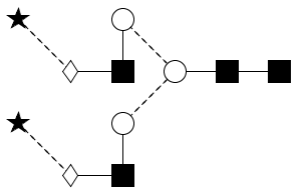
Certificate of Analysis

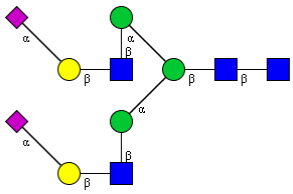
BQ-GPEP-A2G2S2-10U

Cat. #: BQ-GPEP-A2G2S2-10U Batch: B5B3-02 Size: 10 µg (3.49nmol)

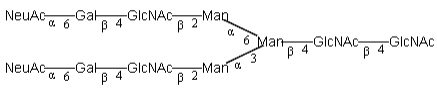
Glycan Structure



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 > 95% by UHPLC, impurities peptide backbone only-ANKT, KVAN.

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 ± 0.48 (3.49nmol)
Monosaccharide based determination (GlcN – HCl hydrolysis)	=	9.00 µg ± 0.70 (3.15nmol)
Sialic acid based determination	=	10.52 µg ± 0.74 (3.67nmol)

Ludger

Quantitative Nuclear Magnetic Resonance (qNMR)

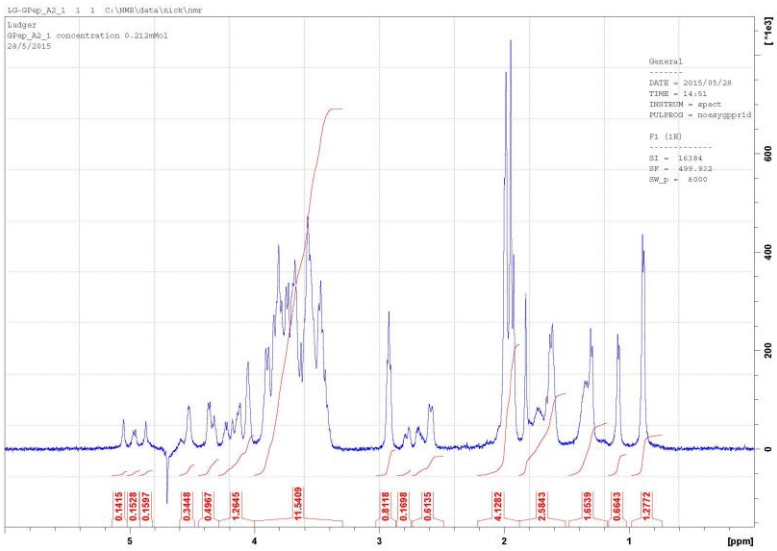


Figure 1. ^{1}H-NMR (500 MHz) of BQ-GPEP-A2G2S2-Bulk in D_2O (Batch Number: B5AS-03)

Sample	Concentration (mM) calculated using a certified quantitative standard.
BQ-GPEP-A2G2S2-Bulk	0.4757 ± 0.0227

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).

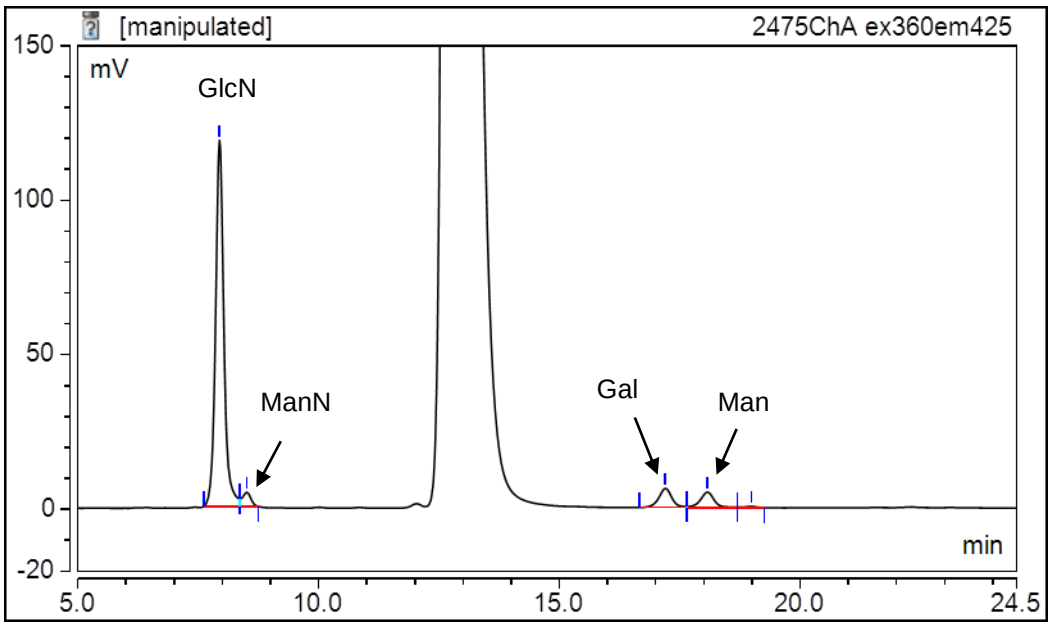


Figure 2. LudgerSep-uR2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of HCl hydrolysed BQ-GPEP-A2G2S2-10U (Batch B5B3-02).

