

Ludger

## Certificate of Analysis

### BQ-GPEP-A2G2S2-10U

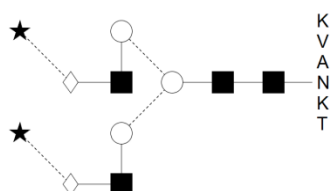
Cat. #: BQ-GPEP-A2G2S2-10U

Batch: B75P-01

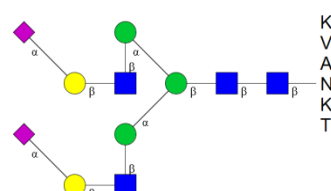
Size: 10 µg (3.49nmol)

Expiry Date: 19 Jun 2022

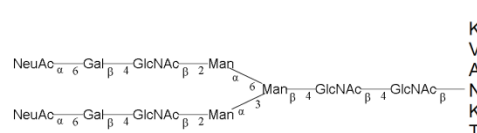
### Glycopeptide Structure



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 LC-MS**

**Monoisotopic mass: 2865.18 [M+H]<sup>+</sup>**

Storage conditions: -20°C

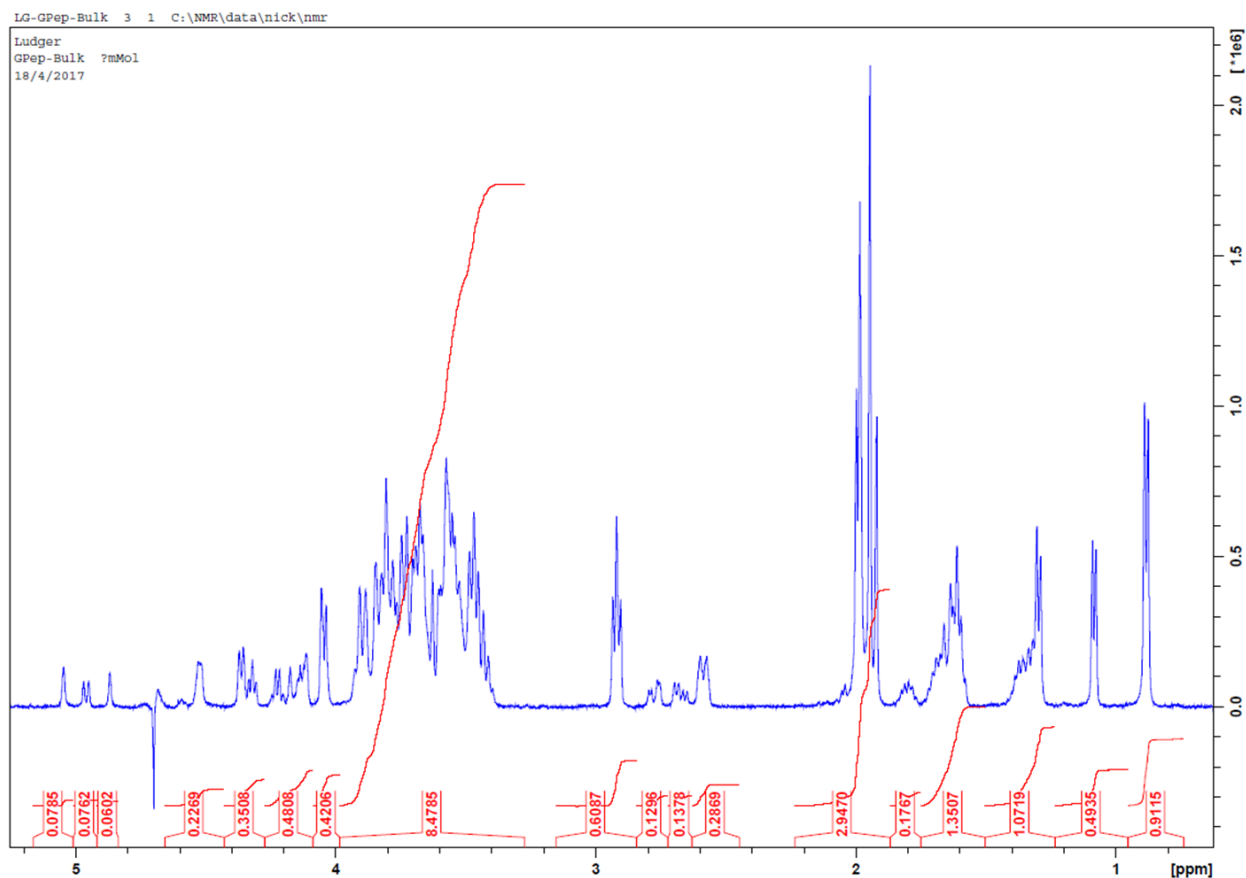
### BQ-GPEP-A2G2S2-10U Quantity Summary

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 verified by monosaccharide analysis and sialic acid analysis. These results 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 ± 0.46 (3.49nmol)
