

## CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® ASIALO-, GALACTOSYLATED, TRIANTENNARY COMPLEX N-GLYCAN (NA3)

PRODUCT CODE: GKC-035300

LOT NUMBER: DP11K0402a

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

PURITY: ≥90% of glycan by UPLC®

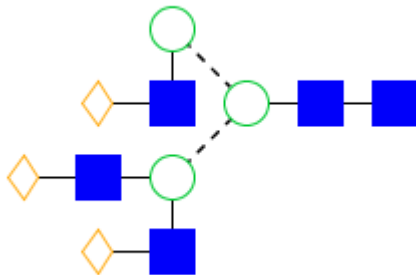
FORM: Dry solid

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

EXPIRATION: November 2021, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)

RE-ASSAY DATE: November 2016

STRUCTURE<sup>1,2,3</sup>:

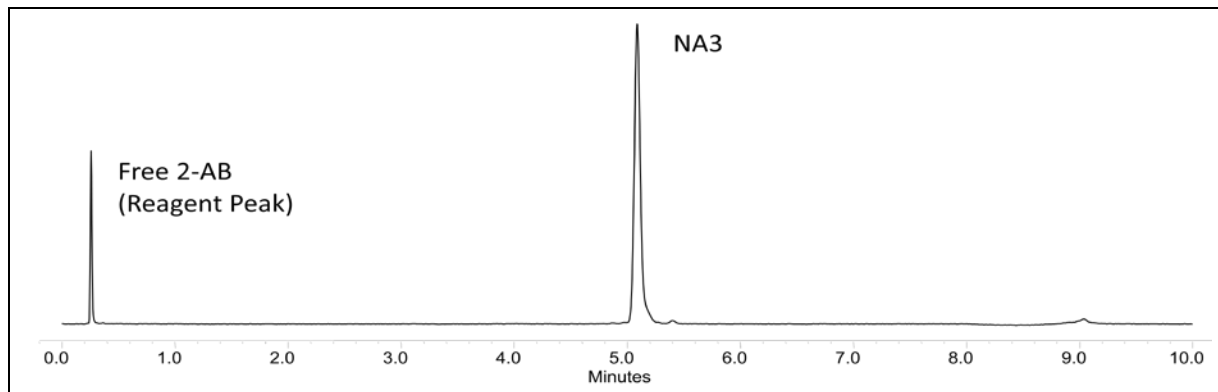


**Structure Key:**

<u>Monosaccharide symbol</u>	<u>Linkage position</u>	<u>Linkage type</u>
Galactose		
Mannose		
N-Acetylglucosamine (GlcNAc)		
		β-linkage
		α-linkage

## Quality Control:

**Sample Preparation:** NA3 was labeled with 2-aminobenzamide (2-AB) by reductive amination<sup>4</sup> using the Signal™ 2-AB Labeling Kit (product code GKK-404).



**Figure 1 - UPLC® Results:** 3 - 6 pmol (1  $\mu$ l, aqueous) of the 2-AB-labeled<sup>4</sup> glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

Time (min)	Flow	%ACN	%Buffer
00.0	1.0	75.0	25.0
8.0	1.0	60.0	40.0
8.1	0.5	40.0	60.0
8.5	0.5	40.0	60.0
8.6	1.0	40.0	60.0
8.8	1.0	75.0	25.0
10.0	1.0	75.0	25.0

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7  $\mu$ m, 2.1 x 100 mm)

ACN: Acetonitrile

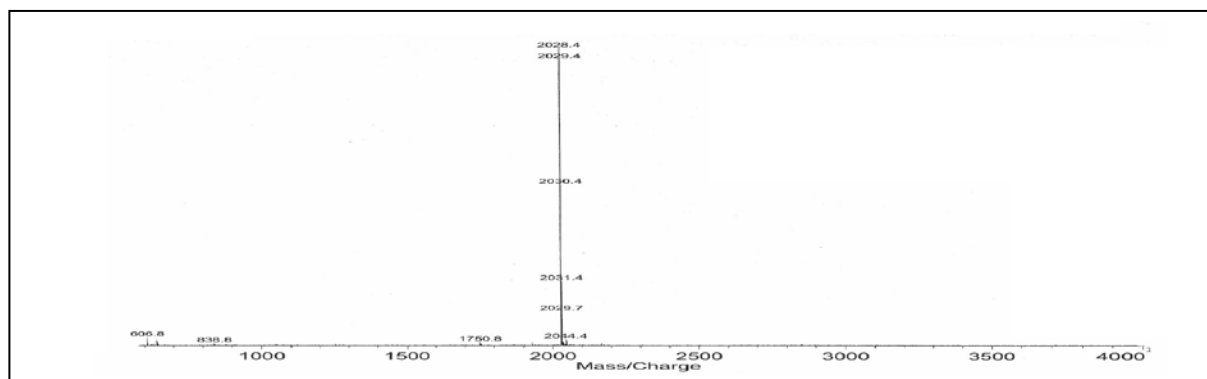
Buffer: 100 mM ammonium formate, pH 4.4

Flow rate: As stated in table, in ml/min

Temperature: 60° C

Max Pressure: 15,000 psi

Fluorescence Detection:  $\lambda_{ex}$  = 330 nm,  $\lambda_{em}$  = 420 nm



**Figure 2 - Mass Spectrum of NA3 [M + Na]<sup>+</sup>**

**Average Mass<sup>5</sup>:** 2006.8

**Monoisotopic Mass<sup>5</sup>:** 2005.7244

**Structural Analysis:** The purity and structural integrity of the glycan was assessed by UPLC<sup>6</sup> (as described above) and MALDI-TOF<sup>7,8</sup>, LC-MS. Agreement was found between the results from mass spectrometry and UPLC.

**Application:**

- 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 oligosaccharide is shipped as a dried solid. Use ultra-pure water or an aqueous buffer to dissolve the materials (see Directions for Use for suggested volumes). 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 material.

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**

1. Ceroni A, Maass K, Geyer H, Geyer R, Dell A, Haslam SM. GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res.* 2008 Apr; 7(4): 150-9.
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.
3. Harvey DJ, Merry AH, Royle L, Campbell MP, Rudd PM. Symbol nomenclature for representing glycan structures: Extension to cover different carbohydrate types. *Proteomics* 2011 Nov;11(22):4291-5.
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 of the glycan 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.
7. James DC, Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry; in *A laboratory guide to glycoconjugate analysis.* BioMethods (P. Jackson and J. T. Gallagher, ed) 1997; 9: 91-112.
8. Papac DI, Wong A, 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.

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Authorized Signature