Neu5Gc/Neu5Ac N-Glycan Array User Manual

Z BIOTECH

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For Research Use Only

Introduction

Neu5Gc is a major mammalian sialic acid synthesized from Neu5Ac by an enzyme no longer present in humans. Regardless, nonhuman, diet-derived Neu5Gc is metabolically integrated into a variety of human tissues. Human cells can biochemically recognize Neu5Gc and incorporate it into cell-surface glycoconjugates, however the immune system recognizes Neu5Gc-containing glycans as alien and produces anti-Neu5Gc antibodies resulting in inflammation. Diseases relating to chronic inflammation such as cancer, cardiovascular diseases, and thyroiditis are associated with higher levels of anti-Neu5Gc antibodies and Neu5Gc antigens, and it is suspected to be involved in other diseases that are exacerbated by a red meat, Neu5Gc-rich diet.

Z Biotech's Neu5Gc/Neu5Ac N-glycan Array represents a broad range of N-glycans found on cell surfaces that have incorporated Neu5Gc. These arrays can be investigated with glycan-binding proteins, antibodies, or cells in order to determine their specific interaction with these Neu5Gc xenoantigens. For comparison, each array includes the precursory, non-xenogenic Neu5Ac sialic acid form of each glycan.

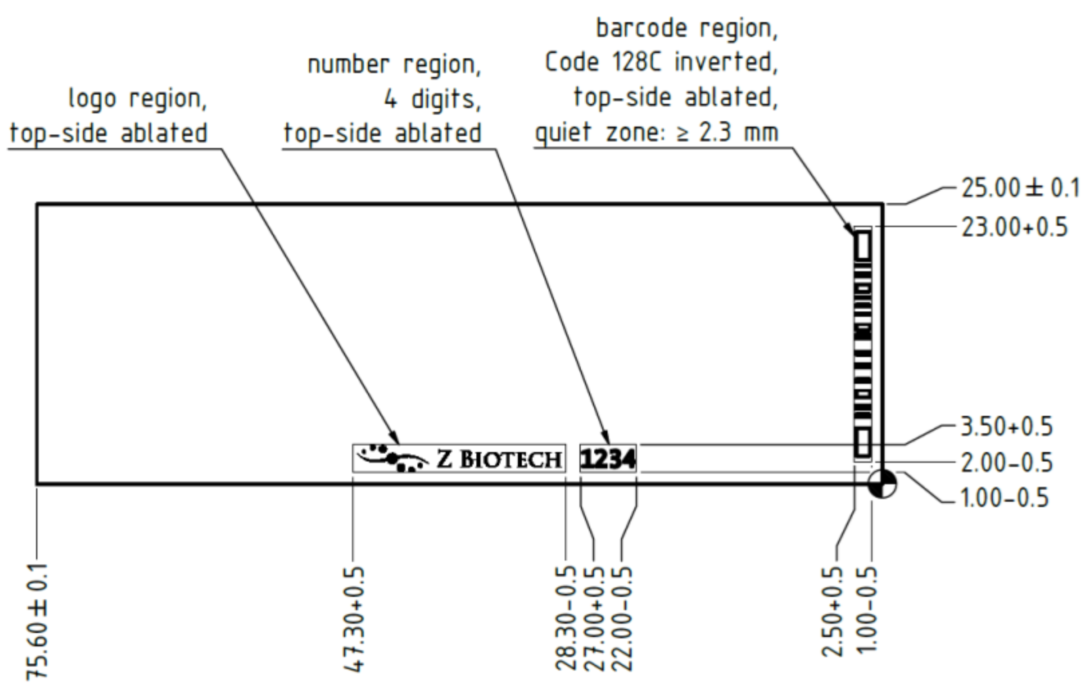
Handling and Storage

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.

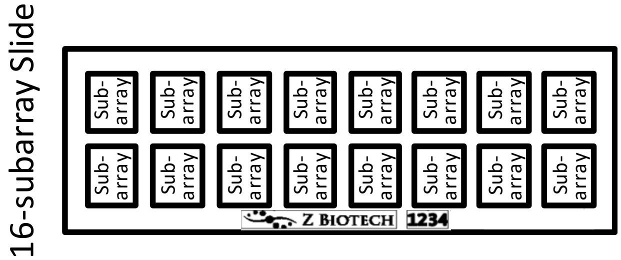
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

Neu5Gc/Neu5Ac N-glycan Array 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.