Monosaccharide based determination (GlcN – HCl hydrolysis)	=	9.81 µg ± 0.48 (3.42nmol)
Sialic acid based determination	=	9.21 µg ± 2.16 (3.21nmol)

## Quantitative Nuclear Magnetic Resonance (qNMR)



**Figure 1.**  $^1\text{H-NMR}$  (500 MHz) of BQ-GPEP-A2G2S2-Bulk in  $\text{D}_2\text{O}$  (Batch Number: B74K-01)

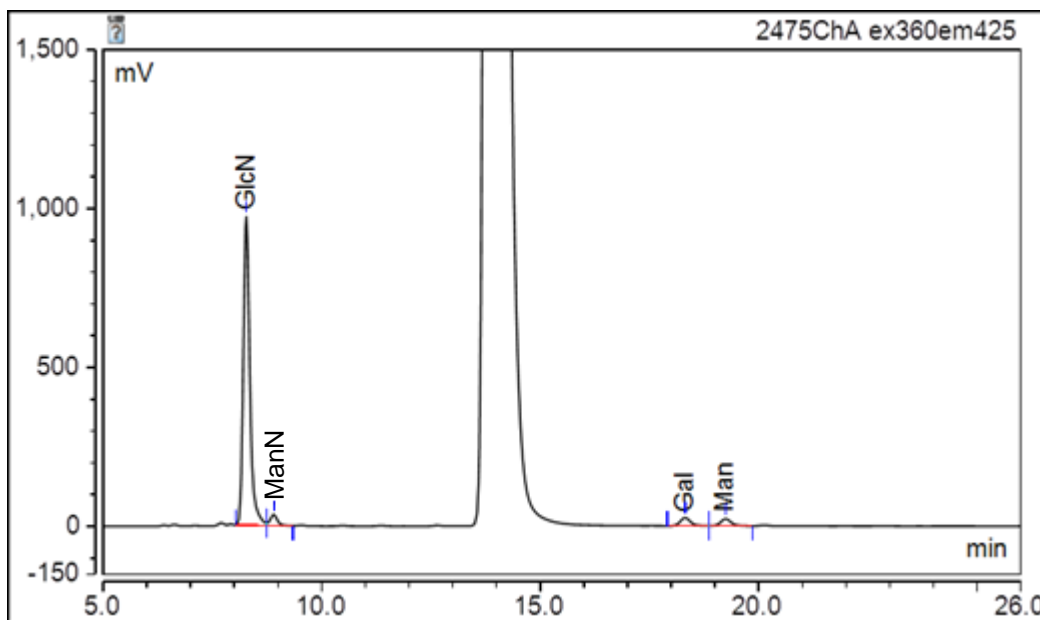
Sample	Concentration (mM) calculated using a certified quantitative standard.
BQ-GPEP-A2G2S2-Bulk	$0.31238 \pm 0.01439$

**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  $\mu\text{g}$  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 UHPLC equipped with a LudgerSep uR2 monosaccharide analysis column (LS-UR2-2.1x50).



