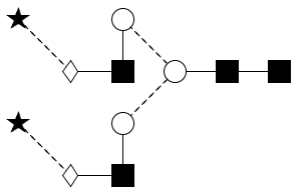
Certificate of Analysis

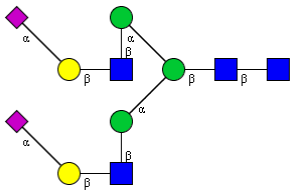
BQ-GPEP-A2G2S2-10U

Cat. #: BQ-GPEP-A2G2S2-10U Batch: B2BE-01 Size: 10 µg

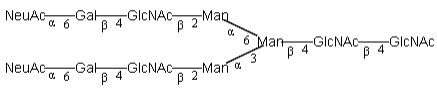
Glycan Structure



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).

Glycopeptide Purity > 91% as determined by UHPLC

Monoisotopic mass: 2865.1763 [M+H]+

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 ± 0.51 µg.
Monosaccharide based determination (GlcN – HCl hydrolysis)	=	10.41 ± 0.94 µg.
Sialic acid based determination	=	10.26 ± 0.54 µg.

Ludger

Quantitative Nuclear Magnetic Resonance (qNMR)

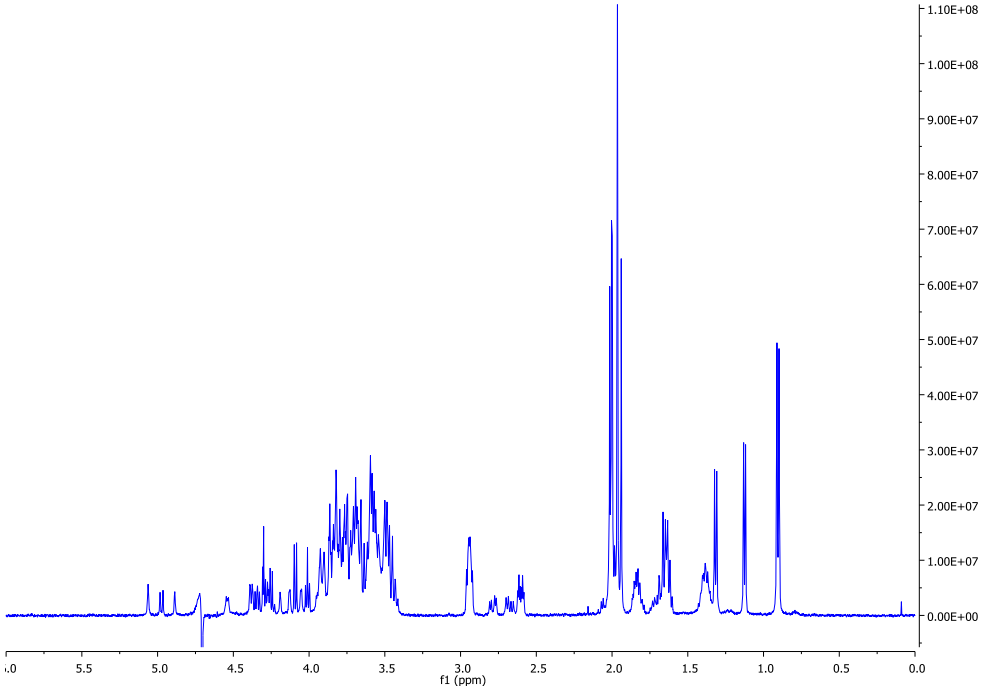


Figure 1. ^{1}H-NMR (500 MHz) of BQ-GPEP-A2G2S2-Bulk in D_2O (Batch Number: B2B6-06)

Sample	Concentration (mM) calculated using a certified quantitative standard.
BQ-GPEP-A2G2S2-bulk	0.2833 ± 0.0144

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 3 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 UHPLC equipped with a LudgerSep uR2 monosaccharide analysis column (LS-UR2-2.1x50).