Array Map (16-sample):



GC-1	GC-1	GC-1	GC-2	GC-2	GC-2	GC-3	GC-3	GC-3	GC-4	GC-4	GC-4	GC-5	GC-5	GC-5	NC1
GC-6	GC-6	GC-6	GC-7	GC-7	GC-7	GC-8	GC-8	GC-8	GC-9	GC-9	GC-9	GC-10	GC-10	GC-10	NC1
GC-11	GC-11	GC-11	GC-12	GC-12	GC-12	GC-13	GC-13	GC-13	GC-14	GC-14	GC-14	GC-15	GC-15	GC-15	NC1
GC-16	GC-16	GC-16	GC-17	GC-17	GC-17	GC-18	GC-18	GC-18	GC-19	GC-19	GC-19	GC-20	GC-20	GC-20	PC1
GC-21	GC-21	GC-21	GC-22	GC-22	GC-22	GC-23	GC-23	GC-23	GC-24	GC-24	GC-24	GC-25	GC-25	GC-25	PC1
GC-26	GC-26	GC-26	GC-27	GC-27	GC-27	GC-28	GC-28	GC-28	GC-29	GC-29	GC-29	GC-30	GC-30	GC-30	PC1
GC-31	GC-31	GC-31	GC-32	GC-32	GC-32	GC-33	GC-33	GC-33	GC-34	GC-34	GC-34	GC-35	GC-35	GC-35	PC2
GC-36	GC-36	GC-36	GC-37	GC-37	GC-37	GC-38	GC-38	GC-38	GC-39	GC-39	GC-39	GC-40	GC-40	GC-40	PC2
AC-1	AC-1	AC-1	AC-2	AC-2	AC-2	AC-3	AC-3	AC-3	AC-4	AC-4	AC-4	AC-5	AC-5	AC-5	PC2
AC-6	AC-6	AC-6	AC-7	AC-7	AC-7	AC-8	AC-8	AC-8	AC-9	AC-9	AC-9	AC-10	AC-10	AC-10	PC3
AC-11	AC-11	AC-11	AC-12	AC-12	AC-12	AC-13	AC-13	AC-13	AC-14	AC-14	AC-14	AC-15	AC-15	AC-15	PC3
AC-16	AC-16	AC-16	AC-17	AC-17	AC-17	AC-18	AC-18	AC-18	AC-19	AC-19	AC-19	AC-20	AC-20	AC-20	PC3
AC-21	AC-21	AC-21	AC-22	AC-22	AC-22	AC-23	AC-23	AC-23	AC-24	AC-24	AC-24	AC-25	AC-25	AC-25	PC4
AC-26	AC-26	AC-26	AC-27	AC-27	AC-27				AC-29	AC-29	AC-29	AC-30	AC-30	AC-30	PC4
AC-31	AC-31	AC-31	AC-32	AC-32	AC-32	AC-33	AC-33	AC-33	AC-34	AC-34	AC-34	AC-35	AC-35	AC-35	PC4
AC-36	AC-36	AC-36							AC-39	AC-39	AC-39	GC-41	GC-41	GC-41	Marker

Array Map (8-sample):

