Certificate of Analysis

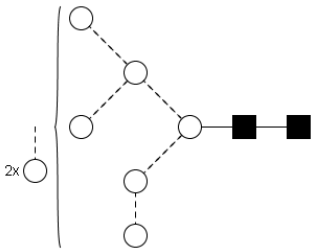
Ludger BioQuant Quantitative Man 8 Glycan

Cat. #: BQ-CN-MAN8-10U
Batch: B72A-01
Size: 10 µg (5.81 nmol)
Expiry date: 10 Jan 2028

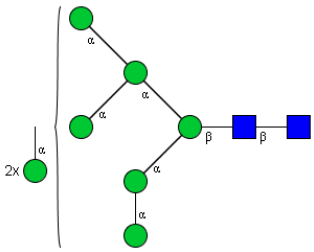
Product Description

The BioQuant Man 8 glycan is a purified and quantified glycan standard which can be used as an internal standard and positive control for quantitative analysis (e.g. monosaccharide analysis)

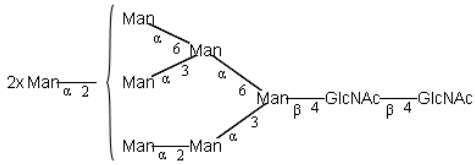
Glycan Structure



Oxford Notation



CFG Notation



Text Notation

Glycan Purity determined as > 90% by UHPLC
Monoisotopic mass: 1721.6 [M+H]+
Storage conditions: -20°C

BQ-CN-MAN8-10U Quantity Summary

The amount of BioQuant Man8 glycan to be dispensed per vial is determined by quantitative Nuclear Magnetic Resonance (qNMR) of the bulk glycan stock. Once dispensed the **amount of glycan per vial** is determined by monosaccharide analysis. These determinations are detailed on the following pages, but a summary is provided below:

		Amount of BQ-CN-MAN8-10U per vial
qNMR based determination: derived from glycan bulk stock	=	10.00 µg (5.81nmol)
Monosaccharide based determination (GlcN – HCl hydrolysis)	=	10.0 µg ± 0.49µg (5.80nmol) (± indicates standard deviation)

Quantitative Nuclear Magnetic Resonance (qNMR)