*Figure 2. LudgerSep-R2 HPLC profile of 2-aminobenzoic acid (2-AA) labeled monosaccharides of HCl hydrolysed BQ-GPEP-A2G2S2-10U (Batch B75P-01).*

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

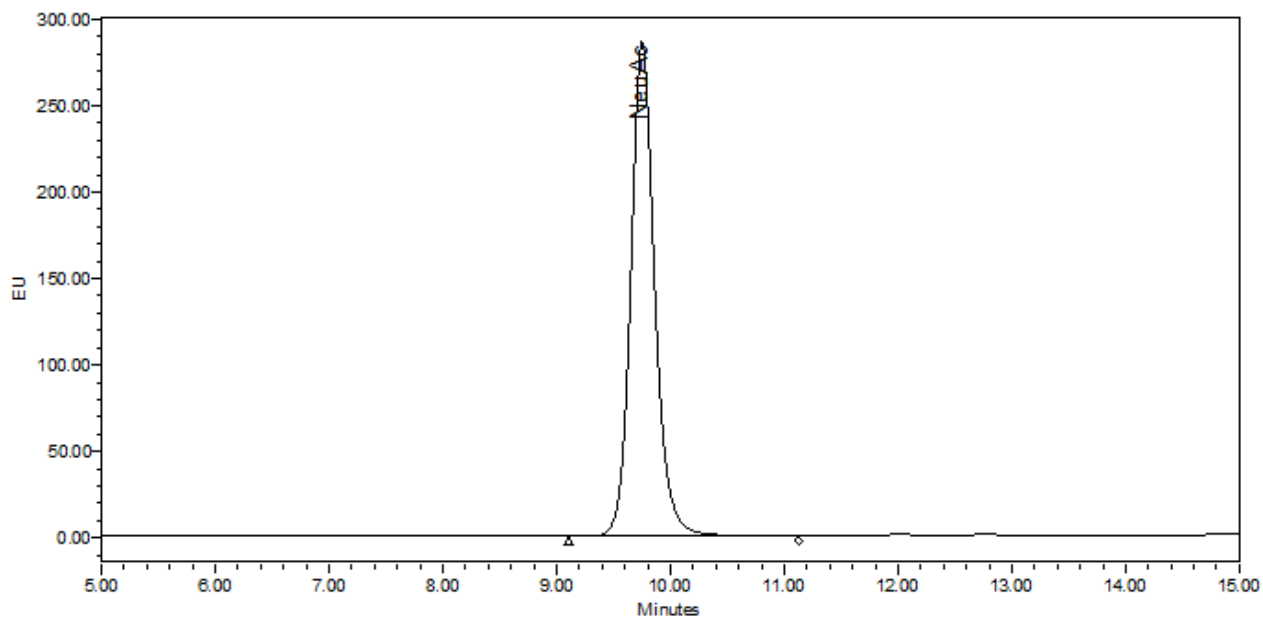
Calculation of the amount of GPEP-A2G2S2 using the GlcN value:

**Quantity of GlcN per vial =  $13.70 \pm 0.67$  nmol**

**Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by GlcN content) =  $9.81 \pm 0.48$   $\mu$ g (3.42 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 UHPLC equipped with a LudgerSep uR2 column (LS-UR2-2.1x100).