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	GC-1	GC-1	GC-1	GC-1	GC-1	GC-1	GC-2	GC-2	GC-2	GC-2	GC-2	GC-2	GC-3	GC-3	GC-3	GC-3	GC-3	GC-3
2	GC-4	GC-4	GC-4	GC-4	GC-4	GC-4	GC-5	GC-5	GC-5	GC-5	GC-5	GC-5	GC-6	GC-6	GC-6	GC-6	GC-6	GC-6
3	GC-7	GC-7	GC-7	GC-7	GC-7	GC-7	GC-8	GC-8	GC-8	GC-8	GC-8	GC-8	GC-9	GC-9	GC-9	GC-9	GC-9	GC-9
4	GC-10	GC-10	GC-10	GC-10	GC-10	GC-10	GC-11	GC-11	GC-11	GC-11	GC-11	GC-11	GC-12	GC-12	GC-12	GC-12	GC-12	GC-12
5	GC-13	GC-13	GC-13	GC-13	GC-13	GC-13	GC-14	GC-14	GC-14	GC-14	GC-14	GC-14	GC-15	GC-15	GC-15	GC-15	GC-15	GC-15
6	GC-16	GC-16	GC-16	GC-16	GC-16	GC-16	GC-17	GC-17	GC-17	GC-17	GC-17	GC-17	GC-18	GC-18	GC-18	GC-18	GC-18	GC-18
7	GC-19	GC-19	GC-19	GC-19	GC-19	GC-19	GC-20	GC-20	GC-20	GC-20	GC-20	GC-20	GC-21	GC-21	GC-21	GC-21	GC-21	GC-21
8	GC-22	GC-22	GC-22	GC-22	GC-22	GC-22	GC-23	GC-23	GC-23	GC-23	GC-23	GC-23	GC-24	GC-24	GC-24	GC-24	GC-24	GC-24
9	GC-25	GC-25	GC-25	GC-25	GC-25	GC-25	GC-26	GC-26	GC-26	GC-26	GC-26	GC-26	GC-27	GC-27	GC-27	GC-27	GC-27	GC-27
10	GC-28	GC-28	GC-28	GC-28	GC-28	GC-28	GC-29	GC-29	GC-29	GC-29	GC-29	GC-29	GC-30	GC-30	GC-30	GC-30	GC-30	GC-30
11	GC-31	GC-31	GC-31	GC-31	GC-31	GC-31	GC-32	GC-32	GC-32	GC-32	GC-32	GC-32	GC-33	GC-33	GC-33	GC-33	GC-33	GC-33
12	GC-34	GC-34	GC-34	GC-34	GC-34	GC-34	GC-35	GC-35	GC-35	GC-35	GC-35	GC-35	GC-36	GC-36	GC-36	GC-36	GC-36	GC-36
13	GC-37	GC-37	GC-37	GC-37	GC-37	GC-37	GC-38	GC-38	GC-38	GC-38	GC-38	GC-38	GC-39	GC-39	GC-39	GC-39	GC-39	GC-39
14	GC-40	GC-40	GC-40	GC-40	GC-40	GC-40	GC-41	GC-41	GC-41	GC-41	GC-41	GC-41						
15	NC1	NC1	NC1	NC1	NC1	NC1	PC1	PC1	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC2	PC2

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	AC-1	AC-1	AC-1	AC-1	AC-1	AC-1	AC-2	AC-2	AC-2	AC-2	AC-2	AC-2	AC-3	AC-3	AC-3	AC-3	AC-3	AC-3
2	AC-4	AC-4	AC-4	AC-4	AC-4	AC-4	AC-5	AC-5	AC-5	AC-5	AC-5	AC-5	AC-6	AC-6	AC-6	AC-6	AC-6	AC-6
3	AC-7	AC-7	AC-7	AC-7	AC-7	AC-7	AC-8	AC-8	AC-8	AC-8	AC-8	AC-8	AC-9	AC-9	AC-9	AC-9	AC-9	AC-9
4	AC-10	AC-10	AC-10	AC-10	AC-10	AC-10	AC-11	AC-11	AC-11	AC-11	AC-11	AC-11	AC-12	AC-12	AC-12	AC-12	AC-12	AC-12
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10							AC-29	AC-29	AC-29	AC-29	AC-29	AC-29	AC-30	AC-30	AC-30	AC-30	AC-30	AC-30
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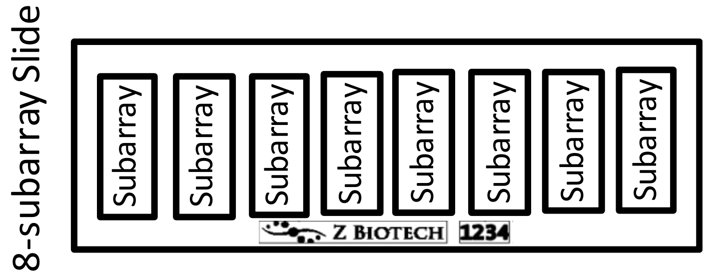
12	AC-34	AC-34	AC-34	AC-34	AC-34	AC-34	AC-35	AC-35	AC-35	AC-35	AC-35	AC-35	AC-36	AC-36	AC-36	AC-36	AC-36	AC-36
13													AC-39	AC-39	AC-39	AC-39	AC-39	AC-39
14																		
15	PC3	PC3	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	PC4	PC4					Marker	Marker