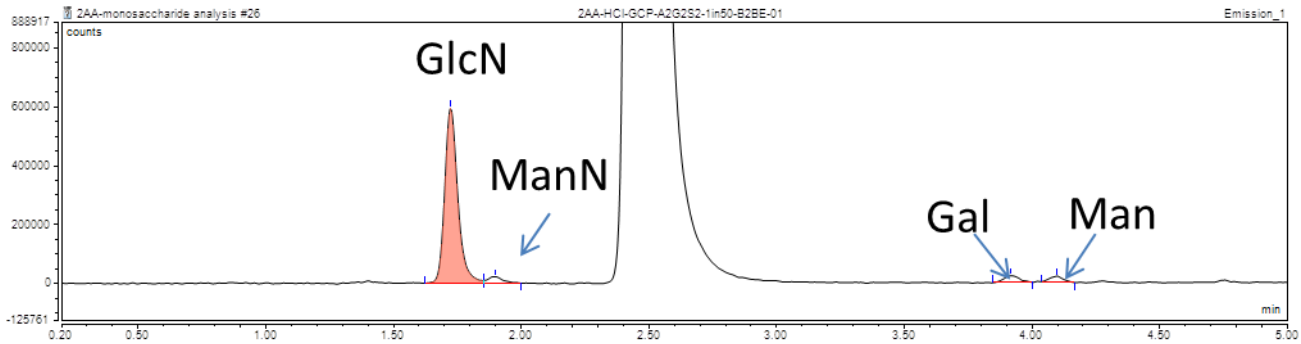


Figure 2. LudgerSep-uR2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of HCl hydrolysed BQ-GPEP-A2G2S2-10U (Batch B2BE-01).

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

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by GlcN content) = 10.41 µg ± 0.94.

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 UHPLC equipped with a LudgerSep uR2 column (LS-UR2-2.1x100).

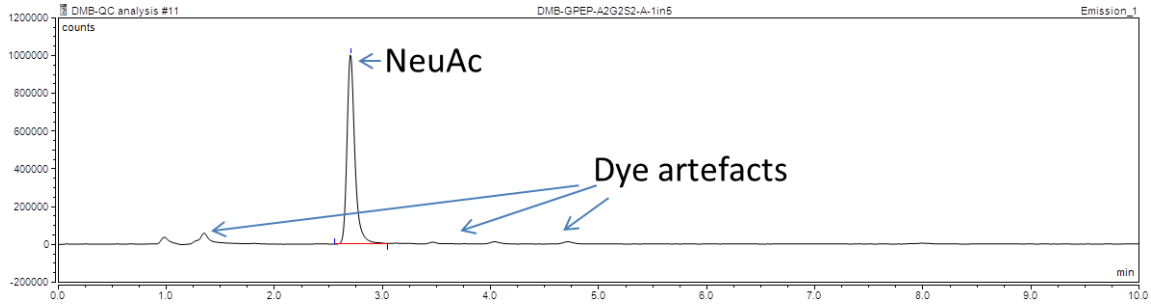


Figure 3. LudgerSep-uR2 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled Neu5Ac of acetic acid hydrolysed BQ-GPEP-A2G2S2-10U (Batch B2BE-01).

Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by NeuAc content) = 10.26 µg ± 0.54

