



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO[®] ASIALO, GALACTOSYLATED, BIANTENNARY COMPLEX N-GLYCAN, CORE-SUBSTITUTED WITH FUCOSE AND BISECTING GlcNAc (NA2FB)

PRODUCT CODE: GKC-024311

LOT NUMBER: DP14C2103a

PACK SIZE: 10 µg (qualitative standard for glycan identification)

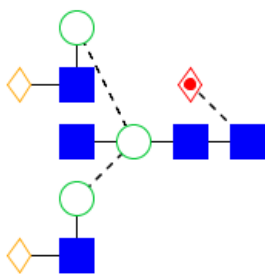
PURITY: ≥90% of glycan by UPLC[®]

FORM: Dry solid

STORAGE: Store at -20°C before and after reconstitution

EXPIRATION: August 2020, may be used for 1 year after reconstitution

STRUCTURE^{1,2,3} :



Structure Key:

Monosaccharide symbol:	Linkage position:	Linkage type:
□ Glucose		— β-linkage
◇ Galactose		- - - α-linkage
○ Mannose		~ Unspecified β-linkage
◇ Fucose		- · - Unspecified α-linkage
△ Xylose		
■ N-Acetylglucosamine (GlcNAc)		
◆ N-Acetylgalactosamine (GalNAc)		
★ N-Acetylneuraminic acid (Neu5Ac or NANA)		
☆ N-Glycolyneuraminic acid (Neu5Gc or NGNA)		

Quality Control:

Sample Preparation: NA2FB was labeled with 2-aminobenzamide (2-AB) by reductive amination⁴ using the Signal™ 2-AB Labeling Kit (product code GKK-404).

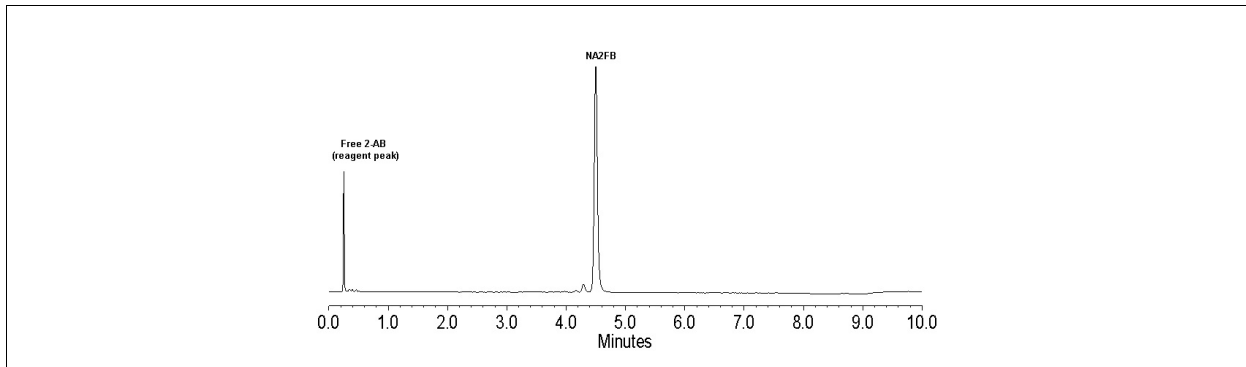


Figure 1 - UPLC® Results: 3 - 6 pmol (1 µl, aqueous) of the 2-AB-labeled glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

Time (min)	Flow (ml/min)	% ACN	% Buffer
0	1.0	75	25
8.0	1.0	60	40
8.1	0.5	40	60
8.5	0.5	40	60
8.6	1.0	40	60
8.8	1.0	75	25
10.0	1.0	75	25

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 mM ammonium formate, pH 4.4
Flow rate: As stated in table, in ml/min
Temperature: 60°C
Max Pressure: 15,000 psi
Fluorescence Detection: $\lambda_{ex} = 330 \text{ nm}$
 $\lambda_{em} = 420 \text{ nm}$