Controls

NC1: Negative control 1 – Print buffer

PC1: Postitive control 1 – Biotinylated mannose (0.01 mg/mL)

PC2: Positive control 2 - Human IgG (0.1 mg/ml)

PC3: Positive control 3 - Mouse IgG (0.1 mg/ml)

PC4: Positive control 4 - Rabbit IgG (0.1 mg/ml)

Marker: Anti-Human IgG, Cy3 (0.01 mg/ml) and anti-Human IgG, Alexa647 (0.01 mg/ml)

Neu5Gc/Neu5Ac N-Glycan Identification List:

Gc Glycan ID	Structure
GC-1	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-2	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-3	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-4	Manα1-6(Manα1-3)Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-5	Manα1-6(Manα1-3)Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-6	Manα1-6(Manα1-3)Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-7	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
GC-8	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
GC-9	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
GC-10	Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-11	Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-12	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
GC-13	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
GC-14	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
GC-15	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-16	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-17	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-18	GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-19	GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-20	GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-21	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-22	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-23	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-24	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-25	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-26	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-27	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-28	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-29	Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-30	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-31	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-32	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-33	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-34	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-35	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-36	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-37	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-38	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-39	Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-40	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
GC-41	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-

Ac Glycan ID	Structure
AC-1	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-2	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-3	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-4	Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-5	Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-6	Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-7	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
AC-8	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
AC-9	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-
AC-10	Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-11	Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-12	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
AC-13	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
AC-14	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-
AC-15	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-16	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-17	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-18	GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-19	GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-20	GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-21	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-22	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-23	Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-24	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-25	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-26	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-27	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-29	Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-30	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-31	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-32	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-33	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-34	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-35	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-36	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
AC-39	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-

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
- Wash Buffer: 50 mM Tris-HCl, 137 mM NaCl, 0.05% Tween 20, pH 7.6
- 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 samples or detection antibodies in a centrifuge tube by diluting with the GAAB buffer. For the fluorescently labelled streptavidin we recommend a concentration of 1 µg/mL. For detection antibodies, we suggest a concentration around 10-1 µg/ml. A range of 100 µg/ml to 0.1 µg/ml concentration for glycan-binding samples works, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This can be accomplished by applying a different dilution of samples to different wells of the array. In addition to testing a dilution range for your sample of interest, we recommend setting up control assays for any additional detection or secondary antibodies to ensure that any binding observed is specific to your sample of interest. A fluorescent signal due to specific binding to your sample of interest should be dose-dependent within the dynamic range of your sample dilution, and should have a positive binding signal after a signal from control assays has been subtracted. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. Use 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. 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 subarrays. 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

Part 1 – Blocking

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

1. Allow the arrayed slides to equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Add blocking buffer to each subarray well. We recommend using 100 µL 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.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on shaker at 85 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Make sure the orbital shaker is completely flat. If the slide is sloped in any direction during incubation it can cause variation in binding.

Part 2 – Binding assay

1. Unless the glycan-binding sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner and remove the blocking buffer. Avoid touching the array surface.
3. Immediately apply the glycan-binding sample of interest to each well. We recommend using 100 µL 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. Avoid leaving air bubbles.
4. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labelled, cover with aluminum foil to keep it in the dark. Incubate on the shaker for 1-3 hours at 100 rpm. If the samples can easily aggregate, shake at higher speed to prevent aggregation. Longer incubation time may increase 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.

If your glycan-binding samples are fluorescently labelled, go directly to Part 6 – Final wash and dry.