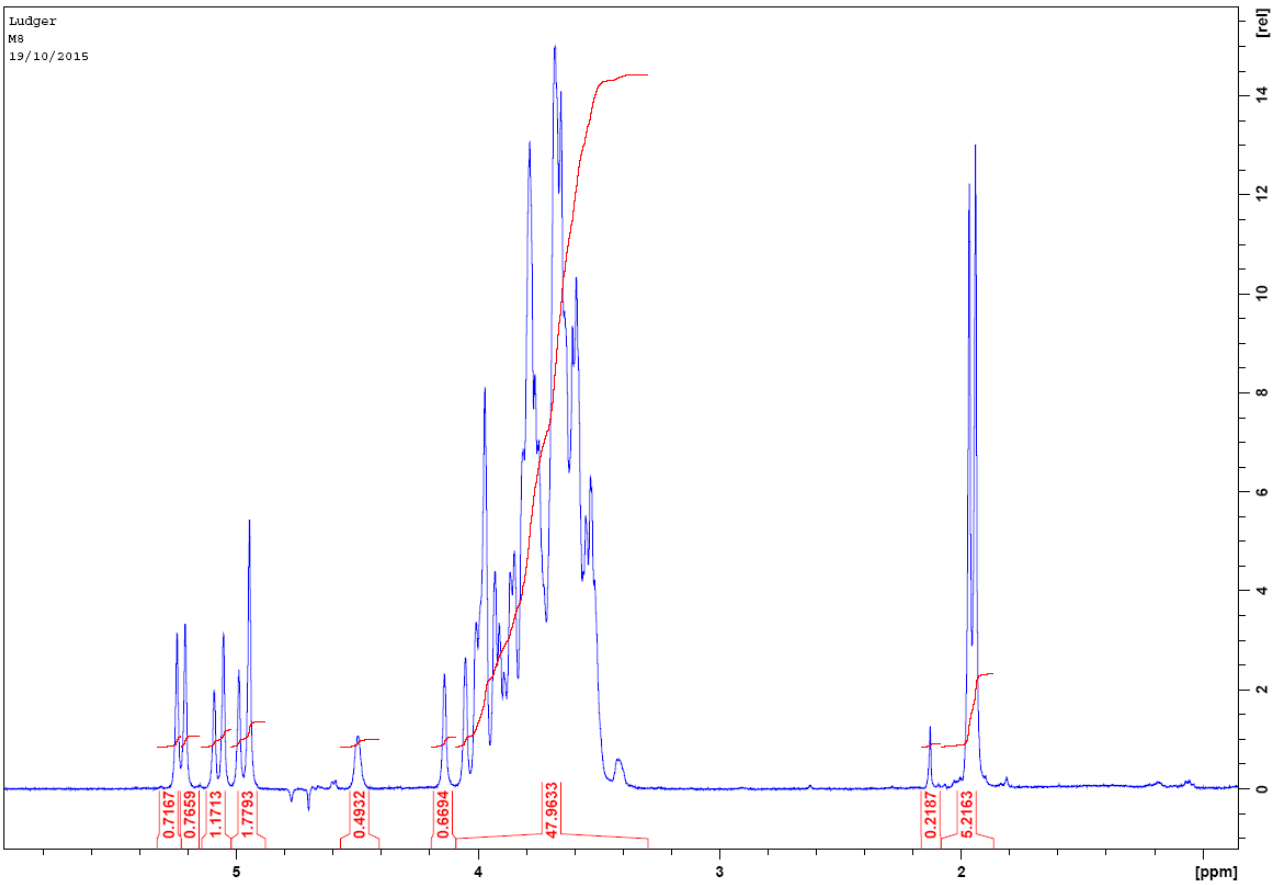


Figure 1. ^{1}H-NMR (500 MHz) of BQ-CN-MAN8-BULK in D_2O (Batch Number: B6BI-08)

Sample	Concentration (mM) calculated using a certified quantitative standard
BQ-CN-MAN8-BULK	2.0866

Table 1. Concentration of BQ-CN-MAN8-BULK calculated by qNMR

The concentration of the BioQuant Man 8 stock was calculated by qNMR in comparison to a certified quantitative standard (Table 1). This value was used to determine the amount of sample to be dispensed to obtain 10 µg of glycan per vial.

Monosaccharide analysis of BQ-CN-MAN8-10U

Quantitative monosaccharide analysis using the Ludger LT-MONO-96 kit was performed on 5 replicates of BQ-CN-MAN8-10U using 6M hydrochloric acid hydrolysis (HCl) to release the N-acetylglucosamine (GlcNAc hydrolysed to GlcN) constituents of the glycan. The GlcN monosaccharides were labelled with 2-aminobenzoic acid and chromatography was performed on a HPLC equipped with a LudgerSep R2 monosaccharide analysis column (LS-R2-4.6x150).