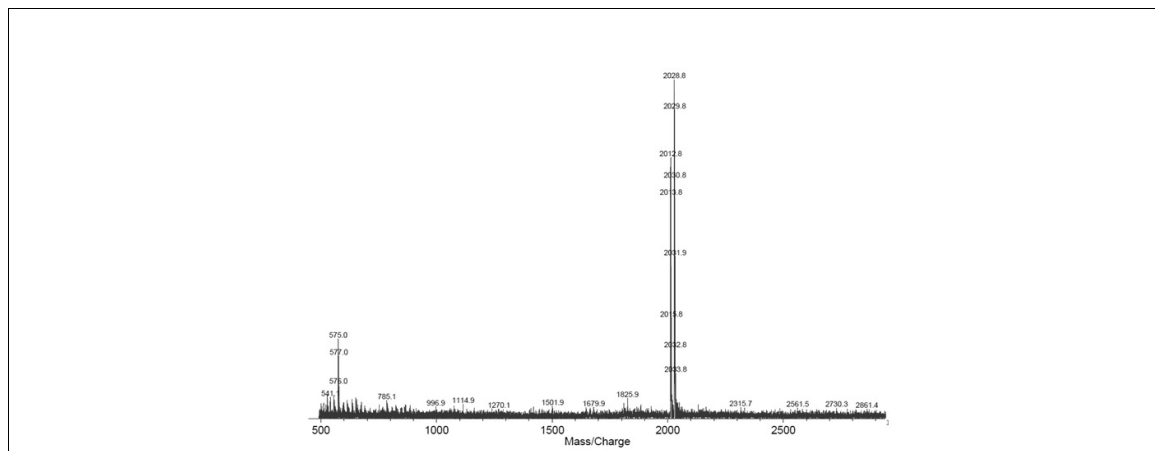


Figure 2 - Mass Spectrum of NA2FB [M + Na]⁺

Average Mass⁵: 1990.8

Monoisotopic Mass⁵: 1989.7295

Structural Analysis: The purity and structural integrity of the glycan was assessed by UPLC⁶ (as described above) and MALDI-TOF^{7,8} or LC-MS. Agreement was found between the results from mass spectrometry and UPLC.

Applications:

- Qualitative standard for various analytical procedures
- Fluorescent-labeling or formation of a variety of oligosaccharide derivatives
- Substrate for glycosidase and glycosyl transferase assays

Handling & Reconstitution: The labeled oligosaccharide is shipped as a dried solid. Use ultra-pure water or an aqueous buffer to dissolve the glycan.

Allow the unopened vial to reach ambient temperature and tap on a solid surface to ensure that most of the material is at the bottom of the vial. Gently remove the cap, add the desired volume of ultra-pure water or aqueous buffer, re-cap and mix thoroughly to redissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed. Centrifuge the reconstituted vial briefly before use.

Make sure that any glassware, plasticware, solvents or reagents used are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH.

Store the reconstituted glycan at -20°C. Allow the vial to equilibrate to ambient temperature before use.

REFERENCES

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2. Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA, Rudd PM. Proposal for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related compounds. *Proteomics* 2009 Aug;9(15):3796-801.
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4. Bigge JC, Patel T, Bruce JA, Goulding PN, Charles SM, Parekh RB. Nonselective and efficient fluorescent labeling of glycans using 2-amino benzamide and anthranilic acid. *Anal Biochem* 1995 Sep 20;230(2):229-238.
5. Average mass and monoisotopic mass were calculated using the ExpASY GlycanMass calculator:
<http://web.expasy.org/glycanmass/>
6. Ahn J, Bones J, Yu YQ, Rudd PM, Gilar M. Separation of 2-aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 microm sorbent. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010 Feb 1;878(3-4):403-8.
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8. Papac DI, Wong A and Jones AJS. Analysis of acidic oligosaccharides by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *Anal Chem* 1996 Sep 15;68(18):3215-3223.

Authorized Signature