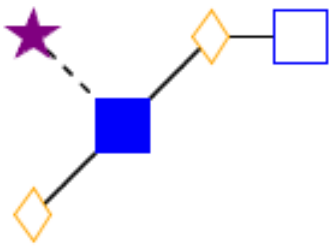
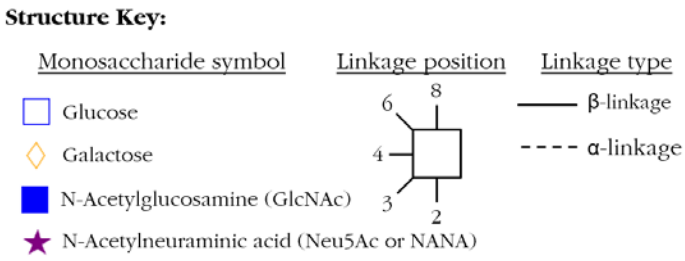
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® LS-TETRASACCHARIDE b (LST b)
PRODUCT CODE:	GKAD-01018
LOT NUMBER:	DP17E0401
PACK SIZE:	0.5 mg (qualitative standard for glycan identification)
PURITY:	≥90% of glycan by UPLC®
FORM:	Dry solid
STORAGE:	Store at -20°C before and after reconstitution
EXPIRATION:	June 2022

STRUCTURE[1,2,3] :





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Quality Control:

Sample Preparation: LST b was labeled with 2-aminobenzamide (2-AB) by reductive amination[4] using the Signal™ 2-AB Labeling Kit (product code GKK-404) under modified labeling conditions.

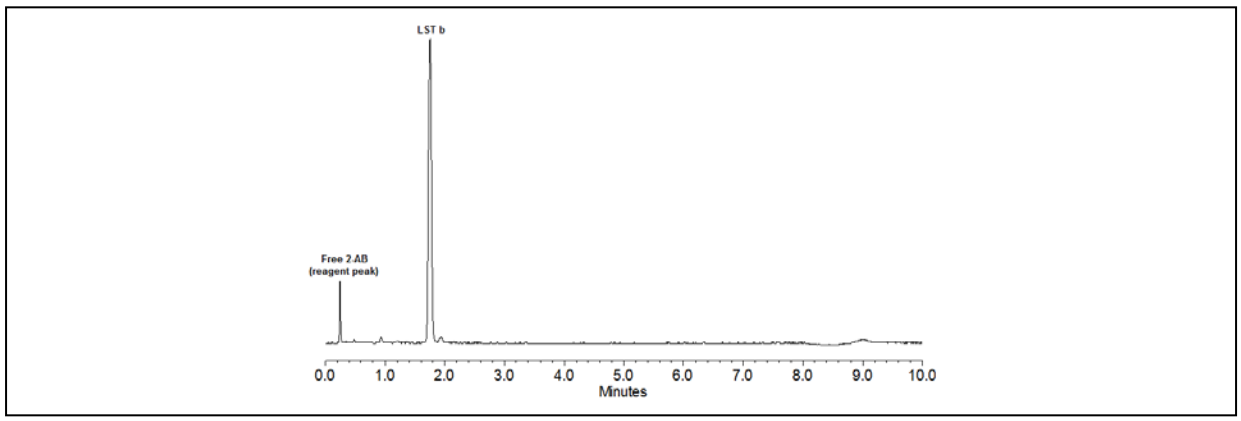

Figure 1 - **UPLC® Results**: 3 – 6 pmol (1 µl, aqueous) of the 2-AB-labeled glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 10-minute method under the conditions below:

Time (min)	Flow (ml/min)	%ACN	%Buffer
00.0	1.0	75.0	25.0
8.0	1.0	60.0	40.0
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} = 330 nm, λ_{em} = 420 nm

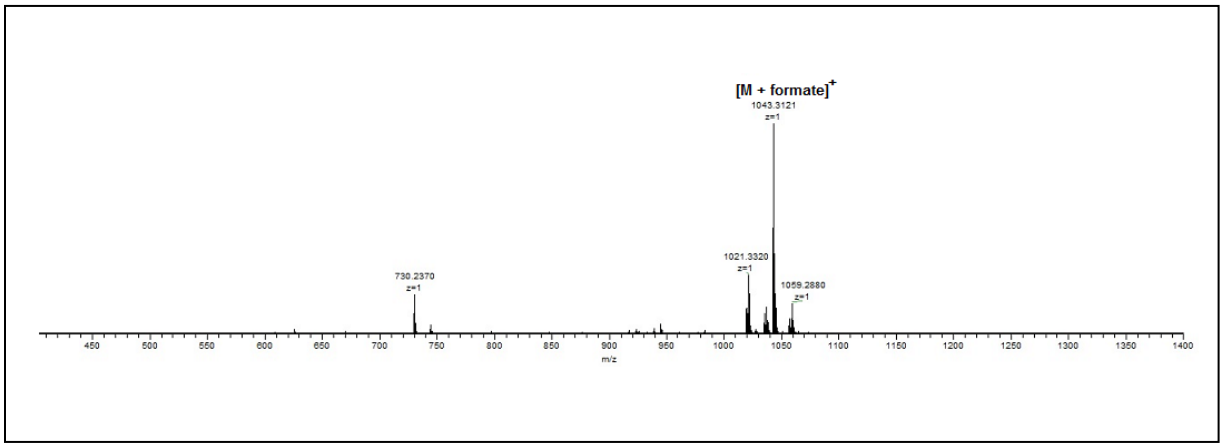

Figure 2 - Mass Spectrum of LST b

Average Mass[5]: 998.9

Monoisotopic Mass[5]: 998.3438

Structural Analysis: The identity of the 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
- Fluorescent-labeling or formation of a variety of oligosaccharide derivatives

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.

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 glycan were calculated using the ExPASy GlycanMass calculator:

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

6. 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.

7. 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.

8. 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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