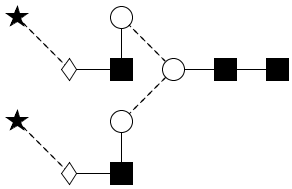
Certificate of Analysis

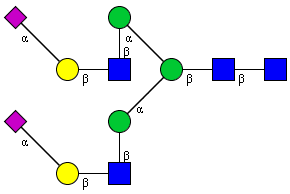
BQ-GPEP-A2G2S2-10U

Cat. #: BQ-GPEP-A2G2S2-10U Batch: B67I-01 Size: 10 µg (3.49nmol)

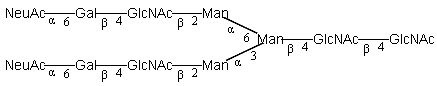
Glycan Structure



Oxford Notation



CFG Notation



Text Notation

The glycopeptide is comprised of an A2G2S2 glycan attached to the asparagine amino acid of a peptide with the sequence Lysine-Valine-Alanine-Asparagine-Lysine-Threonine (KVANKT).

Glycan Purity determined as > 90% by UHPLC

Monoisotopic mass: 2865.1763 [M+H]+

Storage conditions: -20°C

BQ-GPEP-A2G2S2-10U Quantity Summary

The amount of GPEP-A2G2S2 glycopeptide to be dispensed per vial is determined by quantitative Nuclear Magnetic Resonance (qNMR) of the bulk glycopeptide stock. Once dispensed the **amount of glycopeptide per vial** is determined by monosaccharide analysis and sialic acid analysis. These determinations are detailed on the following pages, but a summary is provided below:

		Amount of BQ-GPEP-A2G2S2-10U per vial
qNMR based determination: derived from glycopeptide bulk stock	=	10.00 µg ± 0.14 (3.49nmol)
Monosaccharide based determination (GlcN – HCl hydrolysis)	=	10.26 µg ± 0.29 (3.58nmol)
Sialic acid based determination	=	8.57 µg ± 0.20 (2.99nmol)

Quantitative Nuclear Magnetic Resonance (qNMR)

