CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® INSTANTAB™ RIBONUCLEASE B N-LINKED GLYCAN LIBRARY
PRODUCT CODE:	GKIB-009
LOT NUMBER:	DP12D1201a
PACK SIZE:	70 pmol (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	June 2023, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	June 2018
STRUCTURE[1,2,3]:	The Ribonuclease B (RNase B) N-Linked Glycan Library consists principally of neutral high mannose structures. The reducing termini are derivatized with the fluorescent dye, InstantAB™.

3832 Bay Center Place
Hayward, CA 94545-3619

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

info@prozyme.com
www.prozyme.com

Quality Control:

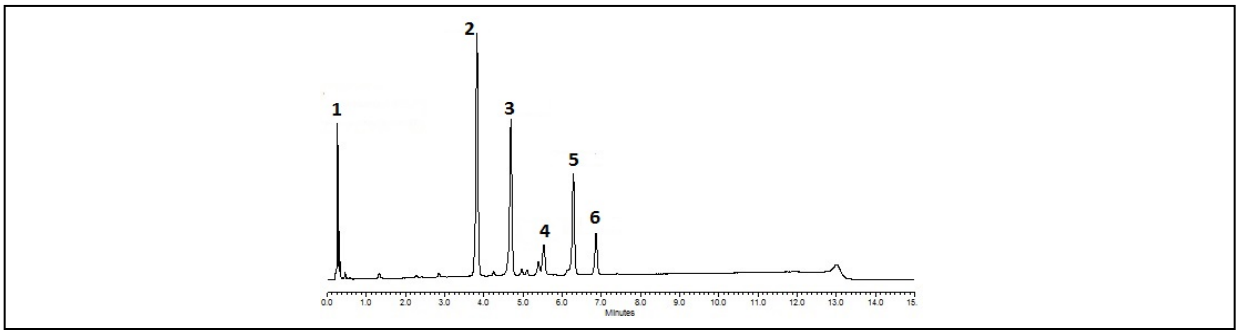
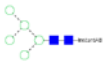
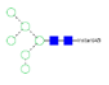
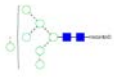


Figure 1 - **UPLC® Results**: 2 -4 pmol (1 µl, aqueous) of the InstantAB-labeled glycan library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below (see Table 1 for peak ID; the number of peaks observed depends on the running conditions employed):

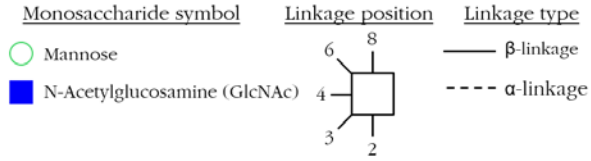
Time (min)	Flow (ml/min)	%ACN	%Buffer
0.0	1.0	75.0	25.0
12.0	0.5	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	1.0	75.0	25.0
15.0	1.0	75.0	25.0

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 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} = 278 nm, λ_{em} = 344 nm

Table 1 - Peak Identification of InstantAB Ribonuclease B N-Linked Glycan Library

Peak Number	Glycan Identification	ProZyme	Oxford (New)	Structure[1,2,3]
1	Free Dye (InstantAB)			
2	Oligomannose 5	Man-5	M5	
3	Oligomannose 6	Man-6	M6	
4	Oligomannose 7	Man-7	M7	
5	Oligomannose 8	Man-8	M8	
6	Oligomannose 9	Man-9	M9	

Structure Key:



Structural Analysis: The identity of the unlabeled glycan is confirmed by MALDI-TOF[4,5] or LC-MS. Agreement was found between the results from mass spectrometry and UPLC[6].

Application:

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

Handling & Reconstitution: The labeled oligo-saccharide 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 library at -20° C. Allow the vial to equilibrate to ambient temperature before use.

Directions For Use: The amount of InstantAB-labeled library standard injected on a UPLC column is typically 2 - 4 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 at:

www.prozyme.com/protocols/

REFERENCES

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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.

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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.

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.

Susan C Fuller
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Authorized Signature