Glycosaminoglycan Microarray User Manual

Z BIOTECH

Innovative Biochemical Analysis Solutions

Website: http://www.zbiotech.com/
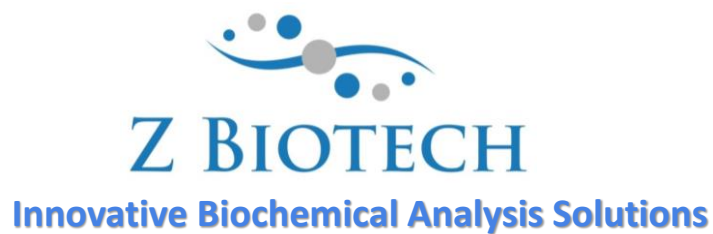
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Introduction

Glycosaminoglycans (GAGs), also known as mucopolysaccharides, are a class of long and linear polysaccharides. There are four major classes of GAGs: hyaluronic acid (HA), heparan sulfate/heparin (HS/Hep), chondroitin sulfate/dermatan sulfate (CS/DS), and keratan sulfate (KS). They are structurally different polysaccharides defined by specific monosaccharides, sulfation modifications, and chemical linkages.

GAGs are ubiquitously found on the mammalian cell surface and in the extracellular matrix. They maintain cell hydration and provide structural support for connective tissues. They are also critical regulators in cell signaling pathways. However, abnormally expressed GAGs have been linked to various diseases. For example, alterations in GAG sulfation patterns have been associated with poor prognosis of breast, ovarian, colorectal, prostate, and gastric cancers. However, it is not fully understood to what extent the degree of GAG sulfation can promote or inhibit cancer progression. One of the hallmarks of Alzheimer's disease is the presence of extracellular plaques composed of fibrillated amyloid-beta (Aβ) protein. Sulfated GAGs act as pathological chaperones to assist Ab aggregation. However, GAGs of a unique N-sulfation pattern can reduce Aβ protein aggregation. GAGs are often targeted by microbial pathogens to enter the cell and establish infections. Many microbial pathogens hijack the host biological process of GAG synthesis to subvert immunity and promote disease. Therefore, identifying and understanding the differences between GAGs expressed in normal and pathological conditions is vital for understanding the pathogenesis of these diseases.

ZBiotech has developed a robust microarray platform that allows researchers to define GAG-binding specificities for various biological samples, such as proteins, antibodies, cells, cell lysate, serum, vesicles, bacteria, or viral particles. The glycosaminoglycan array features 46 distinct GAG structures varying in length, degree of sulfation, and disaccharide sequence. Each array contains 8 or 16 identical subarrays, enabling the simultaneous analysis of multiple samples. The glycosaminoglycan array provides high-throughput and reliable glycan-binding information with a simple assay format that only requires a small sample volume. It can be customized to meet individual client needs. Assay services are available upon request**.**
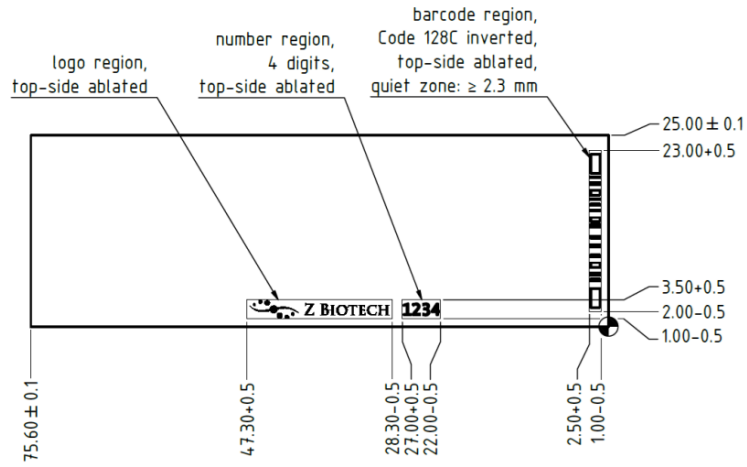
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 3 weeks upon receipt. For long term storage keep the bag of slides at -20°C. Avoid freezing and thawing multiple times. Purchased slides and buffers should be used within 6 months.

Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.
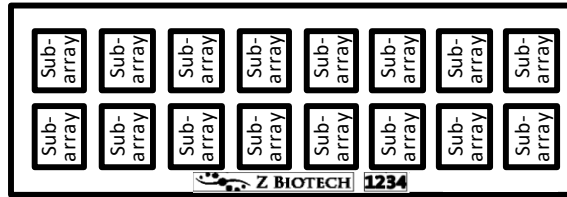
Array Map/Schematic

Glycosaminoglycan Microarray slides have either 8 or 16 subarrays. Arrays are printed on the side with the "Z Biotech" label and 4-digit number ID facing upward. The "Z Biotech" label is located on the bottom center from a landscape view. The number ID is consistent with the barcode ID on the bottom from a portrait view. Dimensions and array maps are shown below.
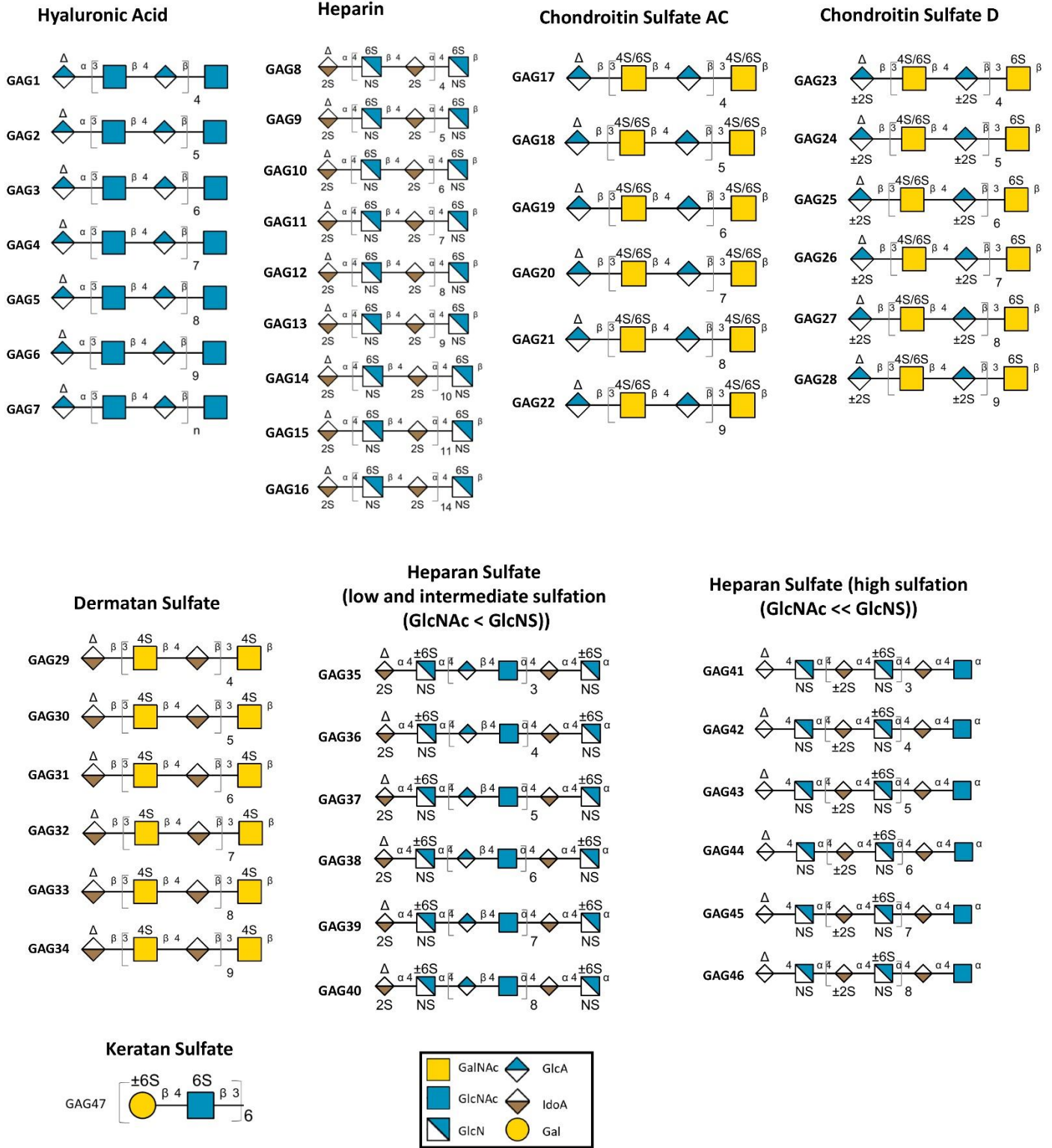
logo region,
top-side ablated

number region,
4 digits,
top-side ablated

barcode region,
Code 128C inverted,
top-side ablated,
quiet zone: ≥ 2.3 mm

Z BIOTECH 1234

25.00 ± 0.1
23.00+0.5

3.50+0.5
2.00−0.5
1.00−0.5

75.60 ± 0.1

47.30−0.5

28.30−0.5
27.00+0.5
22.00−0.5

2.50+0.5
1.00−0.5

Array Map (16-subarray slides):