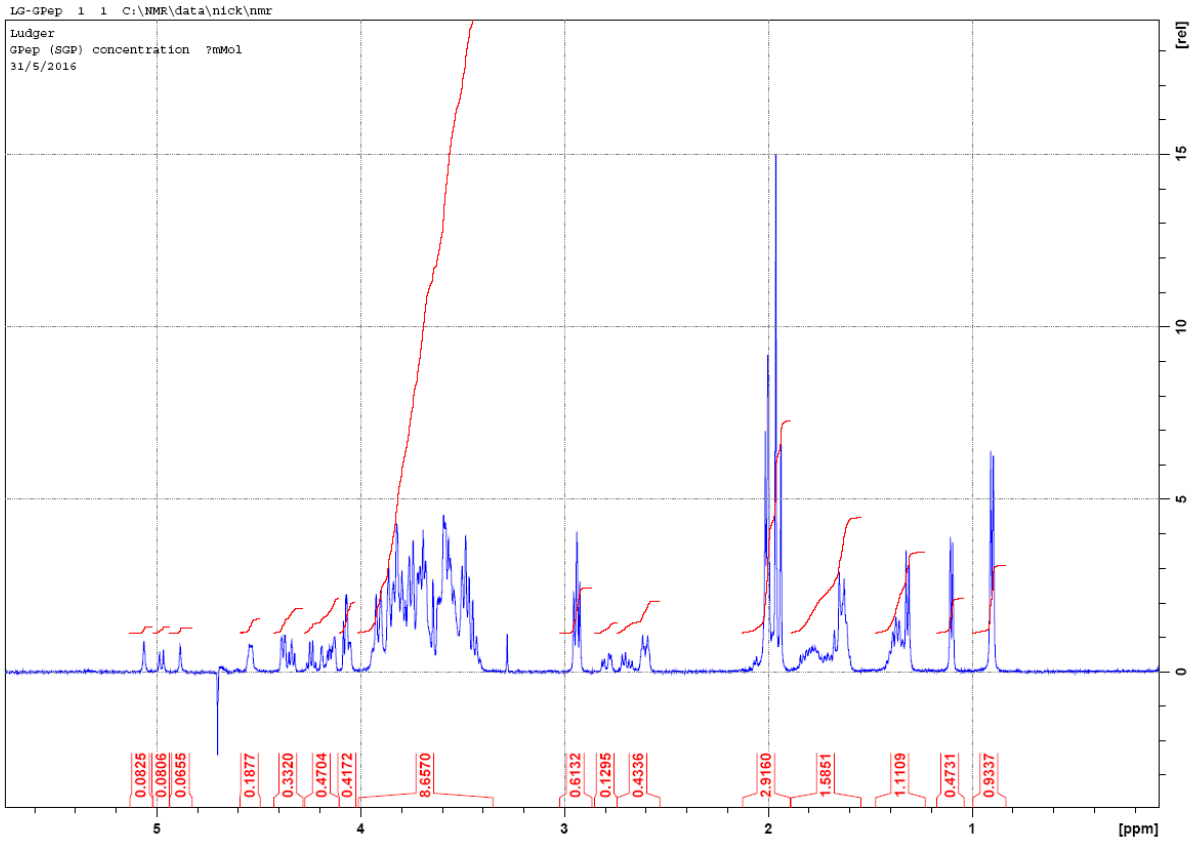


Figure 1. ^{1}H-NMR (500 MHz) of BQ-GPEP-A2G2S2-Bulk in D_2O (Batch Number: B677-01)

Sample	Concentration (mM) calculated using a certified quantitative standard.
BQ-GPEP-A2G2S2-Bulk	0.3111 ± 0.0044

Table 1. Concentration of BQ-GPEP-A2G2S2-Bulk calculated by qNMR

The concentration of the BQ-GPEP-A2G2S2 stock was calculated by qNMR by 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 glycopeptide per vial.

Monosaccharide analysis of BQ-GPEP-A2G2S2-10U

Quantitative monosaccharide analysis using the Ludger LT-MONO-96 kit was performed on 5 replicates of BQ-GPEP-A2G2S2 using 6M hydrochloric acid hydrolysis (HCl) to release the N-acetylglucosamine (GlcNAc – hydrolysed to GlcN) constituents of the glycopeptide. 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).

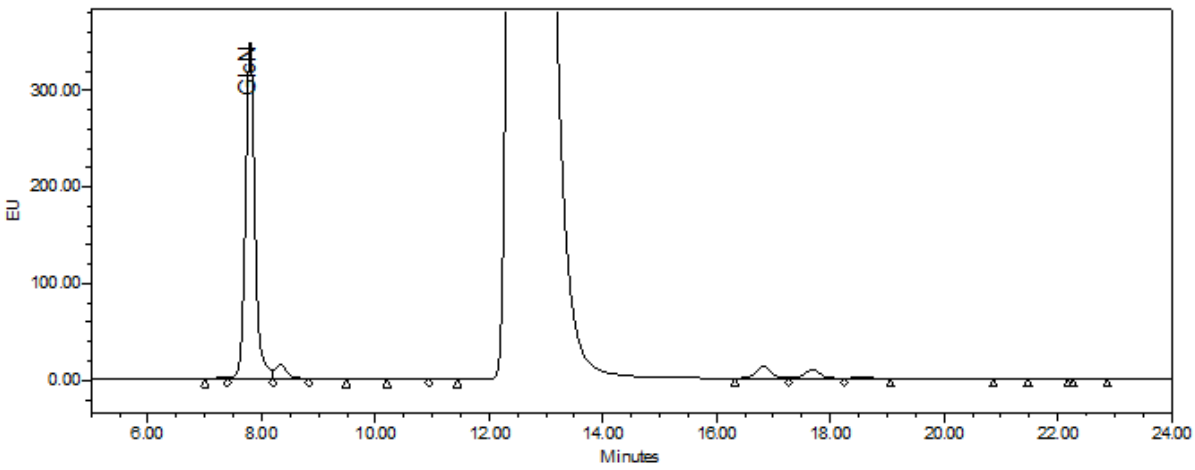


Figure 2. LudgerSep-uR2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of HCl hydrolysed BQ-GPEP-A2G2S2-10U (Batch B67I-01).

