

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® 2-AB-(NA2)

PRODUCT CODE: GKSB-304

GLYCAN NAME: Asialo, galactosylated biantennary (NA2)

LOT NUMBER: DP19C2202

PACK SIZE: 100 pmol (qualitative standard for glycan identification)

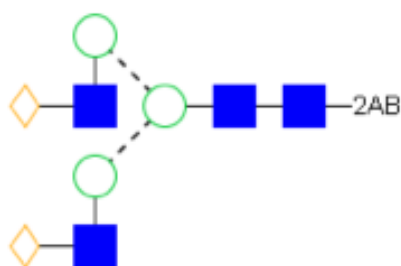
PURITY: ≥90% of glycan by UPLC®

FORM: Dry solid

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

EXPIRATION: May 2024, may be used for 1 year after reconstitution

STRUCTURE^{1,2,3}:



Structure Key:

Monosaccharide symbol	Linkage position	Linkage type
Orange diamond	Galactose	
Green circle	Mannose	
Blue square	N-Acetylglucosamine (GlcNAc)	
		——— β-linkage - - - α-linkage

Quality Control:

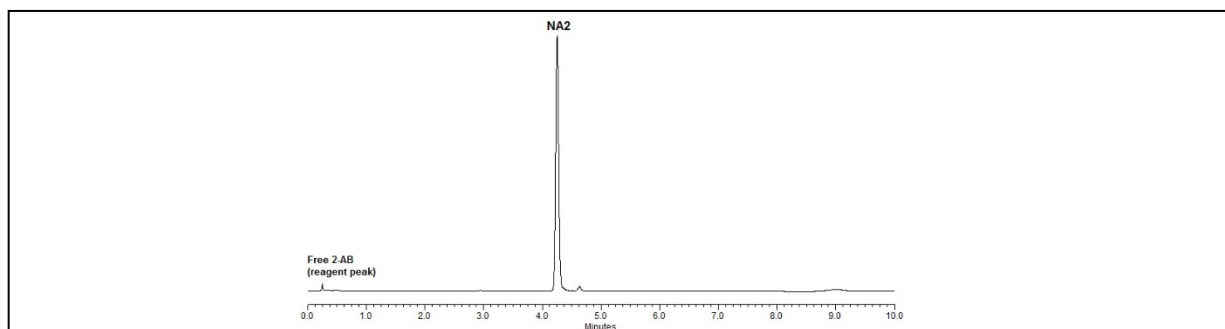


Figure 1 - UPLC® Results: 3 - 6 pmol (1 µl, aqueous) of the 2-AB-labeled⁴ glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

Time (min)	Flow	%ACN	%Buffer
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: λ_{ex} = 330 nm

λ_{em} = 420 nm

Average Mass⁵: 1761.7

Monoisotopic Mass⁵: 1760.6609

Structural Analysis: The identity of the unlabeled glycan is confirmed by MALDI-TOF^{6,7} or LC-MS. Agreement was found between the results from mass spectrometry and UPLC⁸.

Application:

- Qualitative standard for various analytical procedures
- As a migration standard for liquid chromatography

Handling & Reconstitution:

The labeled 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 volume).

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 water or buffer, re-cap and mix thoroughly to redissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed and 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.

Directions For Use: The amount of 2-AB-labeled glycan injected on a UPLC column is typically 3 - 6 pmol of total glycan. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY column. For larger injection volumes or other LC systems we recommend further dilution as necessary for compatibility with the mobile phase. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans (TNGP101) available at:

www.prozyme.com/tech_notes.html

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): 1650-9.
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 unlabeled glycan were calculated using the ExPASy GlycanMass calculator:

<http://web.expasy.org/glycanmass/>

The average mass of the 2-AB labeled glycan is obtained using the following formula:

$$\text{Average Mass}_{\text{Glycan}} + \text{Average Mass}_{2\text{-AB}} (136.2) - 16$$

The monoisotopic mass of the 2-AB labeled glycan is obtained using the following formula (result rounded to 4 decimal places):

$$\text{Monoisotopic Mass}_{\text{Glycan}} + 120.06875$$
6. 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.
7. 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.
8. 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.

Authorized Signature