16-subarray Slide

GAG1	GAG1	GAG1	GAG1	GAG2	GAG2	GAG2	GAG2	GAG3	GAG3	GAG3	GAG3	GAG4	GAG4	GAG4	GAG4
GAG5	GAG5	GAG5	GAG5	GAG6	GAG6	GAG6	GAG6	GAG7	GAG7	GAG7	GAG7	GAG8	GAG8	GAG8	GAG8
GAG9	GAG9	GAG9	GAG9	GAG10	GAG10	GAG10	GAG10	GAG11	GAG11	GAG11	GAG11	GAG12	GAG12	GAG12	GAG12
GAG13	GAG13	GAG13	GAG13	GAG14	GAG14	GAG14	GAG14	GAG15	GAG15	GAG15	GAG15	GAG16	GAG16	GAG16	GAG16
GAG17	GAG17	GAG17	GAG17	GAG18	GAG18	GAG18	GAG18	GAG19	GAG19	GAG19	GAG19	GAG20	GAG20	GAG20	GAG20
GAG21	GAG21	GAG21	GAG21	GAG22	GAG22	GAG22	GAG22	GAG23	GAG23	GAG23	GAG23	GAG24	GAG24	GAG24	GAG24
GAG25	GAG25	GAG25	GAG25	GAG26	GAG26	GAG26	GAG26	GAG27	GAG27	GAG27	GAG27	GAG28	GAG28	GAG28	GAG28
GAG29	GAG29	GAG29	GAG29	GAG30	GAG30	GAG30	GAG30	GAG31	GAG31	GAG31	GAG31	GAG32	GAG32	GAG32	GAG32
GAG33	GAG33	GAG33	GAG33	GAG34	GAG34	GAG34	GAG34	GAG35	GAG35	GAG35	GAG35	GAG36	GAG36	GAG36	GAG36
GAG37	GAG37	GAG37	GAG37	GAG38	GAG38	GAG38	GAG38	GAG39	GAG39	GAG39	GAG39	GAG40	GAG40	GAG40	GAG40
GAG41	GAG41	GAG41	GAG41	GAG42	GAG42	GAG42	GAG42	GAG43	GAG43	GAG43	GAG43	GAG44	GAG44	GAG44	GAG44
GAG45	GAG45	GAG45	GAG45	GAG46	GAG46	GAG46	GAG46	GAG47	GAG47	GAG47	GAG47	NC	NC	NC	NC
PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Marker	Marker	Marker	Marker

Array Map (8-subarray slides):

