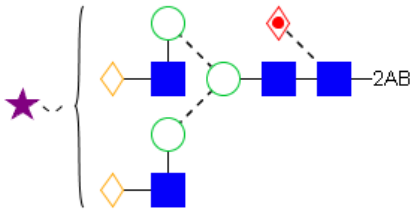
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® 2-AB-(A1F)
PRODUCT CODE:	GKSB-315
GLYCAN NAME:	Mono-sialylated, galactosylated biantennary, core-substituted with fucose (A1F)
LOT NUMBER:	DP17H1101
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:	September 2022, may be used for 1 year after reconstitution

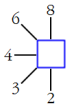
STRUCTURE[1,2,3] :



Structure Key:

Monosaccharide symbol:
- Glucose
- Galactose
- Mannose
- Fucose
- Xylose
- N-Acetylglucosamine (GlcNAc)
- N-Acetylgalactosamine (GalNAc)
- N-Acetylneuraminic acid (Neu5Ac or NANA)
- N-Glycolyneuraminic acid (Neu5Gc or NGNA)

Linkage position:



Linkage type:
- β-linkage
- α-linkage
- Unspecified β-linkage
- Unspecified α-linkage

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Quality Control:

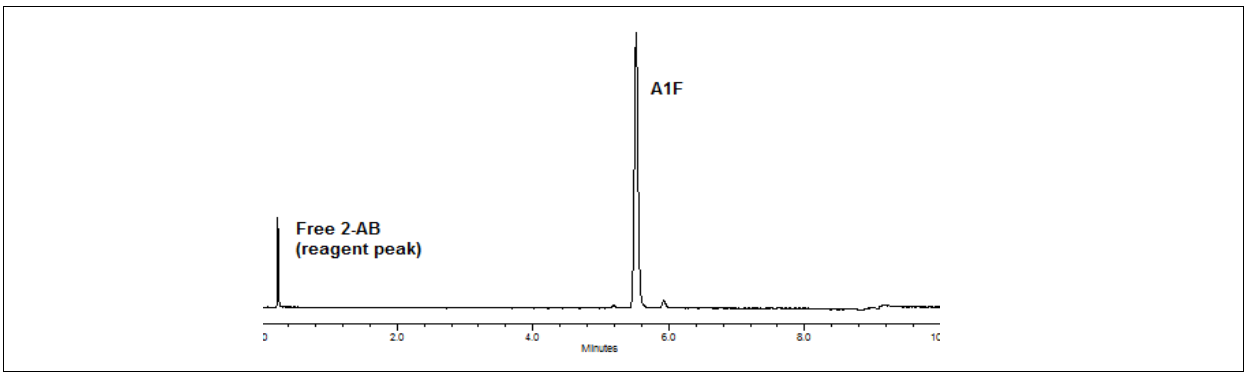

Figure 1 - UPLC® Results: 3 - 6 pmol (1 µl) of the 2-AB-labeled[4] glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

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 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} = 330 nm
λ_{em} = 420 nm

Average Mass[5]: 2199.1

Monoisotopic Mass[5]: 2197.8142

Structural Analysis: The identity of the unlabeled glycan is confirmed by MALDI-TOF[6,7], ESI-MS or LC-MS. Agreement was found between the results from mass spectrometry and UPLC[8].

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 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

LICENSE TO USE

Purchase of the Signal™ 2-AB Labeling Kit or 2-AB-labeled standards from ProZyme or its authorized distributors grants a Use Sublicense under Glyko's 2-AB patents. By accepting delivery of the 2-AB Kit or labeled standards [Material(s)] and by subsequently using them in glycan analysis, Recipient agrees to be bound by the following terms and restrictions:

1. A Use Sublicense is granted Recipient for in-house use of Material(s) only.

2. The Material(s) will not be made available by Recipient to any outside parties in any form, separately or in combinations, for any monetary or other consideration or at no charge, except that the Materials may be made available to outside parties who agree to be bound by all the terms and restrictions of this Agreement for purposes of evaluation only.

3. Recipient will not make commercial use of the Material(s) unless it first secures a license agreement from ProZyme, Inc. for such commercial use.

4. Recipient is solely responsible for qualification of the products for the Recipient's specific use.

5. The Material(s) will not be used *in vivo* in humans.

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:

   $\text{Average Mass}_{\text{Glycan}} + \text{Average Mass}_{\text{2-AB}}$ (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 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.

7. 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.

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.

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