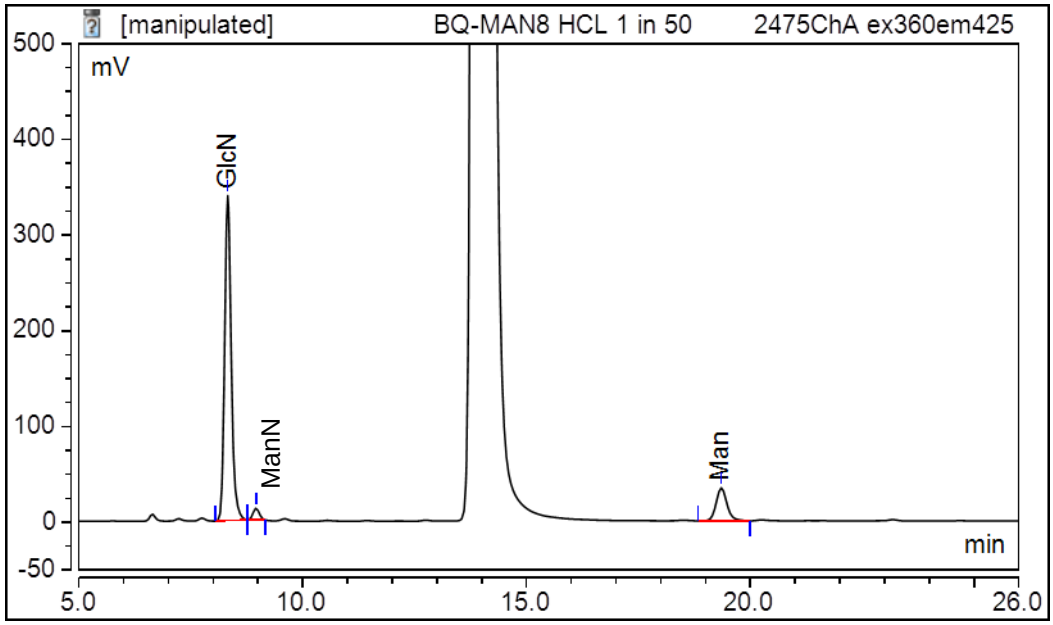


Figure 2. LudgerSep-R2 HPLC profile of 2-aminobenzoic acid (2-AA) labelled monosaccharides of HCl hydrolysed BQ-CN-MAN8-10U (Batch B72A-01).

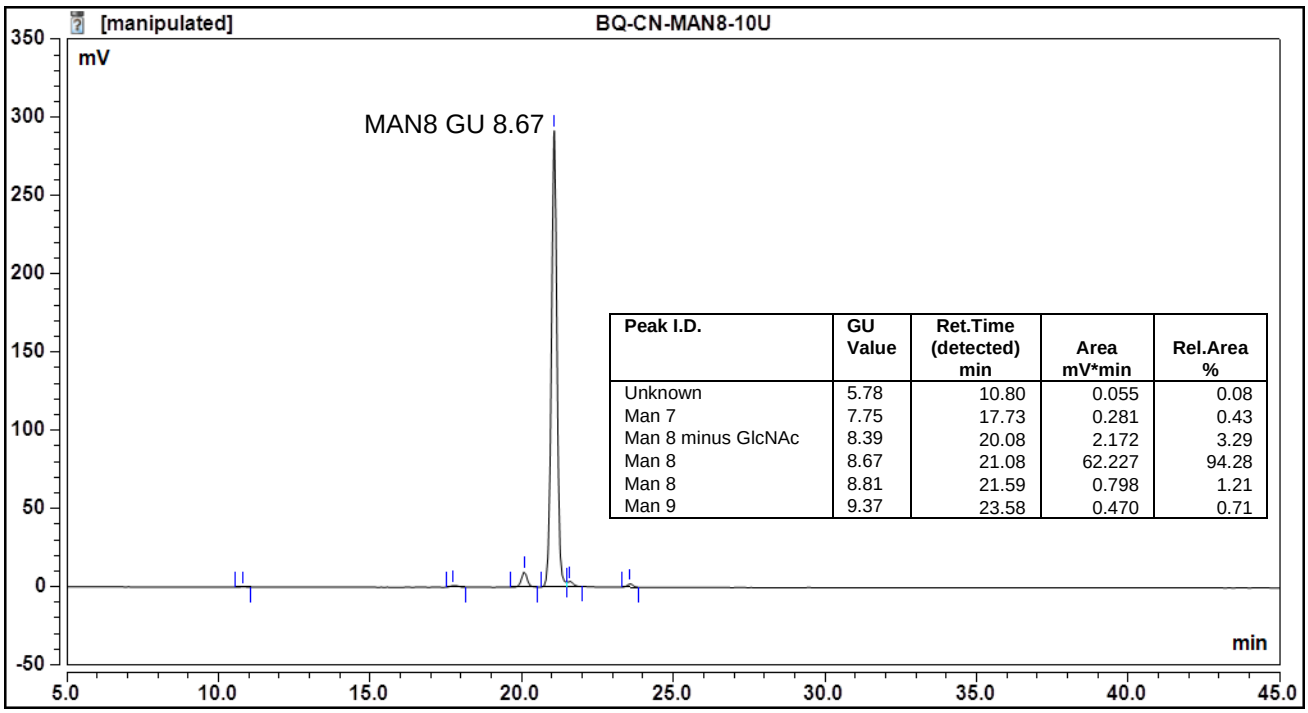
The ManN monosaccharide is due to epimerisation of the GlcN monosaccharide during sample processing.

