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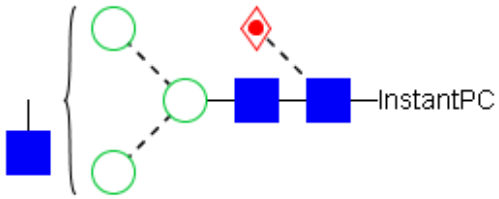
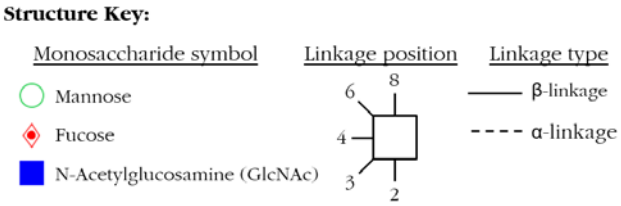
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® INSTANTPC™ NGA2F-N (G0F-N)
PRODUCT CODE:	GKPC-402
GLYCAN NAME:	Asialo-, agalacto-, biantennary complex N-Glycan with core fucose, -1 N-Acetylglucosamine (NGA2F-N)
LOT NUMBER:	DP17I1103a
PACK SIZE:	~25 injections (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:	May 2020 (extended from prior exp. date based on re-assay) May be used for 6 months after reconstitution in 100 m*M* ammonium formate, pH 4.4 – 5.0, or for 1 month after reconstitution in water.
RE-ASSAY DATE:	February 2019
STRUCTURE[1,2,3]:	The glycosylamine form of the glycan is labeled with the fluorescent dye, InstantPC.





3832 Bay Center Place
Hayward, CA 94545-3619

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

info@prozyme.com
www.prozyme.com

Quality Control:

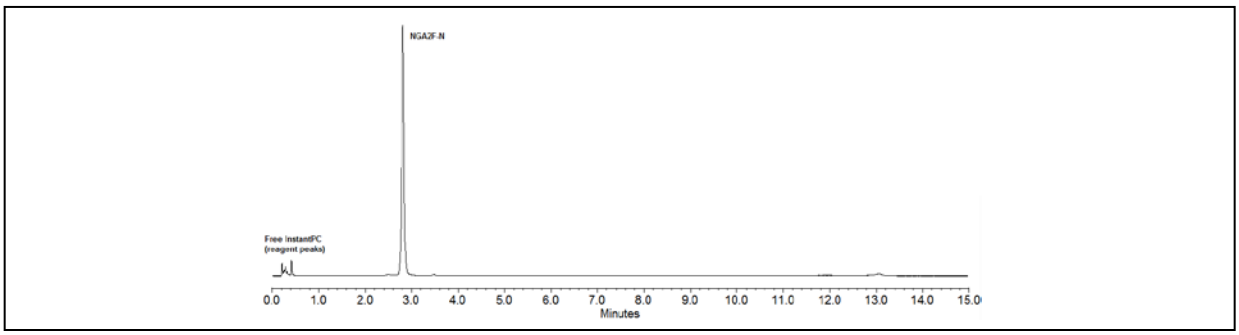


Figure 1 - **UPLC® Results**: 1 µl, aqueous of the InstantPC-labeled glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below:

Time (min)	Flow	%ACN	%Buffer
00.0	1.0	75.0	25.0
12.0	1.0	50.0	50.0
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
13.0	1.0	75.0	25.0
15.0	1.0	75.0	25.0

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 m*M* 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} = 285 nm, λ_{em} = 345 nm

Average Mass[4]: 1521.5

Monoisotopic Mass[4]: 1520.6128

Structural Analysis: The identity of the glycan is confirmed by MALDI-TOF[5,6] or LC-MS. Agreement was found between the results from mass spectrometry and UPLC[7].

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 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.

Directions For Use: The amount of InstantPC-labeled glycan standard injected on a UPLC column is typically 1 µl. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY BEH Glycan column

We suggest reconstituting with 100 m*M* ammonium formate, pH 4.4 – 5.0 for storage at -20° C for up to 6 months. This buffer is often used as a HILIC mobile phase. Water may also be used for reconstitution, but the recommended storage period is shorter, -20° C for up to 1 month.

For larger injection volumes of InstantPC-labeled glycans (> 1 µl), do not use ACN alone to dilute the glycan to match the high organic % at the start of HILIC methods, as this may cause sialylated InstantPC glycans to precipitate. Use 1 part glycan in ammonium formate or water to 3 parts 1:1 [v/v] ACN:DMF, for a final concentration of 25% aqueous buffer, 37.5 % DMF, 37.5% ACN. Dilute only as much as is needed, and freeze the main stock at -20° C. For example, for a 10 µl injection, dilute 5 µl of glycan stock in ammonium formate or water with 15 µl 1:1 [v/v] ACN:DMF.

For further information on LC and LC-MS methods for InstantPC-labeled glycans, please contact ProZyme:

info@prozyme.com

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 (free reducing end form) were calculated using the ExPASy GlycanMass calculator:

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

   Calculating the Mass of Glycans Labeled with InstantPC.

   Mass added to glycan with a free reducing end:
   $Mass_{Glycan\ (free\ reducing\ end)} + C_{14}N_3O_2H_{19} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

   Mass added by $C_{14}N_3O_2H_{19}$ (Da):
   Monoisotopic: 261.14773
   Average: 261.3

   Mass added to glycosylamine:
   $Mass_{Glycan\ (glycosylamine)} + C_{14}N_2O_3H_{18} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

   Mass added by $C_{14}N_2O_3H_{18}$ (Da):
   Monoisotopic: 262.13174
   Average: 262.3

5. 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.

6. 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.

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.

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