GAG1	GAG1	GAG1	GAG1	GAG1	GAG1	GAG2	GAG2	GAG2	GAG2	GAG2	GAG2	GAG3	GAG3	GAG3	GAG3	GAG3	GAG3	GAG4	GAG4	GAG4	GAG4	GAG4	GAG4	GAG5	GAG5	GAG5	GAG5	GAG5	GAG5	GAG6	GAG6	GAG6	GAG6	GAG6	GAG6
GAG7	GAG7	GAG7	GAG7	GAG7	GAG7	GAG8	GAG8	GAG8	GAG8	GAG8	GAG8	GAG9	GAG9	GAG9	GAG9	GAG9	GAG9	GAG10	GAG10	GAG10	GAG10	GAG10	GAG10	GAG11	GAG11	GAG11	GAG11	GAG11	GAG11	GAG12	GAG12	GAG12	GAG12	GAG12	GAG12
GAG13	GAG13	GAG13	GAG13	GAG13	GAG13	GAG14	GAG14	GAG14	GAG14	GAG14	GAG14	GAG15	GAG15	GAG15	GAG15	GAG15	GAG15	GAG16	GAG16	GAG16	GAG16	GAG16	GAG16	GAG17	GAG17	GAG17	GAG17	GAG17	GAG17	GAG18	GAG18	GAG18	GAG18	GAG18	GAG18
GAG19	GAG19	GAG19	GAG19	GAG19	GAG19	GAG20	GAG20	GAG20	GAG20	GAG20	GAG20	GAG21	GAG21	GAG21	GAG21	GAG21	GAG21	GAG22	GAG22	GAG22	GAG22	GAG22	GAG22	GAG23	GAG23	GAG23	GAG23	GAG23	GAG23	GAG24	GAG24	GAG24	GAG24	GAG24	GAG24
GAG25	GAG25	GAG25	GAG25	GAG25	GAG25	GAG26	GAG26	GAG26	GAG26	GAG26	GAG26	GAG27	GAG27	GAG27	GAG27	GAG27	GAG27	GAG28	GAG28	GAG28	GAG28	GAG28	GAG28	GAG29	GAG29	GAG29	GAG29	GAG29	GAG29	GAG30	GAG30	GAG30	GAG30	GAG30	GAG30
GAG31	GAG31	GAG31	GAG31	GAG31	GAG31	GAG32	GAG32	GAG32	GAG32	GAG32	GAG32	GAG33	GAG33	GAG33	GAG33	GAG33	GAG33	GAG34	GAG34	GAG34	GAG34	GAG34	GAG34	GAG35	GAG35	GAG35	GAG35	GAG35	GAG35	GAG36	GAG36	GAG36	GAG36	GAG36	GAG36
GAG37	GAG37	GAG37	GAG37	GAG37	GAG37	GAG38	GAG38	GAG38	GAG38	GAG38	GAG38	GAG39	GAG39	GAG39	GAG39	GAG39	GAG39	GAG40	GAG40	GAG40	GAG40	GAG40	GAG40	GAG41	GAG41	GAG41	GAG41	GAG41	GAG41	GAG42	GAG42	GAG42	GAG42	GAG42	GAG42
GAG43	GAG43	GAG43	GAG43	GAG43	GAG43	GAG44	GAG44	GAG44	GAG44	GAG44	GAG44	GAG45	GAG45	GAG45	GAG45	GAG45	GAG45	GAG46	GAG46	GAG46	GAG46	GAG46	GAG46	GAG47	GAG47	GAG47	GAG47	GAG47	GAG47	NC	NC	NC	NC	NC	NC
PC1	PC1	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	PC4	PC4	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M	M	M	M

Glycosaminoglycan SNFG Structures:

Glycosaminoglycan Identification List:

Type	ID	Name	Structure and Molecular Weight
Hyaluronic Acid (HA)	GAG1	Hyaluronic Acid dp10 (HA10)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_4$ GlcNAc, Mw 1,950 Da
	GAG2	Hyaluronic Acid dp12 (HA12)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_5$ GlcNAc, Mw 2,350 Da
	GAG3	Hyaluronic Acid dp14 (HA14)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_6$ GlcNAc, Mw 2,700 Da
	GAG4	Hyaluronic Acid dp16 (HA16)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_7$ GlcNAc, Mw 3,150 Da
	GAG5	Hyaluronic Acid dp18 (HA18)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_8$ GlcNAc, Mw 3,650 Da
	GAG6	Hyaluronic Acid dp20 (HA20)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_9$ GlcNAc, Mw 3,900 Da
	GAG7	Hyaluronic Acid Polymer (HA93)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_n$ GlcNAc, Mw 93 kDa
Heparin	GAG8	Heparin dp10 (H10)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_4$, Mw 3,000
	GAG9	Heparin dp12 (H12)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_5$, Mw 3,550
	GAG10	Heparin dp14 (H14)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_6$, Mw 4,100
	GAG11	Heparin dp16 (H16)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_7$, Mw 4,650
	GAG12	Heparin dp18 (H18)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_8$, Mw 5,200
	GAG13	Heparin dp20 (H20)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_9$, Mw 5,750
	GAG14	Heparin dp22 (H22)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{10}$, Mw 6,300
	GAG15	Heparin dp24 (H24)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{11}$, Mw 6,850
	GAG16	Heparin dp30 (H30)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{14}$, Mw 9,000
Chondroitin Sulfate (CS)	GAG17	Chondroitin Sulphate Oligosaccharide dp10 (CSO10)	ΔUA - [GalNAc,6S or 4S - GlcA]$_4$ - GalNAc,6S or 4S, Mw 2,480
	GAG18	Chondroitin Sulphate Oligosaccharide dp12 (CSO12)	ΔUA - [GalNAc,6S or 4S - GlcA]$_5$ - GalNAc,6S or 4S, Mw 2,976
	GAG19	Chondroitin Sulphate Oligosaccharide dp14 (CSO14)	ΔUA - [GalNAc,6S or 4S - GlcA]$_6$ - GalNAc,6S or 4S, Mw 3,472
	GAG20	Chondroitin Sulphate Oligosaccharide dp16 (CSO16)	ΔUA - [GalNAc,6S or 4S - GlcA]$_7$ - GalNAc,6S or 4S, Mw 3,968
	GAG21	Chondroitin Sulphate Oligosaccharide dp18 (CSO18)	ΔUA - [GalNAc,6S or 4S - GlcA]$_8$ - GalNAc,6S or 4S, Mw 4,464
	GAG22	Chondroitin Sulphate Oligosaccharide dp20 (CSO20)	ΔUA - [GalNAc,6S or 4S - GlcA]$_9$ - GalNAc,6S or 4S, Mw 4,960
	GAG23	Chondroitin Sulphate D Oligosaccharide dp10 (CSDO10)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_4$ – GalNAc,6S, Mw 2,480
	GAG24	Chondroitin Sulphate D Oligosaccharide dp12 (CSDO12)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_5$ – GalNAc,6S, Mw 2,976
	GAG25	Chondroitin Sulphate D Oligosaccharide dp14 (CSDO14)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_6$ – GalNAc,6S, Mw 3,472
	GAG26	Chondroitin Sulphate D Oligosaccharide dp16 (CSDO16)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_7$ – GalNAc,6S, Mw 3,968

