

CERTIFICATE OF ANALYSIS

PRODUCT NAME: InstantQ CHO mAb N-Linked Glycan Library

PRODUCT CODE: GKSQ-020

LOT NUMBER: DP18E0102a

PACK SIZE: 1 each (contains InstantQ glycans from 20 µg purified CHO mAb)
Qualitative standard for glycan identification

FORM: Dry solid

STORAGE: Store at -20°C in the dark. The glycan may be stored on the Gly-Q™ instrument at room temperature for up to 18 hours without significant impact on performance (cap securely and return to -20°C for longer term storage).

EXPIRATION: July 2020 (extended from prior exp. date based on re-assay)

RE-ASSAY DATE: July 2019

STRUCTURE: The InstantQ CHO mAb Library contains N-glycans whose reducing termini are derivatized with the proprietary fluorescent dye, InstantQ.

Quality Control:

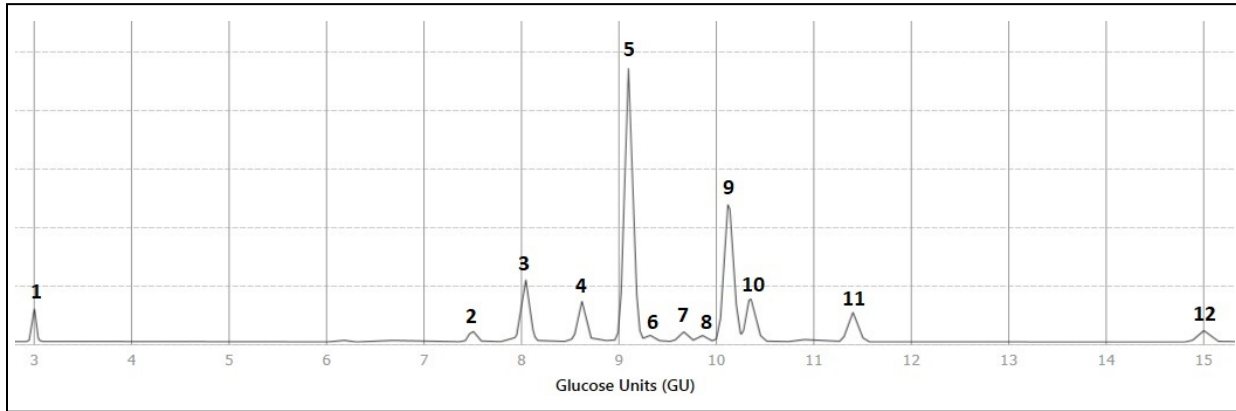


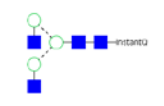
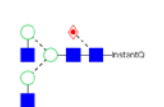
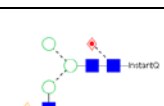
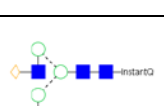
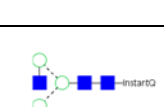
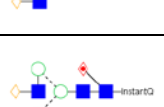
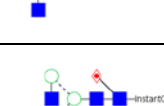
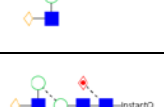
Figure 1 – Gly-Q Results: The InstantQ-labeled standard is injected onto a Gly-Q CE Instrument (GQ2100) under the conditions and method below (see Directions for Use for recommended amounts). Gly-Q Migration Standards (GKSQ-500) are co-injected with the glycan:

METHOD:

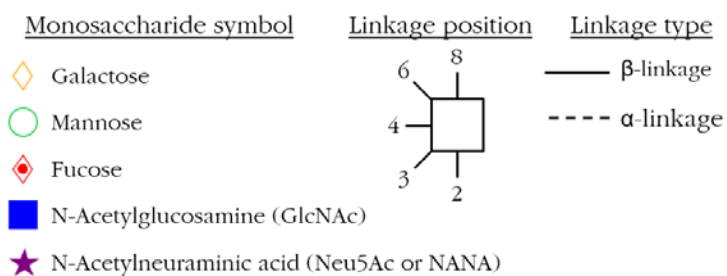
Action	High Voltage	Duration, seconds	Position	Sampling Interval, Seconds
High Voltage Purge	4.00	10.00	Wash	0.04
Pause		2.00	Clean	
Reagent Block Injection	2.00	2.00	MA02	0.04
Pause		2.00	Clean	
Well Plate Injection	2.00	2.00	Sample	0.04
Pause		2.00	Clean	
Separation & Detection	10.00	120.00	Separation	0.04

Table 1 - Peak Identification of InstantQ CHO mAb Library

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{4,5}
1	Lower Migration Standard, DP3 (maltotriose)				
2	Asialo-, agalacto-, biantennary, -1 N-Acetylglucosamine	NGA2-N	G0-N[3]	A1[3]	
3	Asialo-, agalacto-, biantennary with core fucose, -1 N-Acetylglucosamine + Oligomannose 5	NGA2F-N + Man-5	G0F-N + Man5	F(6)A1 + M5	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{4,5}
4	Asialo-, agalacto- biantennary	NGA2	G0	A2	
5	Asialo-, agalacto- biantennary with core fucose	NGA2F	G0F	F(6)A2	
6	Asialo-, monogalactosylated biantennary with core fucose, - 1 N-Acetylglucosamine	NA2G1F-N	G1F-N[3]	F(6)A1[3]G(4)1	
7	Asialo-, monogalactosylated biantennary	NA2G1	G1[6]	A2[6]G(4)1	
8	Asialo-, monogalactosylated biantennary	NA2G1	G1[3]	A2[3]G(4)1	
9	Asialo-, monogalactosylated biantennary with core fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	
10	Asialo-, monogalactosylated biantennary with core fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
11	Asialo-, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	
12	Upper Migration Standard, DP15 (maltopentadecaose)				

Structure Key⁴:



Structural Analysis: The purity and structural integrity of the glycan standard was assessed by capillary electrophoresis (CE).

Application: Qualitative reference standard for the separation and identification of N-glycan structures labeled with InstantQ™ on the Gly-Q CE system.

Handling: The labeled oligosaccharide standard is shipped as a dried solid from a solution of 50 mM Trehalose. Use ultra-pure water or an aqueous buffer to dissolve the material (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.

Aliquot out the desired quantity and return remaining solution to -20°C. The glycan may undergo up to four freeze-thaws without significant effect on performance.

Directions For Use: The amount of labeled glycan used with the Gly-Q Instrument is typically 50 µl. For our Quality Control testing, the standard was reconstituted in 100 µl water, a 50 µl aliquot was transferred to a PCR tube, and replicate runs were processed. Typically, ~30 injections are obtained from a 50 µl aliquot; aliquots of less than 50 µl are not recommended. Signal decrease can occur over repeated injections from the same aliquot. For further information on using InstantQ labeled glycans with the Gly-Q system, please contact ProZyme:

info@prozyme.com

REFERENCES

1. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. A recombinant IgG Fc that recapitulates the anti-inflammatory activity of IGIV. *Science* 2008 Apr 18; 320(5874): 373–376.
2. Lee EU, Roth J, Paulson JC. Alteration of terminal glycosylation sequences on N-Linked oligosaccharides of Chinese hamster ovary cells by expression of β-Galactoside α2,6-Sialyltransferase. *J Biol Chem.* 1989 August 15; 264(23): 13848-13855.
3. Gly-Q: An Integrated Solution for High-throughput, User-friendly Glycoanalysis Using Rapid Separation by Capillary Electrophoresis. ProZyme Tech Note TN2004. www.prozyme.com/pages/tech-notes
4. 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.
5. 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.

Authorized Signature