Part 3 – Wash

1. Remove glycan-binding samples from each well by gently touching the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid touching the array surface, but a gentle touch is okay to ensure no sample is left pooled in the corners.
2. Add wash buffer to each well. We recommend using 100 μL per well for 16 subarray cassettes and 200 μL for 8 subarray cassettes. Cover the wells with adhesive film and incubate on the shaker for 5 minutes at 85 rpm. Completely remove the wash buffer by pipette and repeat this step. Avoid allowing the slide to dry out and have your next wash or sample ready before you remove the wash buffer.

If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent staining.

Part 4 – Binding of biotinylated antibody (Sandwich Assay Format)

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add the biotinylated antibody to each well. We recommend using 100 μL per well for 16 subarray cassettes and 200 μL for 8 subarray cassettes. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 100 rpm. Longer incubation time is acceptable, but not necessary.
3. After incubation repeat Part 3 – Wash

Part 5 – Fluorescent staining

1. Centrifuge fluorescent-labeled streptavidin samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add the fluorescently labelled streptavidin sample. 100 μL per well is recommended for 16 subarray cassettes and 200 μL for 8 subarray cassettes. Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 85 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Part 6 – Final wash and dry

1. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner and remove it. Avoid touching the array surface.
2. Briefly rinse each well with wash buffer. 100 μL per well is recommended for 16 subarray cassettes and 200 μL for 8 subarray cassettes.
3. Completely remove the wash buffer by pipette. Avoid touching the array surface. Repeat steps 2 and 3.
4. Disassemble the cassette 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. Immediately immerse the slide in a coplin jar or beaker full of wash buffer. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar.
6. Place the jar or beaker on the 60 rpm shaker for 10 minutes.
7. Decant the wash buffer from the jar or beaker while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide.
8. Place the jar or beaker on the 60 rpm shaker for 2 minutes.
9. Decant the water from the jar or beaker.
10. Allow the slide to dry completely in a clean, dust free environment before scanning.

Analysis

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 any being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there is specific binding the signal intensity should be higher than the background signal (area where there are no printed spots). Fluorescent signal due to specific binding to your sample of interest should be both dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan binding is saturated), and should have positive binding signal after signal from control assays has been subtracted. Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signal 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.

Interpretation of Control Signals:

<u>Negative Control (Print Buffer):</u> 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. Subtracting the negative control's signal from the other binding signals will give more accurate specific binding data.

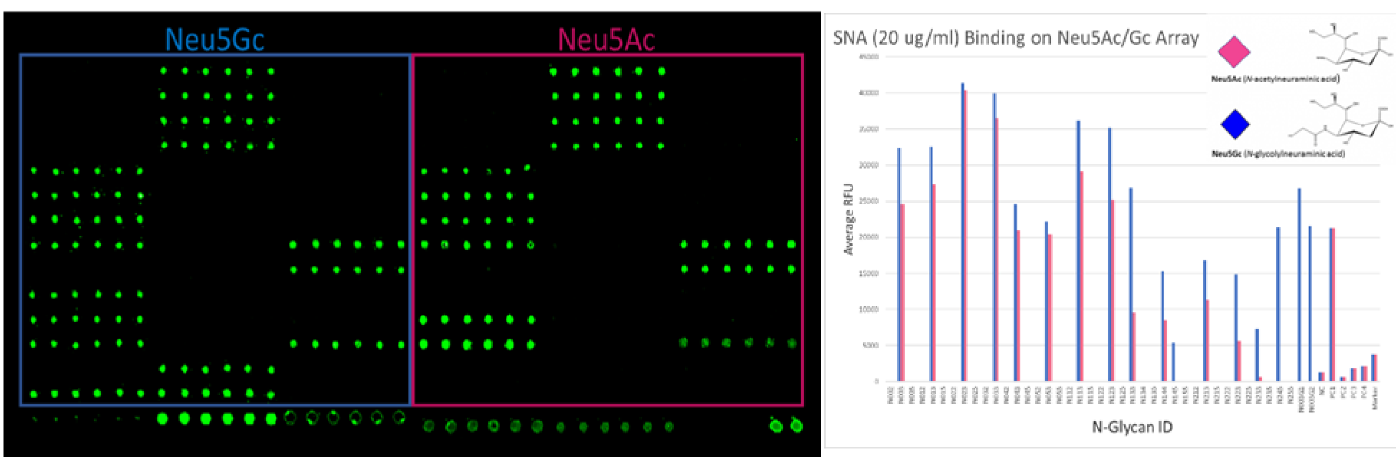
<u>Positive Control 1 (biotinylated mannose):</u> This positive control will bind directly to the fluorescent labelled streptavidin. If your glycoprotein sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 – Fluorescent staining) this positive control will not be reactive.