	GAG27	Chondroitin Sulphate D Oligosaccharide dp18 (CSDO18)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_8$ – GalNAc,6S, Mw 4,464
	GAG28	Chondroitin Sulphate D Oligosaccharide dp20 (CSDO20)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_9$ – GalNAc,6S, Mw 4,960
Dermatan Sulfate (DS)	GAG29	Dermatan Sulphate dp10 (DS10)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_4$, Mw 2,480
	GAG30	Dermatan Sulphate dp12 (DS12)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_5$, Mw 2,976
	GAG31	Dermatan Sulphate dp14 (DS14)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_6$, Mw 3,472
	GAG32	Dermatan Sulphate dp16 (DS16)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_7$, Mw 3,968
	GAG33	Dermatan Sulphate dp18 (DS18)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_8$, Mw 4,464
	GAG34	Dermatan Sulphate dp20 (DS20)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_9$, Mw 4,960
Heparan Sulfate (HS)	GAG35	Heparan Sulphate Oligosaccharide dp10 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_3$ – IdoA - GlcNS, Mw 2,800
	GAG36	Heparan Sulphate Oligosaccharide dp12 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_4$ – IdoA - GlcNS, Mw 3,500
	GAG37	Heparan Sulphate Oligosaccharide dp14 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_5$ – IdoA - GlcNS, Mw 4,000
	GAG38	Heparan Sulphate Oligosaccharide dp16 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_6$ – IdoA - GlcNS, Mw 4,400
	GAG39	Heparan Sulphate Oligosaccharide dp18 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_7$ – IdoA - GlcNS, Mw 5,000
	GAG40	Heparan Sulphate Oligosaccharide dp20 (Hep I, low and intermediate sulphation)	ΔUA2S - GlcNS – [GlcA - GlcNAc]$_8$ – IdoA - GlcNS, Mw 5,400
	GAG41	Heparan Sulphate Oligosaccharide dp10 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_3$ – IdoA - GlcNAc, Mw 2,800
	GAG42	Heparan Sulphate Oligosaccharide dp12 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_4$ – IdoA - GlcNAc, Mw 3,500
	GAG43	Heparan Sulphate Oligosaccharide dp14 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_5$ – IdoA - GlcNAc, Mw 4,200
	GAG44	Heparan Sulphate Oligosaccharide dp16 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_6$ – IdoA - GlcNAc, Mw 4,800
	GAG45	Heparan Sulphate Oligosaccharide dp18 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_7$ – IdoA - GlcNAc, Mw 5,500
	GAG46	Heparan Sulphate Oligosaccharide dp20 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_8$ – IdoA - GlcNAc, Mw 6,200
Keratan Sulfate (KS)	GAG47	Keratan Sulfate Oligosaccharide	[Gal +/- 6S – GlcNAc, 6S]$_6$, Mw 3,000

