



## CERTIFICATE OF ANALYSIS

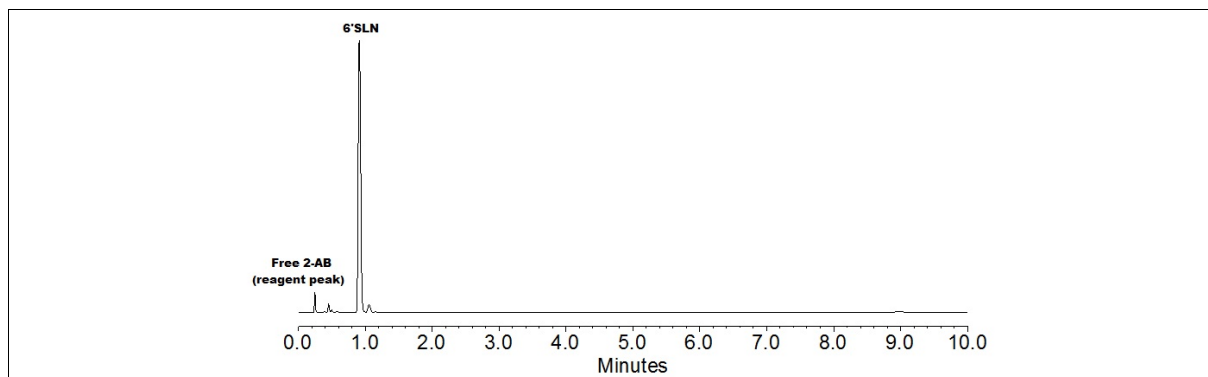
PRODUCT NAME: GLYKO® 6'-SIALYL-N-ACETYLLACTOSAMINE O-GLYCAN (6'-SLN)  
PRODUCT CODE: GKAD-01015  
LOT NUMBER: DP14D0701  
PACK SIZE: 500 µg (qualitative standard for glycan identification)  
PURITY: ≥90% of glycan by UPLC®  
FORM: Dry solid  
STORAGE: Store at -20°C before and after reconstitution  
EXPIRATION: April 2019, may be used for 1 year after reconstitution

STRUCTURE:



## Quality Control:

**Sample Preparation:** 6'-SLN was labeled with 2-aminobenzamide (2-AB) by reductive amination<sup>1</sup> using the Signal™ 2-AB Labeling Kit (product code GKK-404) under modified labeling conditions.



**Figure 1 - UPLC® Results:** 5 pmol (1 µl) 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
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 mM ammonium formate, pH 4.4

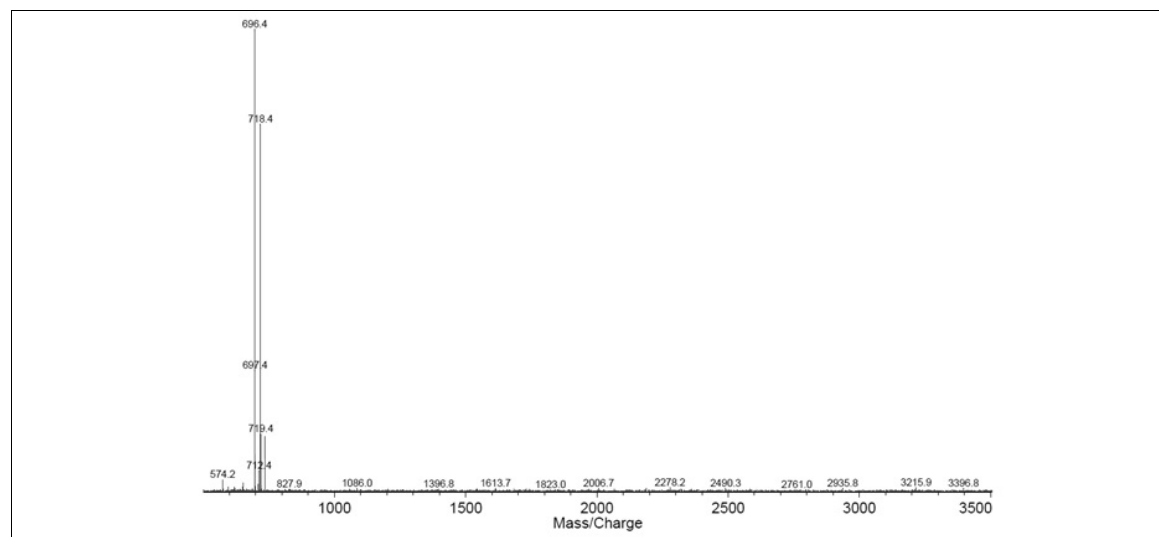
Flow rate: as stated in table, in ml/min

Temperature: 60°C

Max Pressure: 15,000 psi

Fluorescence Detection:  $\lambda_{ex} = 330$  nm

$\lambda_{em} = 420$  nm



**Figure 2 - Mass Spectrum of 6'-SLN**

**Molecular Weight:** 674.6 (free acid, average)<sup>2</sup>

**Structural Analysis:** The purity and structural integrity of the glycan was assessed by UPLC<sup>3</sup> (as described above) and MALDI-TOF mass spectrometry<sup>4,5</sup>, ESI-MS or LC-MS. Agreement was found between the results from mass spectrometry and UPLC.

**Applications:**

- qualitative standard for various analytical procedures
- fluorescent-labeling or formation of a variety of oligosaccharide derivatives
- substrate for glycosidase and glycosyl transferase assays

**Reconstitution:** Use HPLC-grade water or an aqueous buffer to dissolve the glycan. Store the reconstituted glycan at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

**Handling:** The oligosaccharide is shipped as a dried solid. 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 which come into contact with the glycan are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH.

## REFERENCES

1. 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.
2. Average molecular weight was calculated using the ExPASy GlycanMass calculator: <http://web.expasy.org/glycanmass/>
3. 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.
4. 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.
5. 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.

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Authorized Signature