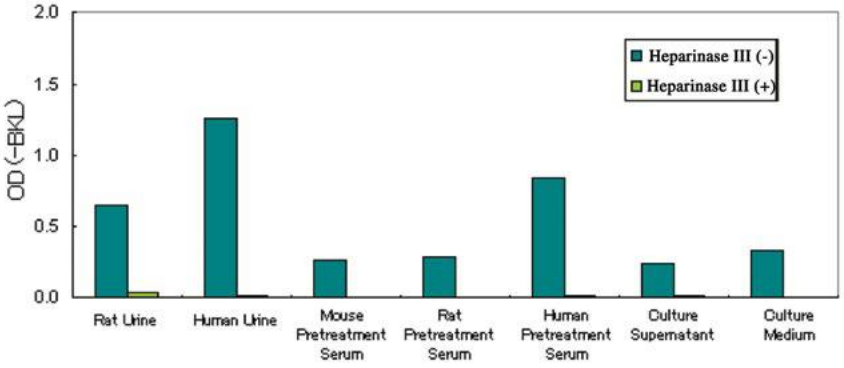
Procedure

At room temperature (15-25°C), the samples prepared was measured according to the protocol, prior to following heparinase III digestion for 2 hours. For the serum sample, Heparinase III digestion was performed after the serum had been pretreated with Actinase E.

* Culture supernatant: Raw 264.7 (Abelson murine leukemia virus-induced tumor).
**Culture Medium: 10% FBS in alpha-MEM.

Results

All samples had significant reactivity (OD value) prior to heparinase III digestion. However, this disappeared almost completely following the enzyme digestion with heparinase III as measured by the Heparan Sulfate ELISA Kit.



Selected Citations

10E4, 3G10 and 3B3 Antibodies

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10E4 and JM-403 Antibodies

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10E4, 3G10 and JM-403 Antibodies

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- Van den Born, J., Gunnarsson, K., Bakker, M. A., Kjellén, L., Kusche-Gullberg, M., Maccarana, M., ... & Lindahl, U. (1995). Presence of N-unsubstituted glucosamine units in native heparan sulfate revealed by a monoclonal antibody. Journal of Biological Chemistry, 270(52), 31303-31309.
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- 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, 239-251. (Also cites our Chondroitinase ABC).
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CONTACT US:

AMS Biotechnology (Europe) Ltd - UK & Rest of World
184 Park Drive, Milton Park, Abingdon OX14 4SE, U.K.
T: +44 (0) 1235 828 200 | F: +44 (0) 1235 820 482

AMS Biotechnology (Europe) Ltd – Switzerland
Centro Nord-Sud 2E CH-6934 Bioggio-Lugano.
T: +41 (0) 91 604 55 22 | F: +41 (0) 91 605 17 85

AMS Biotechnology (Europe) Ltd - Deutschland
T: +49 (0) 69 779099 | F: +49 (0) 69 13376880

amsbio LLC - United States
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or +1.(800) 987-0985 | F: +1 (617) 945-8218