Controls

NC: Negative control, Print Buffer

PC1: Positive control 1, a biotinylated probe (0.01 mg/ml)

PC2: Postitive control 2, Human IgG (0.1 mg/ml)

PC3: Postitive control 3, Mouse IgG (0.1 mg/ml)

PC4: Postitive control 4, Rabbit IgG (0.1 mg/ml)

Array Marker: Anti-Human IgG, Cy3 (0.01 mg/ml) and anti-Human IgG, Alexa555 (0.01 mg/ml)

Materials Required

- Arrayed glass slides
- 16 or 8 cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Glycan Array Assay Buffer (GAAB, Item #10107), add 1% BSA (10 mg/ml) if needed
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

Preparation of assay samples:

Prepare glycan-binding protein samples or secondary antibodies of interest in a centrifuge tube by diluting with the Glycan Array Assay Buffer. We recommend a range of 50 μg/ml to 0.1 μg/ml concentration for protein samples, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This is often accomplished by applying a different dilution of samples to different wells of the array. For the fluorescently labelled streptavidin we recommend a concentration of 1 μg/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. **We recommend using 100 μL volume of sample per well for 16 subarray cassettes and 200 μL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation for every step of the assay.** If necessary, the assay can be done successfully with a minimal volume of 60 μL per well for 16 subarray cassettes and 80 μL for 8 subarray cassettes. We caution that using a minimal volume in the wells has an increased risk of the array drying out during the assay and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

Assay Protocol

Considerations Before Starting the Experiment

1. **Preparation of Buffers:**
    - Ensure that Glycan Array Blocking Buffer (GABB) and Glycan Array Assay Buffer (GAAB) are ready for glycan microarray analysis.
2. **BSA Addition:**
    - If needed, add BSA to GAAB or LAAB to reduce non-specific binding.
    - Prepare a 1% BSA assay buffer by adding BSA to GAAB or LAAB and filter through a 0.2 μm PVDF membrane filter.
3. **Avoiding Dryness:**
    - The array surface is extremely sensitive to dryness. Ensure the array does not dry at any point during the assay.
    - Avoid handling multiple subarrays simultaneously to prevent drying out.
4. **Array Formats and Volumes:**
    - Common array formats: 8, 16, or 24 subarrays.

- - - For 8-subarray format: Use 200 µL per subarray.
    - For 16-subarray format: Use 100 µL per subarray.
    - For 24-subarray format: Use 50-80 µL per subarray.
  - Minimal volumes: 60 µL per well for 16-subarray cassettes and 80 µL for 8-subarray cassettes.
  - Caution: Using minimal volumes increases the risk of drying out and may cause signal variation. Ensure samples are homogeneous and thoroughly mixed.
5. **Sample Preparation:**
  - Dilute glycan-binding protein samples or secondary antibodies in Glycan Array Assay Buffer.
  - Recommended concentration range for protein samples: 50 µg/mL to 0.1 µg/mL. Experiment to find the optimal concentration for highest binding signals with the lowest background.
  - For fluorescently labeled streptavidin, use a concentration of 0.2 µg/mL.
6. **Storage of Microarray Slides and Buffers:**
  - Store microarray slides and buffers at 4°C if assayed within 24 hours of receipt.
  - For long-term storage, keep microarray slides at -20°C. Avoid multiple freeze-thaw cycles.
  - Use slides and buffers within 12 months. Allow slides to equilibrate to room temperature for at least 20 minutes before opening.
  - After opening, reseal unused slides in a moisture barrier bag with a desiccant and refreeze. Handle slides in a dust-free environment, wearing gloves and holding slides by the edges.
  - When adding samples, avoid touching the pipette tip to the array surface. When removing samples, gently touch the pipette tip to the corner of the well and tip the slide.

Analyzing Biological Samples with Glycan Microarray

Part 1: Blocking

Handle the slide in a clean, dry environment. Use gloves and avoid touching the slide surface.

1. Let the microarray slide equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Assemble the slide into a hybridization chamber device.
3. Add the Glycan Array Blocking Buffer (GABB) to each subarray well of the assembled hybridization chamber device:
   - 100 µL for each subarray of a 16-subarray chamber device
   - 200 µL for each subarray of an 8-subarray chamber device
4. Cover the hybridization chamber device with adhesive film to prevent evaporation and incubate the slide on a shaker at 80 rpm for 30 minutes. Ensure the orbital shaker is completely flat to avoid variations in binding and detection. Longer incubation times are acceptable but not necessary.
5. After 30 minutes, add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Aspirate the liquid out from each well, ensuring that some liquid remains to cover the surface.
6. Add another 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
7. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
8. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.

