Certificate of Analysis

Human IgG Glycoprotein Standard

Cat. #: GCP-IGG-100U Batch: B32Q-04 Nominal size: 100µg

Description: A glycoprotein standard for use during glycan release and labeling.

Source: This product is purified from human blood. Human IgG glycoprotein standard contains a mixture of gamma-type antibodies.

Glycans: The majority of glycans present on human antibodies are N-link bi-antennary types (Table 1). These glycans exist in both fucosylated and non-fucosylated forms, with and without bisecting GlcNAcs, and terminate either with 0, 1 or 2 galactoses (the G0, G1 and G2 glycan forms). Sialic acid terminating glycans are also present on native human IgGs.

Form: Dry. Lyophilised powder.

Molecular Weight: ~150kDa (protein weight only)

Amount: 123µg protein (In comparison to BSA standard, determined by BCA assay. Value rounded to nearest µg)

Storage: Refrigerate (-20°C) both before and after dissolving. This product is stable for at least 5 years as supplied.

Shipping: The product is shipped at ambient temperature.

Handling: Once dissolved avoid repeated thawing and refreezing, storage over 3 h at room temperature or above, exposure to light and long term exposure to acid as these will cause glycan desialylation.

Safety: This product is non-hazardous and has been purified from natural sources certified to be free of all hazardous material including pathogenic biological agents.

For research use only. Not for human or drug use

Analysis:

IgG glycans were released from human IgG Glycoprotein (Cat# GCP-IGG-100U) using PNGase F.

Following release the glycans were labeled using 2-Aminobenzamide (2-AB) using the LudgerTag™ 2-AB Glycan Labeling Kit (Cat# LT-KAB-A2).

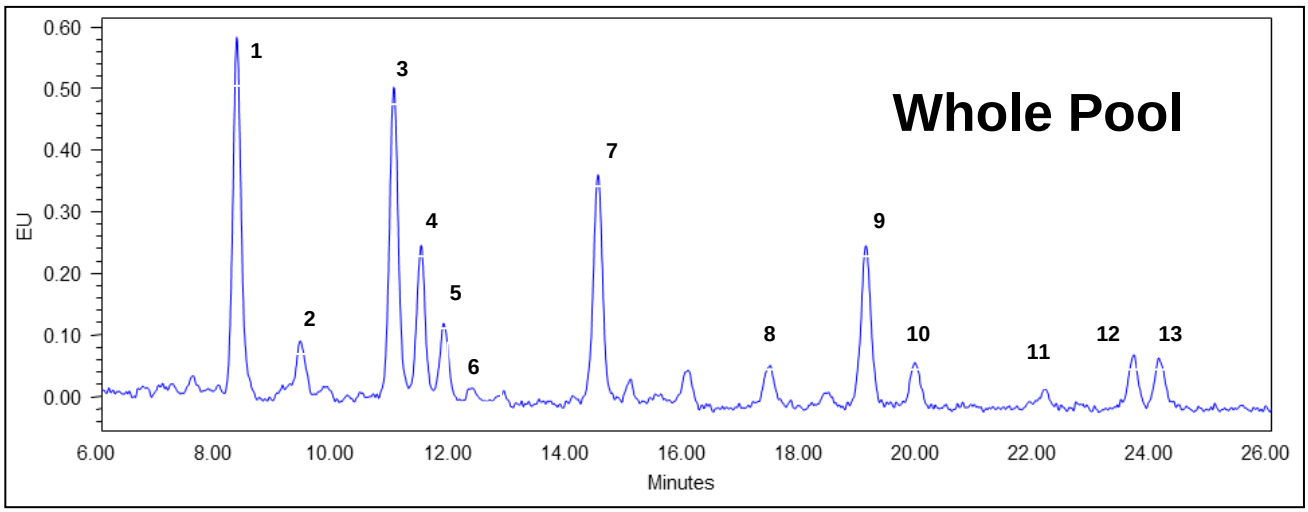

Figure 1: **HILIC HPLC profile of 2-AB labelled IgG N-glycans, released by Hydrazinolysis from GCP-IGG-100U batch B32Q-04 run on Waters BEH Glycancolumn.** See Table 1 for peak assignment.

