



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO[®] INSTANTAB[™]-(NA2G1F)

PRODUCT CODE: GKIB-316

GLYCAN NAME: Asialo, monogalactosylated biantennary complex N-glycan, core substituted with fucose

LOT NUMBER: DP11J3003a

PACK SIZE: 60 pmol (qualitative standard for glycan identification)

PURITY: ≥90% of glycan by UPLC[®]

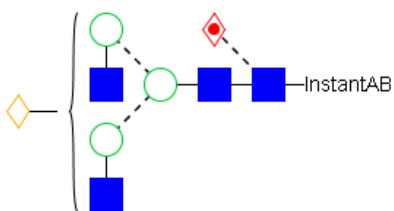
FORM: Dry solid

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

EXPIRATION: February 2023 (extended from prior exp. date based on re-assay), may be used for 1 year after reconstitution

RE-ASSAY DATE: February 2018

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-Glycolylneuraminic acid (Neu5Gc or NGNA)		

Quality Control:

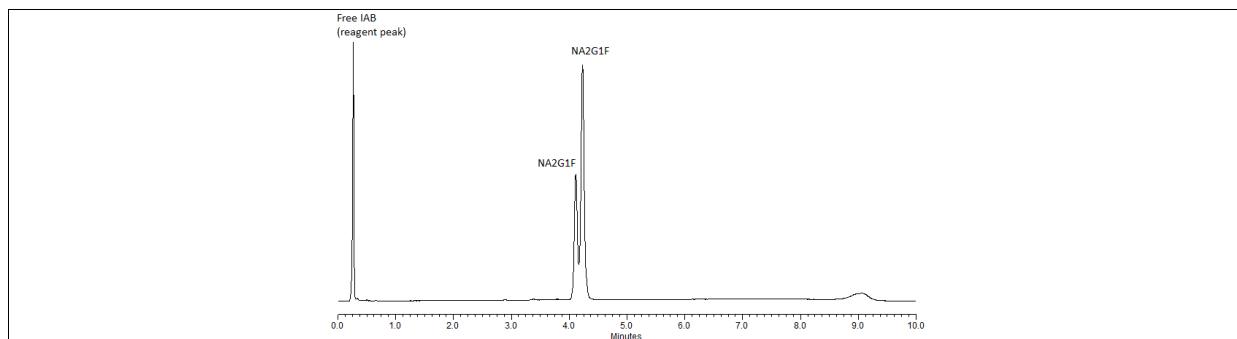


Figure 1 - UPLC® Results: 2 - 4 pmol (1 μ l, aqueous) of the IAB-labeled glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

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 μ 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: λ_{ex} = 278 nm
 λ_{em} = 344 nm

Average Mass⁴: 1786.6

Monoisotopic Mass⁴: 1785.6562

Structural Analysis: The identity of the unlabeled glycan is confirmed by MALDI-TOF^{5,6} or LC-MS. Agreement was found between the results from mass spectrometry and UPLC⁷.

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 glycan (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 InstantAB-labeled glycan injected on a UPLC column is typically 2 - 4 pmol of total glycan. For our Quality Control testing, one vial was dissolved in 30 μ l of water and 1 μ l 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. 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 IAB labeled glycan is obtained using the following formula:
$$\text{Average Mass}_{\text{Glycan}} + \text{Average Mass}_{\text{IAB}} (161.1)$$

The monoisotopic mass of the IAB labeled glycan is obtained using the following formula (result rounded to 4 decimal places):
$$\text{Monoisotopic Mass}_{\text{Glycan}} + 161.05891$$
5. James DC and Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry. In: Jackson P, Gallagher JT, editors. A laboratory guide to glycoconjugate analysis, BioMethods Vol. 9. Basel: Birkhäuser; 1997. p. 91-112.
6. 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.
7. 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