Part 2: Binding Assay

1. Unless the glycan-binding protein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Remove the blocking buffer from each well by gently touching a pipette tip to the corner of the well. We recommend doing this one subarray at a time, not handling multiple subarrays simultaneously to avoid drying out the microarray slide surface.
3. Immediately apply the glycan-binding protein sample of interest to each well.
4. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labeled, cover it with aluminum foil to keep it in the dark.
5. Incubate on the shaker for 1 hour at 80 rpm. Longer incubation times may increase the binding signal, especially for weakly binding samples. Avoid allowing the slides to dry out at any point during the assay, especially during long incubation times. Make sure the adhesive film is sealed around each well.
6. If your glycan-binding protein samples are fluorescently labeled, go directly to Part 6 – Final Wash and Dry.

Part 3: Wash

We recommend doing the following procedure using a multi-channel pipette.

1. **Initial Wash:**
   o After incubating the samples for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   o Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
   o Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
   o After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   o Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   o Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
   o After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   o Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   o Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Proceed to the Next Step:**
   o If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

Part 4: Binding of Biotinylated Antibody (Sandwich Assay Format)

1. **Adding Secondary Antibody:**
   o After removing all the GAAB using a multi-channel aspirating needle device, immediately add the secondary biotinylated antibody to each well using a multi-channel pipette.
2. **Incubation:**

- o Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Longer incubation times are acceptable but not necessary.

Part 5: Fluorescent Staining

1. **Adding Streptavidin:**
   - o After completely removing the GAAB using a multi-channel aspirating needle device, immediately add the fluorescently labeled streptavidin sample using a multi-channel pipette.
2. **Incubation:**
   - o Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 80 rpm for 1 hour. Longer incubation times are acceptable but not necessary.

Part 6: Final Wash and Dry

1. **Initial Wash:**
   - o After incubating the secondary antibody or streptavidin for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - o Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
   - o Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
   - o After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   - o Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - o Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
   - o After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   - o Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - o Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Disassembling the Hybridization Chamber:**
   - o Disassemble the hybridization chamber device from the slide. For the provided cassette, this can be done by holding the slide with one hand at the top and bottom edges and sliding out the cassette clips one by one with the other hand. If your provided cassette has metal clips, they can be removed by rotating the clip outwards from the bottom of the slide. When the clips have been removed, place the slide on the table and hold a small outer edge of the slide to the table as you gently peel the cassette off.
5. **Immersing the Slide:**
   - o Immediately immerse the slide in a Coplin jar full of GAAB. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
6. **Rinsing with Water:**
   - o Decant the buffer from the jar while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide. Place the jar on the shaker at 80 rpm for 5 minutes.
7. **Repeat Rinsing:**
   - o Decant the water from the jar. Repeat once more with fresh de-ionized water.

8. **Drying the Slide:**
   o Allow the slide to dry by using a microarray slide centrifuge completely in a clean, dust-free environment before scanning.

Part 7: Data Acquisition and Analysis

1. **Scanning the Slide:**
   o Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without being saturated (saturated positive control signal is okay).
2. **Analyzing Data:**
   o Analyze data with microarray analysis software. If there is specific binding, the signal intensity should be higher than the background signal (the area where there are no printed spots). The fluorescent signal due to specific binding to your sample of interest should be dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan-binding is saturated) and should have a positive binding signal after the signal from control assays has been subtracted.
3. **Quantifying Signal Intensities:**
   o Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signals from negative control spots as well as the same spots on a negative control assay (assay with only detection antibodies and fluorophore) will give more accurate specific binding data.
4. **Interpreting Control Signals:**
   o **Negative Control (Print Buffer):** The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.
   o **Marker:** The array marker should show a fluorescence signal regardless of the assay. It is there primarily to aid with the orientation of the array map during analysis.
   o **Biotinylated Mannose (PC1):** This positive control will bind directly to the fluorescent-labeled streptavidin. If your glycan-binding protein sample is already fluorescently labeled, or in any case where the addition of fluorescently labeled streptavidin to the array was not performed (Part 5 – Fluorescent Staining), this positive control will not be reactive.
   o **IgG (PC2, PC3, PC4):** IgG is an antibody found in the blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from a human, rabbit, or mouse, it should bind to the respective IgG control.