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

Calculation of the amount of GPEP-A2G2S2 using the GlcN value:

Quantity of GlcN per vial = 14.30 ± 0.41 nmol

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by GlcN content) = 10.26 ± 0.29µg (3.58 nmol)

Sialic acid analysis of BQ-GPEP-A2G2S2-10U

Quantitative sialic acid analysis was performed on 3 separate vials of BQ-GPEP-A2G2S2-10U using the LudgerTag™ DMB sialic acid labelling kit (LT-KDMB-A1). The labelled sialic acid chromatography was performed on a HPLC equipped with a LudgerSep R1 column (LS-R1-4.6x150).

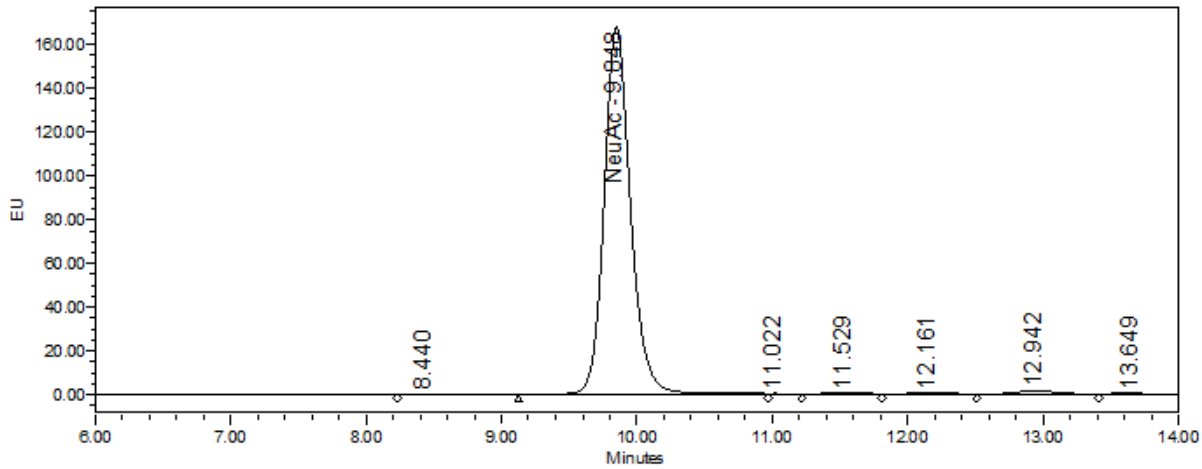


Figure 3. LudgerSep-uR2 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled Neu5Ac of acetic acid hydrolysed BQ-GPEP-A2G2S2-10U (Batch B67I-01).

Quantity of NeuAc per vial = 5.98 ± 0.14 nmol

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by NeuAc content) = 8.57 ± 0.20 (2.99 nmol)

Glycopeptide Purity and Identity of BQ-GPEP-A2G2S2-10U

Retention time	Area (µV*sec)	% Area
7.570	145242	0.29
7.797	333588	0.68
8.826	38944	0.08
9.104	1258199	2.55
9.527	46118513	93.38
9.811	377909	0.77
9.989	281574	0.57
10.599	52589	0.11
10.771	33260	0.07
11.505	481730	0.98
11.853	266449	0.54

