CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio 2-AA Bovine Fetuin N-Glycan Library
PRODUCT CODE:	GKSA-002
LOT NUMBER:	DP15J3001
PACK SIZE:	200 pmol (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at –20 °C in the dark before and after reconstitution
EXPIRATION:	July 2025, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	July 2020
STRUCTURE:	The Bovine Fetuin N-Glycan Library is composed of a mixture of sialylated N-linked glycans (core non-fucosylated), consistent with N-glycans previously reported for bovine fetuin.[1,2] The biantennary glycans can have one or two of the sialic acid residues (NeuAc); the triantennary glycans can have from one to four of the sialic acid residues. The sialic acid residues are found in both α(2,3) and α(2,6) linkages. Triantennary glycans constitute ~85% of total glycans present. A small amount of penta-sialylated glycans can be detected, but their structure has not been determined. The reducing termini are derivatized with the fluorescent dye 2-AA (2-aminobenzoic acid).

LABORATORY REAGENT

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Quality Control:

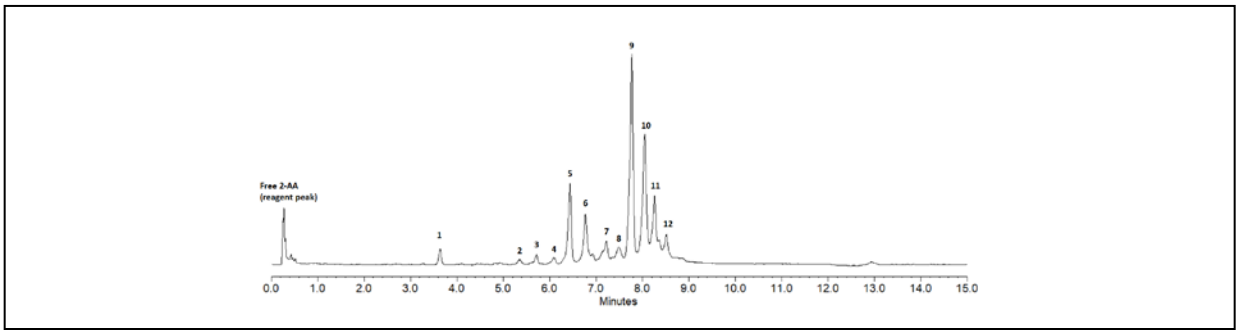


Figure 1 - **UPLC Results**: 6–9 pmol (1 µL, aqueous) of the 2-AA-labeled[3] glycan library was injected on a Waters ACQUITY UPLC H Class System utilizing a 15-minute method under the conditions below:

Time (min)	Flow (mL/min)	%ACN	%Buffer
00.0	1.0	75.0	25.0
12.0	1.0	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	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 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} = 352 nm, λ_{em} = 435 nm

Table 1 - Peak Identification of 2-AA Labeled Bovine Fetuin N-Linked Glycan Library

Peak	Glycan Identification
1	Oligomannose 5 (Man-5)
2	Mono-sialylated-, galactosylated biantennary [A1 (2,3) / A2G2S1]
3	Mono-sialylated-, galactosylated biantennary [A1 (2,6) / A2G2S1]
4	Di-sialylated-, galactosylated biantennary [A2 (2,3) / A2G2S2], S1*
5	Di-sialylated-, galactosylated biantennary [A2 (2,3/2,6) / A2G2S2], S1* (trace amount)
6	Di-sialylated-, galactosylated biantennary [A2 (2,6/2,6) / A2G2S2], S2* and S3* (shoulder)
7	S2*, S3*

Peak	Glycan Identification
8	Tri-sialylated, galactosylated triantennary [A3 (2,3) / A3G3S3] ,A3G3S2 (trace amount)
9	Tri-sialylated, galactosylated triantennary [A3 (2,3/2,3/2,6) / A3G3S3], A3G3S2 (trace amount)
10	Tri-sialylated, galactosylated triantennary [A3 (2,3/2,6/2,6) / A3G3S3], A3G3S4 (trace amount)
11	A3G3S4, Tri-sialylated, galactosylated triantennary [A3 (2,6/2,6/2,6) / A3G3S3]
12	A3G3S4, S3* and S5* (trace amount)

*S1-S5 structures denote the number of sialic acids; these structures have not been further characterized

Structural Analysis: UPLC[4,5] analysis of the 2-AA Bovine Fetuin N-Glycan Library indicates a characteristic profile consistent with N-glycans previously reported for bovine fetuin.

Application:

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

Handling & Reconstitution: The labeled glycan library 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 UPLC column is typically 6–9 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.

REFERENCES

1. Green ED, Adelt G, Baenziger JU, Wilson S, Van Halbeek H. The asparagine-linked oligosaccharides on bovine fetuin. Structural analysis of N-glycanase-released oligosaccharides by 500-megahertz 1H NMR spectroscopy. J Biol Chem. 1988 Dec 5;263(34):18253–18268.

2. Townsend RR, Hardy MR, Cumming DA, Carver JP, Bendiak B. Separation of branched sialylated oligosaccharides using high-pH anion-exchange chromatography with pulsed amperometric detection. Anal Biochem. 1989 Oct;182(1):1-8

3. 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.

4. 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.

5. 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.

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