**Typical Binding Assay Result from the Glycosaminoglycan Microarray**

Using glycosaminoglycan array to determine the binding specificity of mouse CD44

The glycosaminoglycan array was assayed with mouse CD44 hFc (5 μg/mL), followed by an anti-human IgG antibody (Cy3). The array was scanned with a microarray scanner at 532nm wavelength. Positive control showed binding signals as expected. CD44 interacts with hyaluronic acid (GAG7).
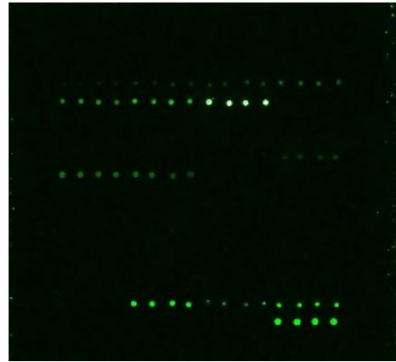
Array Scan Image
_____

The microarray slide was scanned by a microarray slide scanner at high laser intensity.
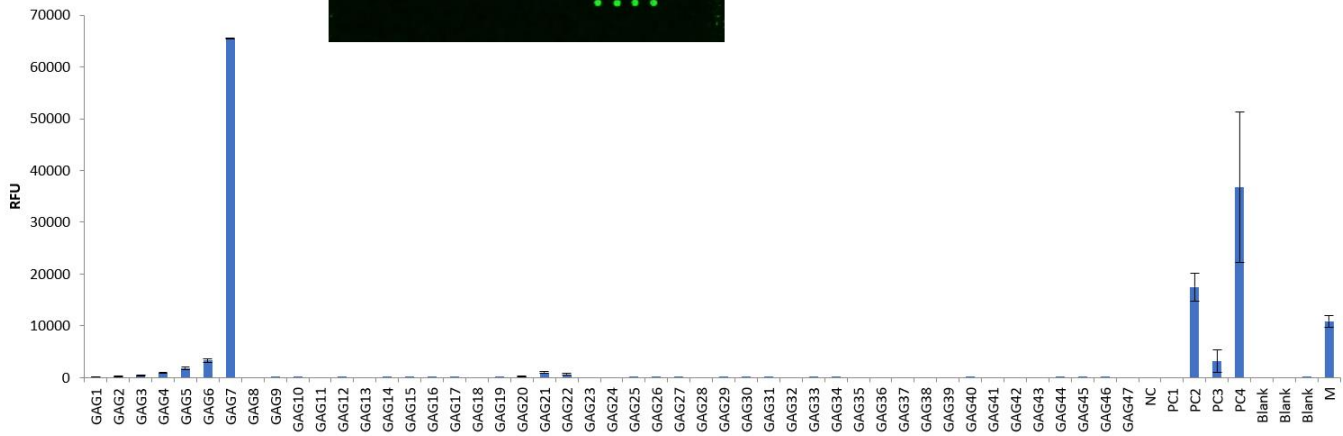
Quantitative Data
_____

Data was generated by analyzing scanned microarray images.

Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of sample of interest is too high • Concentration of fluorescent samples is too high • Arrays are not thoroughly washed. • Slide drying out during assay • Excessive particles in the samples due to sample aggregation, dust, etc.	• Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is. Use control assays to determine which sample is causing high background. • Apply longer times for washing steps and use a higher shaking rate • Make sure wash buffer and sample is completely removed before the next step • Make sure adhesive film fully seals the wells to avoid evaporation • Centrifuge the samples prior to assay to avoid adding irrelevant particles. Make sure buffers are filtered. • If you think that the protein is aggregating during incubation, try shaking at a higher speed
Signal Variation	• Slide drying out during assay • Binding samples are not equally distributed in the wells • Glycan-binding protein aggregation during incubation • Bubbles during incubation	• Make sure wells are sealed to prevent evaporation during incubation • Apply a larger volume of sample to each well to ensure equal distribution • Use a higher shaking rate during incubation • Make sure samples are homogeneous, mixed thoroughly, and do not leave bubbles on the array surface
Unexpected Binding	• Cross contamination between wells or other sources • Sample contamination	• Make sure to use sterilized pipette tips and tubes used for sample application and preparation • Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells • Be careful not to cross contaminate samples when applying to the wells, even during wash steps