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

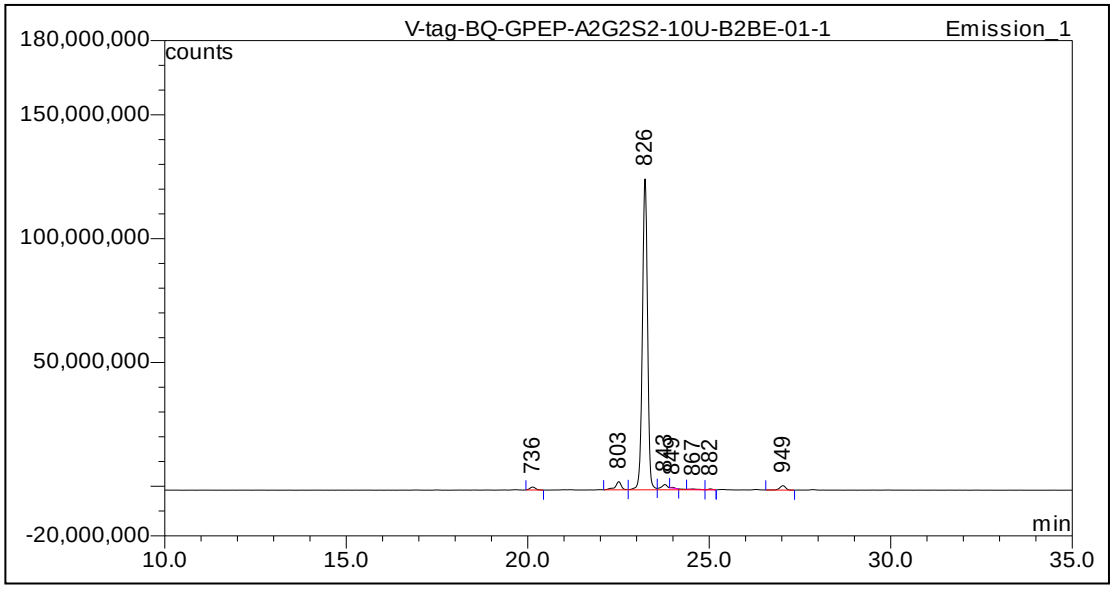


Figure 4. HILIC UHPLC profile of V-Tag (Ludger fluorophore tag) labelled BQ-GPEP-A2G2S2-10U (Batch B2BE-01).

Sample purity determined as 91% by HILIC chromatography of fluorescence tagged glycopeptide.

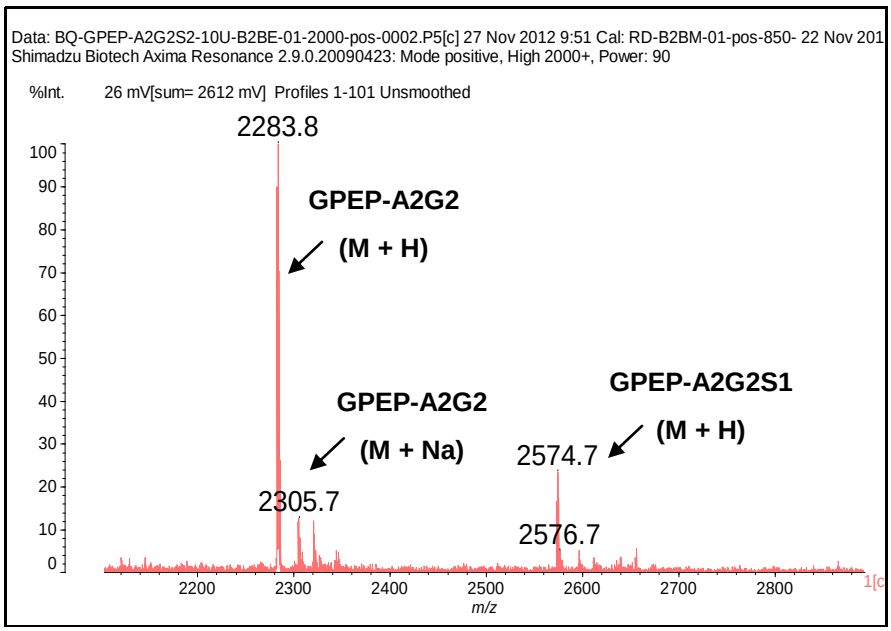


Figure 5. Positive ion MALDI mass spectrum of BQ-GPEP-A2G2S2-10U (Batch B2BE-01).

MALDI mass spectrometry analysis identified the non-sialylated form of the glycopeptide. Sialic acids are not stable in this type of mass spectrometry technique, although a small amount of monosialylated glycopeptide was identified (mass 2574 Da). Protonated, sodiated and potassiated adducts of the glycopeptide were observed.