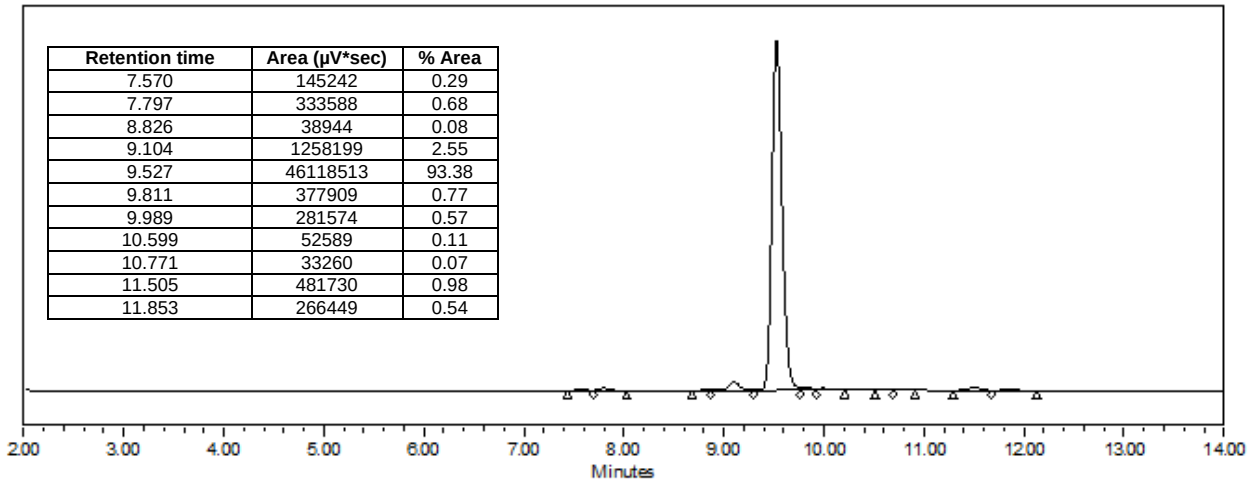


Figure 4. HILIC UHPLC profile of V-Tag (Ludger fluorophore tag) labelled BQ-GPEP-A2G2S2-10U (Batch B67I-01).

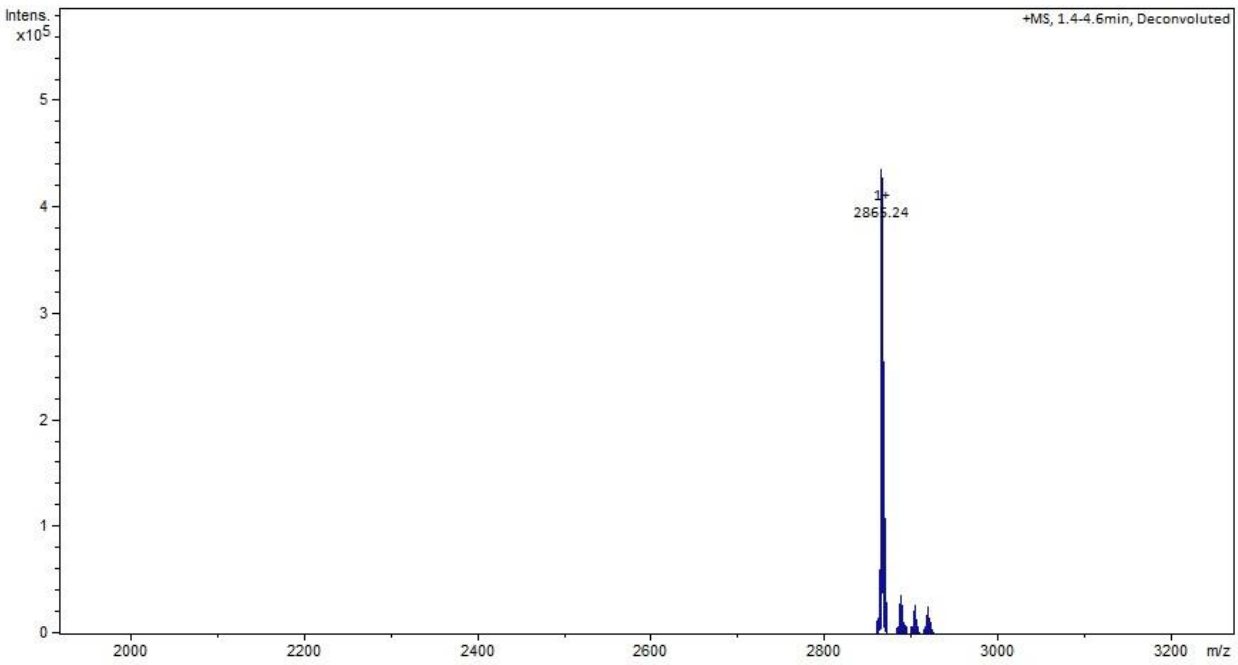


Figure 5. Positive ion ESI mass spectrum of BQ-GPEP-A2G2S2-10U (Batch B67I-01). KVANKT-A2G2S2 theoretical mass: 2865.74 Da