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 = 12.6 ± 0.98 nmol

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by GlcN content) = 9.00 ± 0.70µg (3.15 nmol)

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

Quantitative sialic acid analysis was performed on 5 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 UHPLC equipped with a LudgerSep uR2 column (LS-UR2-2.1x100).

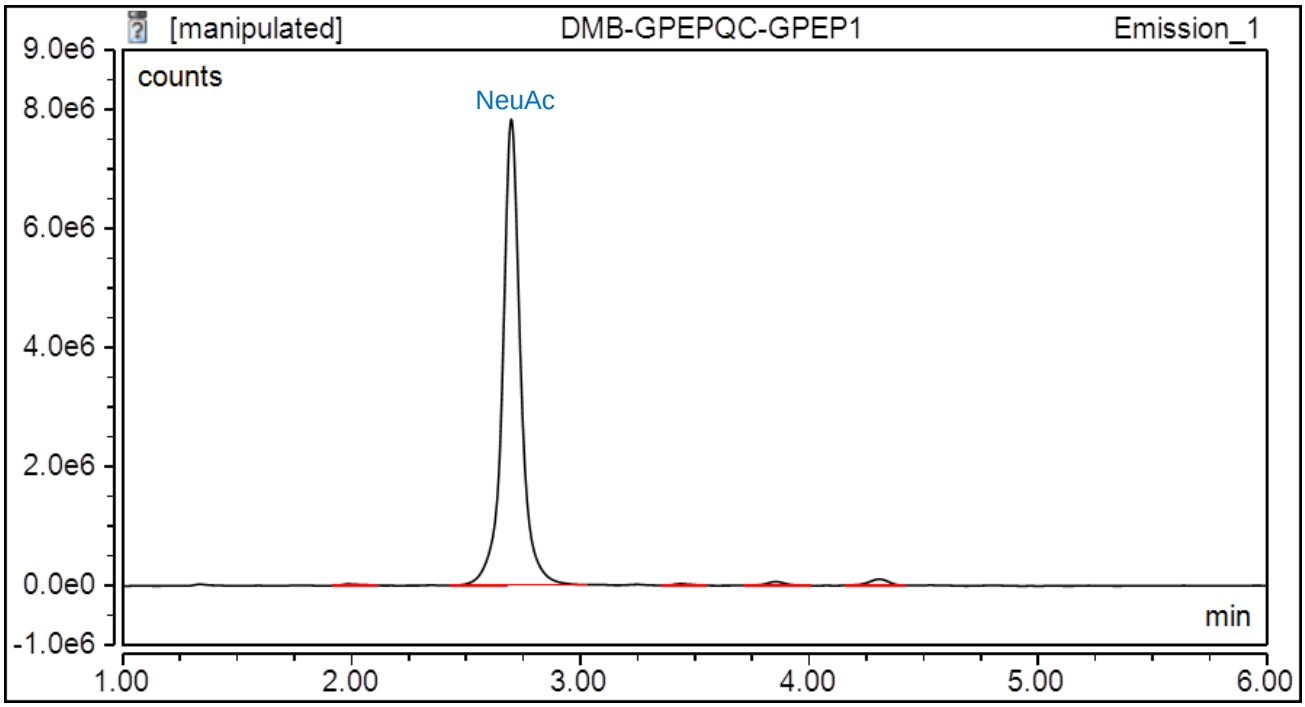


Figure 3. LudgerSep-uR2 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled Neu5Ac of acetic acid hydrolysed BQ-GPEP-A2G2S2-10U (Batch B5B3-02).

Quantity of NeuAc per vial = 7.34 ± 0.58 nmol

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by NeuAc content) = 10.52 ± 0.74 (3.67 nmol)