<u>IgG (PC2, PC3, PC4):</u> IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control.

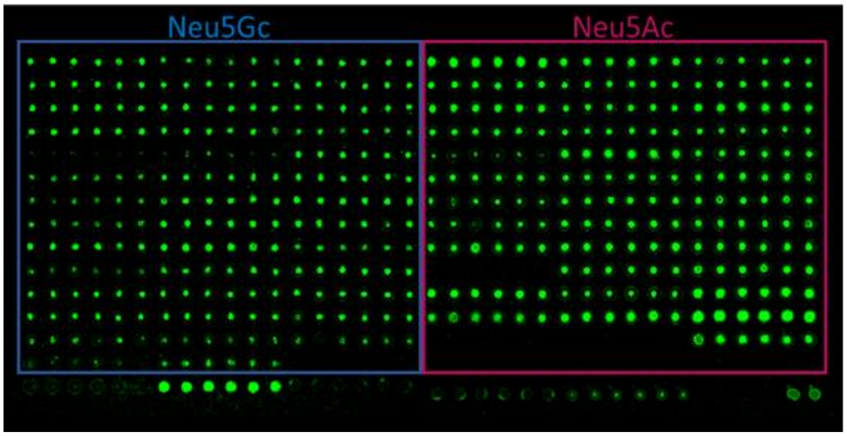
<u>Marker:</u> The array marker should show fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

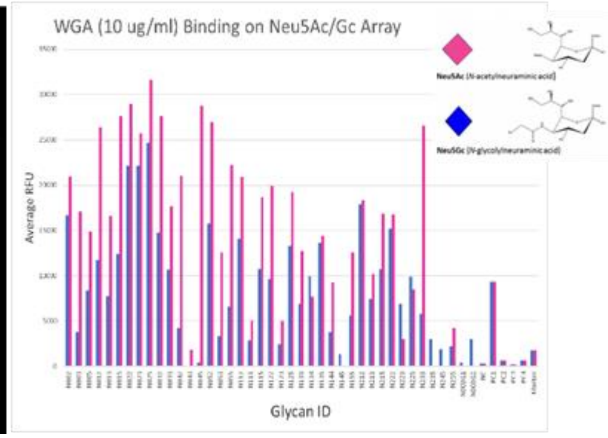
Typical Binding Assay Results from the Neu5Gc/Neu5Ac N-Glycan Array

Example 1: Neu5Gc/Neu5Ac array on 8 subarray formats. A subarray assayed with a biotinylated SNA target (20 µg/ml), followed by streptavidin-Cy3 (1 µg/ml). The array was scanned with GenePix scanner at 500 PMT and 100% laser power at 532 nm wavelength. The positive control shows binding as expected. N-glycans containing α-2,6 Neu5Gc and α-2,6 Neu5Ac show binding as expected. Analysis of the fluorescence intensity reveals that Neu5Gc-sialylated glycans bind more strongly.



Example 2: Neu5Gc/Neu5Ac array on 8 subarray formats. A subarray assayed with a biotinylated WGA target (10 µg/ml), followed by streptavidin-Cy3 (1 µg/ml). The array was scanned with GenePix scanner at 450 PMT and 100% laser power at 532 nm wavelength. The positive control shows binding as expected. Most N-glycans show binding as expected. Analysis of the fluorescence intensity reveals that Neu5Ac-sialylated glycans bind more strongly.





Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of protein samples 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