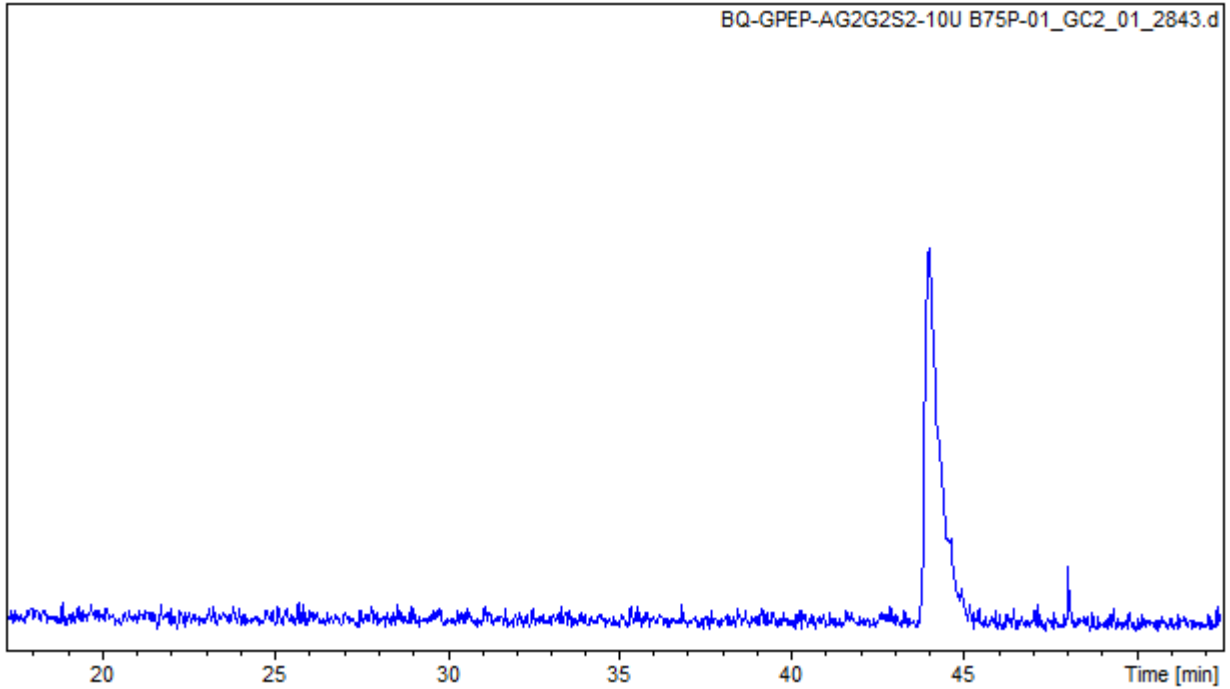


*Figure 3. LudgerSep-R1 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled Neu5Ac of acetic acid hydrolysed BQ-GPEP-A2G2S2-10U (Batch B75P-01).*

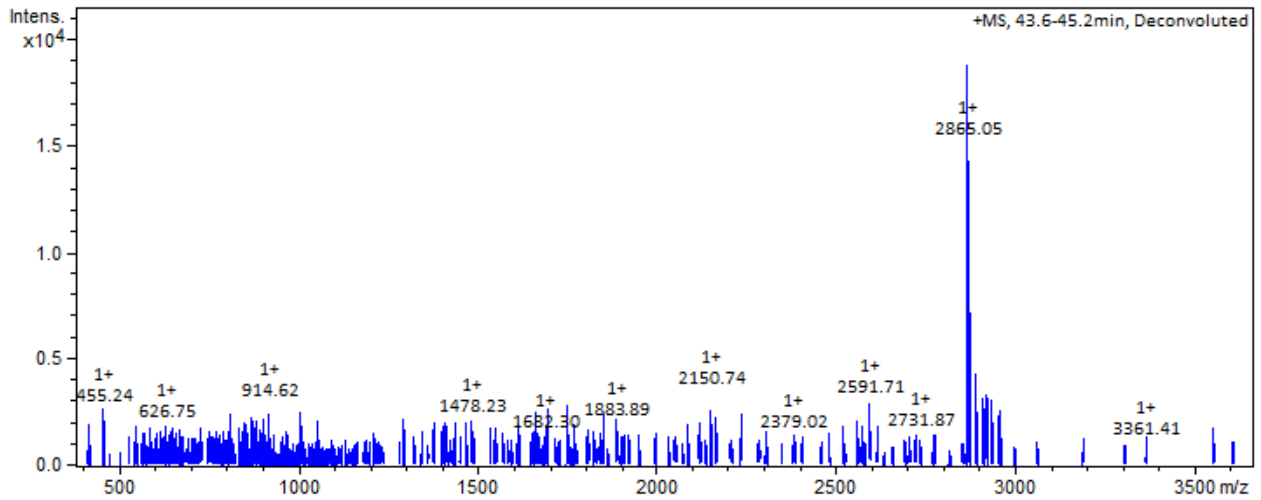
**Quantity of NeuAc per vial =  $6.43 \pm 1.51$  nmol**

**Quantity of BQ-GPEP-A2G2S2-10U per vial (determined by NeuAc content) =  $9.21 \pm 2.16$  (3.21 nmol)**

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



*Figure 4. Base Peak Chromatogram-Positive ESI mass spectrum of BQ-GPEP-A2G2S2-10U (Batch B75P-01).*



*Figure 5. Positive ion ESI mass spectrum of BQ-GPEP-A2G2S2-10U (Batch B75P-01). KVANKT-A2G2S2 theoretical mass: 2865.18 Da;*