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

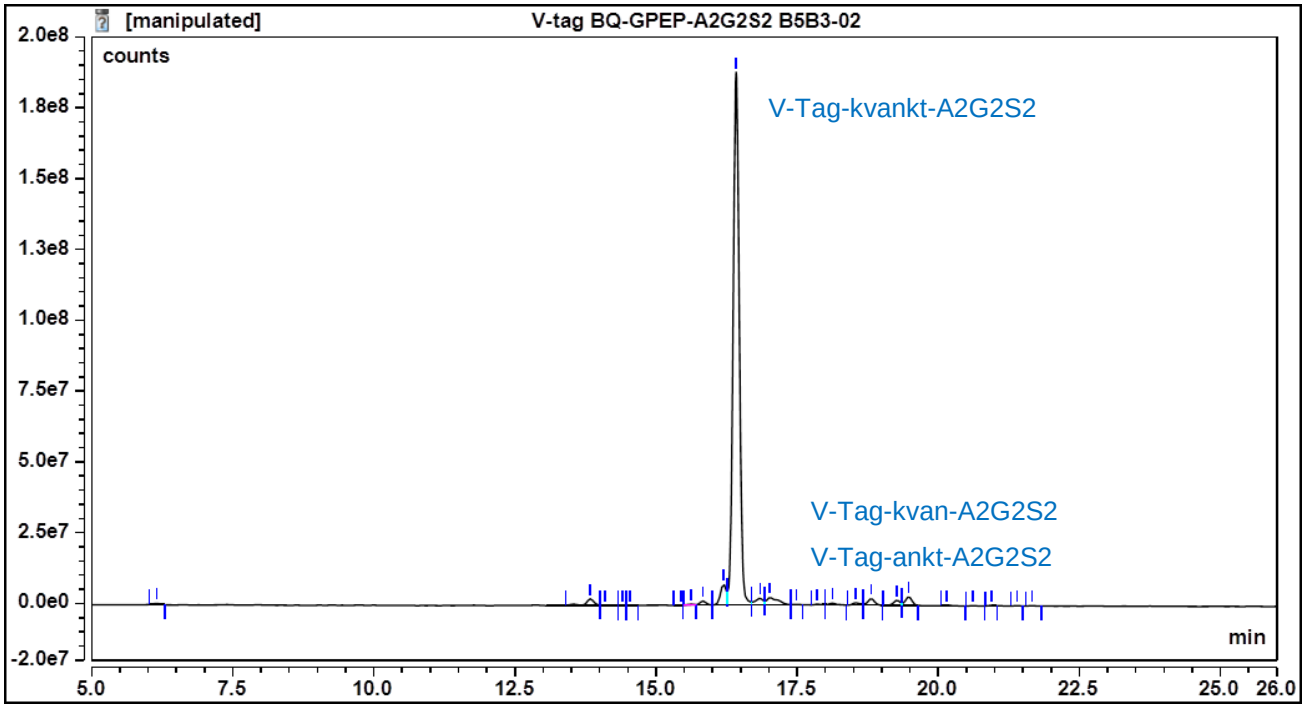


Figure 4. HILIC UHPLC profile of V-Tag (Ludger fluorophore tag) labelled BQ-GPEP-A2G2S2-10U (Batch B5B3-02).

Glycan Purity determined as > 95% by HILIC chromatography of fluorescence tag glycopeptide, impurities peptide backbone only-ANKT, KVAN.

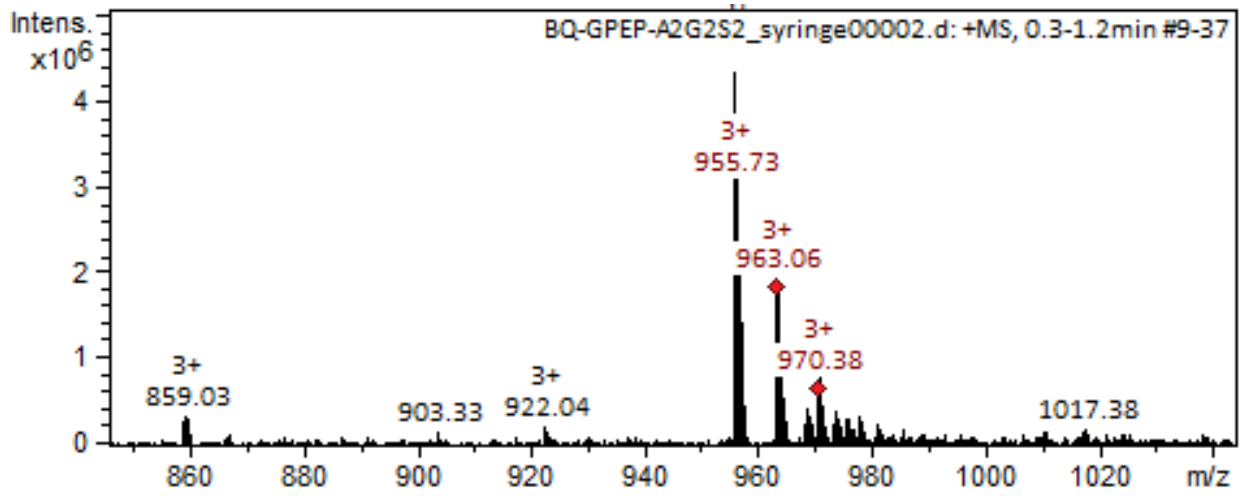


Figure 5. Positive ion ESI mass spectrum of BQ-GPEP-A2G2S2-10U (Batch B5B3-02). KVANKT-A2G2S2 theoretical mass: 955.73 $[M+H]^{3+}$Da. De-convoluted theoretical mass would be 2865.17 $[M+H]^{+}$