Calculation of the amount of MAN8 glycan using the GlcN value:

Quantity of GlcN per vial = 11.59 ± 0.57 nmol

Quantity of BQ-CN-MAN8-10U per vial (determined by GlcN content) = 10.0 ± 0.49µg (5.80 nmol)

Glycan Purity and Identity of BQ-CN-MAN8-10U



Peak I.D.	GU Value	Ret.Time (detected) min	Area mV*min	Rel.Area %
Unknown	5.78	10.80	0.055	0.08
Man 7	7.75	17.73	0.281	0.43
Man 8 minus GlcNAc	8.39	20.08	2.172	3.29
Man 8	8.67	21.08	62.227	94.28
Man 8	8.81	21.59	0.798	1.21
Man 9	9.37	23.58	0.470	0.71

Figure 3. HILIC UHPLC profile of Procainamide (Ludger fluorophore tag) labelled BQ-CN-MAN8-10U (Batch B72A-01).

Glycan Purity determined as > 90% by HILIC chromatography of fluorescently labelled glycan.

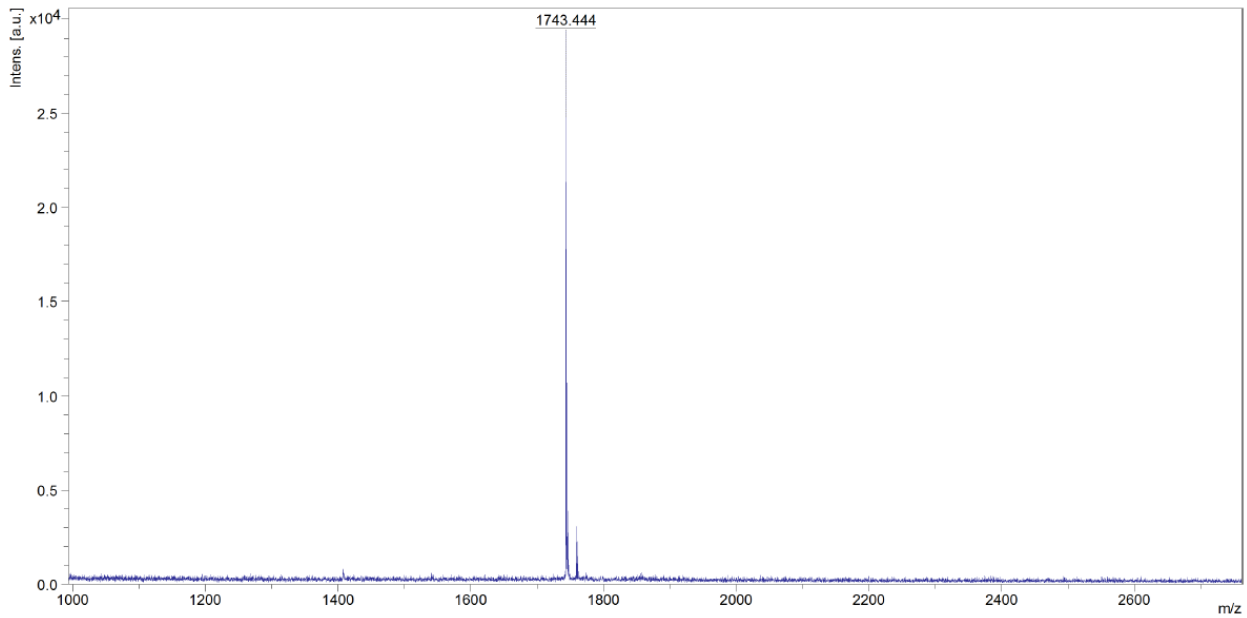
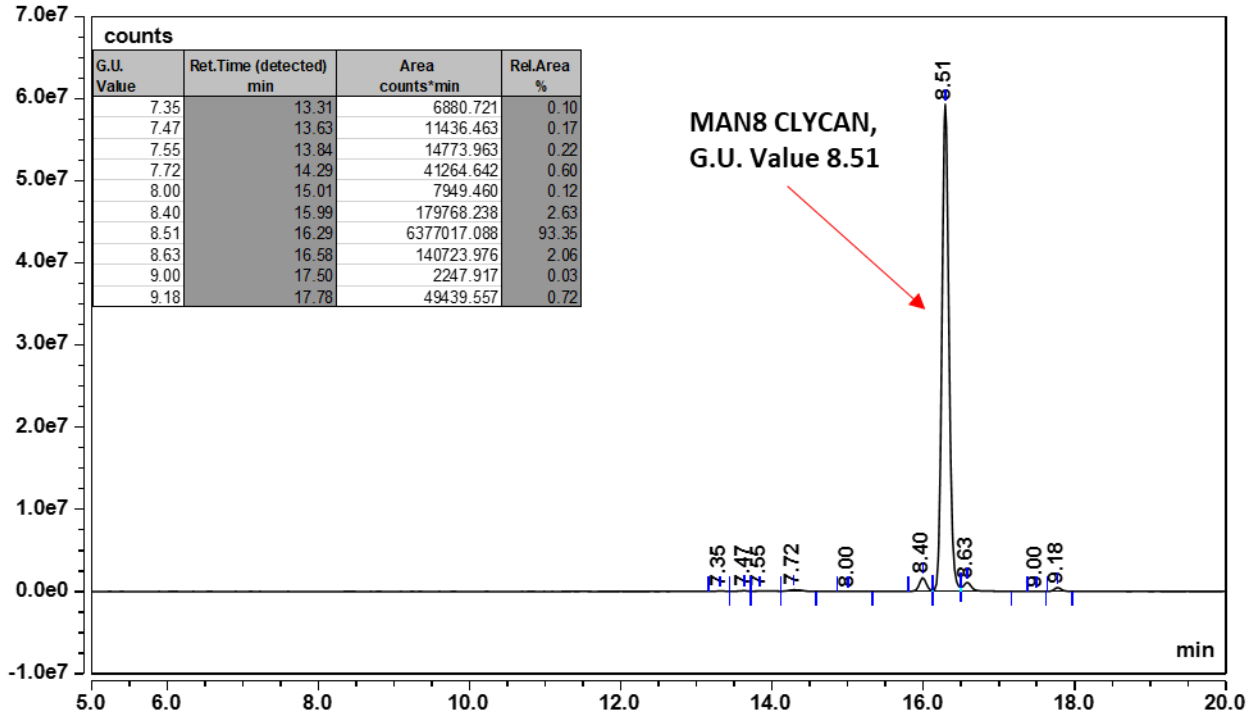


Figure 4. Positive ion mass spectrum of BQ-CN-MAN8-10U (Batch B72A-01). Theoretical mass: 1743.6 Da $[M+Na]^+$

