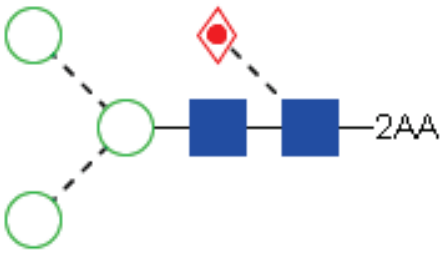
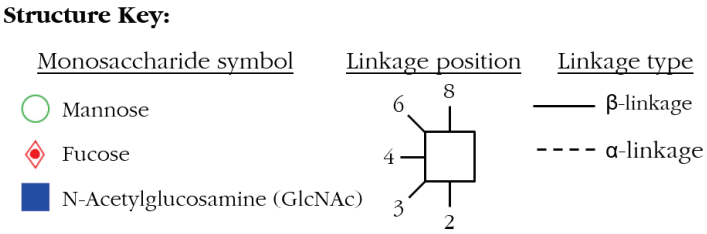
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio 2-AA Man3F / FM3 N-Glycan Standard
PRODUCT CODE:	GKSA-102
GLYCAN NAME:	Conserved tri-mannosyl core with core fucose (M3N2F)
LOT NUMBER:	DP12G2501
PACK SIZE:	100 pmol (qualitative standard for glycan identification)
PURITY:	≥90% of glycan by UHPLC
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	July 2027, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	July 2022
STRUCTURE[1,2,3]:	The reducing terminus of the glycan is derivatized with the fluorescent dye, 2-aminobenzoic acid (2-AA).





Quality Control:

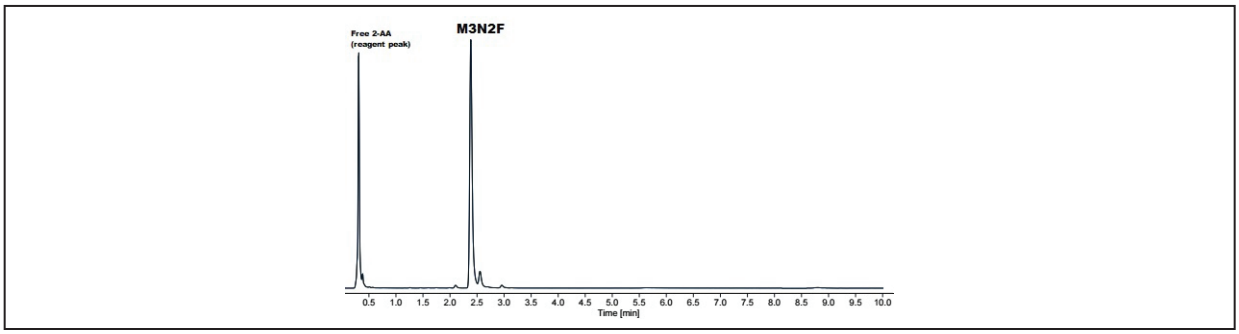


Figure 1 - UHPLC Results: 3 - 6 pmol (1 µl, aqueous) of the 2-AA-labeled[4] glycan was injected on a UHPLC System (Agilent 1290 Infinity II) utilizing a 10-minute method under the conditions below:

Time (min)	Flow	%ACN	%Buffer
00.0	1.0	75.0	25.0
8.0	1.0	52.5	47.5
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 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} = 352 nm, λ_{em} = 435 nm

Average Mass[5]: 1178.1

Monoisotopic Mass[5]: 1177.4384

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 UHPLC[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 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 2-AA-labeled glycan standard injected on a UHPLC 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 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 at:

www.prozyme.com/protocols/

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-AA labeled glycan is obtained using the following formula:

   $\text{Average Mass}_{\text{Glycan}} + \text{Average Mass}_{\text{2-AA}} (137.1) - 16$

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

   $\text{Monoisotopic Mass}_{\text{Glycan}} + 121.05276$

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.

Christopher J. Miller Digitally signed by Christopher J. Miller Date: 2022.07.20 15:31:55 -07'00'

Authorized Signature