Glycan Purity, qauntity and Identity of BQ-CN-MAN8-10U



G.U. Value	Ret.Time (detected) min	Area counts*min	Rel.Area %
7.35	13.31	6880.721	0.10
7.47	13.63	11436.463	0.17
7.55	13.84	14773.963	0.22
7.72	14.29	41264.642	0.60
8.00	15.01	7949.460	0.12
8.40	15.99	179768.238	2.63
8.51	16.29	6377017.088	93.35
8.63	16.58	140723.976	2.06
9.00	17.50	2247.917	0.03
9.18	17.78	49439.557	0.72

Figure 5: HILIC column UPLC chromatogram of 2AB labeled MAN8 glycan (BQ-CN-MAN8-10U, Batch B72A-01)

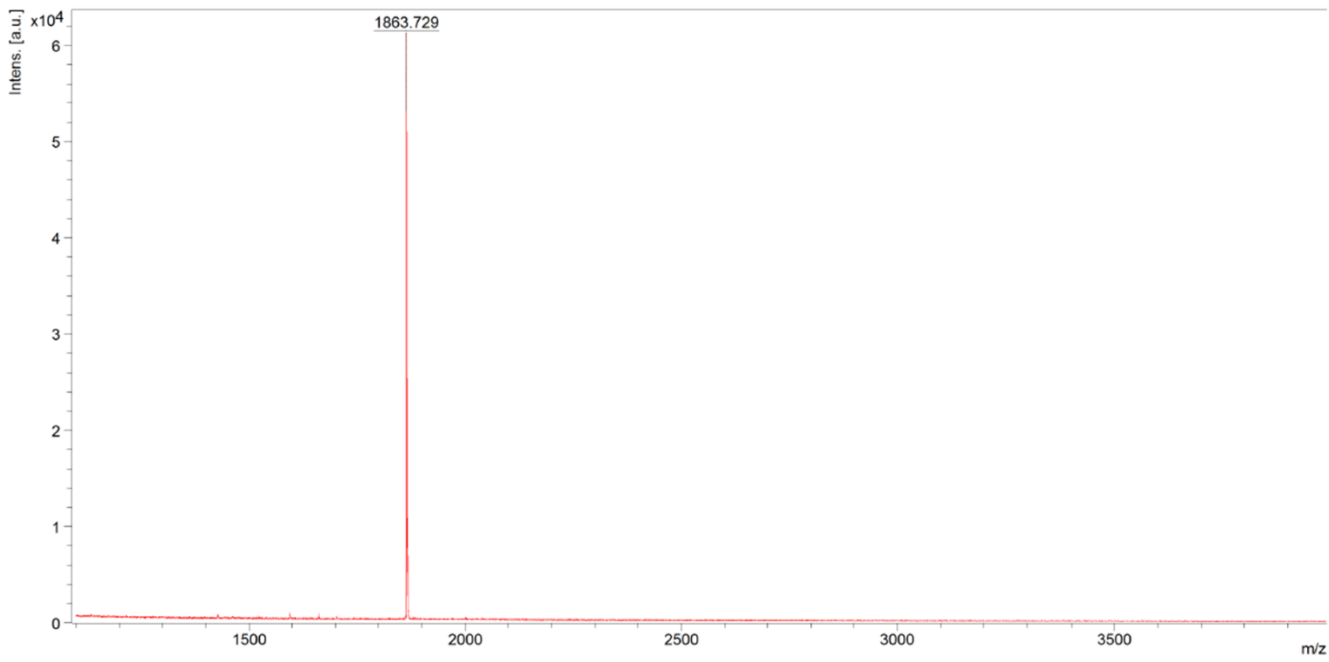


Figure 6: Positive ion mass spectrum of 2AB labelled MAN8 glycan (BQ-CN-MAN8-10U, Batch B72A-01). Theoretical mass 1863.65 Da [M+Na]+