Figure 1 shows a LudgerSepN2 HPLC profile of IgG N-glycans. To thoroughly investigate the N-glycans we first separate them based on charge on a LudgerSepC3 column (Figure 2) and then run each fraction on a LudgerSepN2 column. From these studies, combined with exoglycosidase investigation (Figure 3) ,we identified the glycans shown in Table 1. For further information on Glycoprofiling please contact us at info@ludger.com

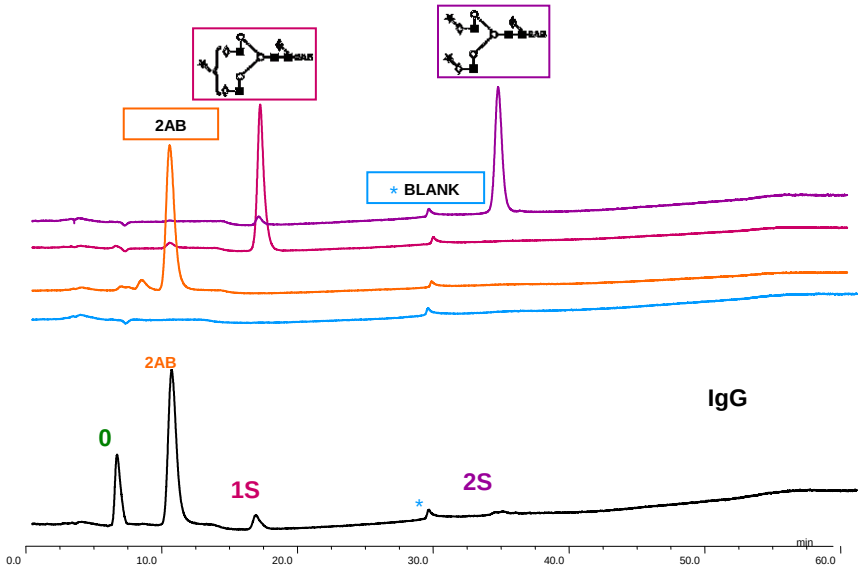

Figure 2: LudgerSep-C3 profile analysis of human IgG PNGaseF released N-glycans from a similar batch of GCP-IGG. *2-AB labelled glycans were separated on the LudgerSepC3 column. It is evident from this analysis that the proportion of sialylated glycans is relatively low (see 1S and 2S peaks above). A combination of LudgerSepC3/N2 and exoglycosidase digestion is required to identify the glycans and their relative abundance, see Table 1.*

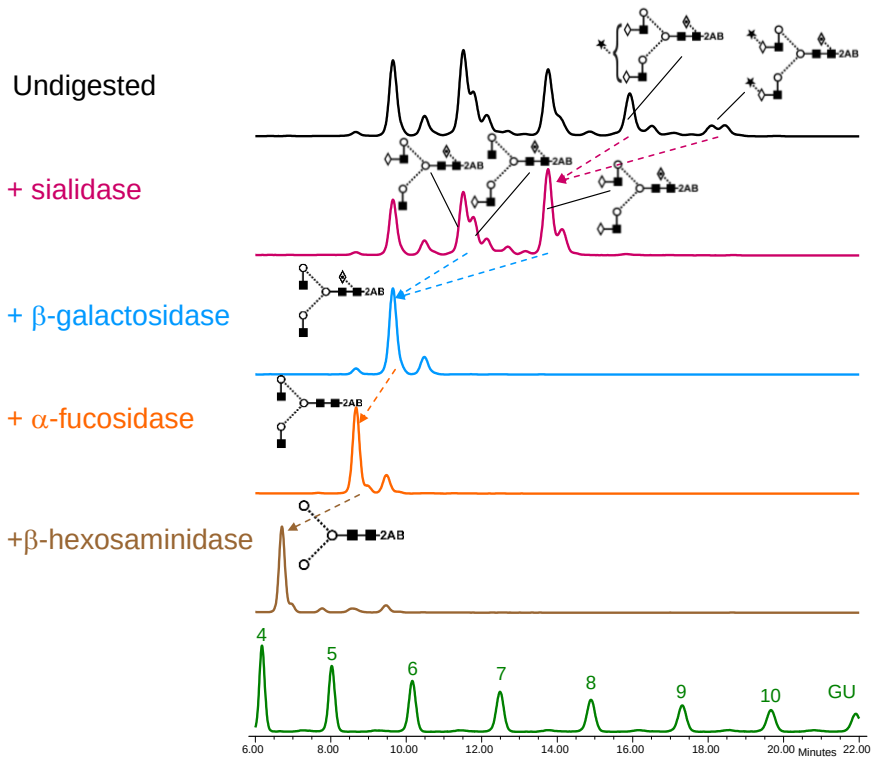

Figure 3: Example exoglycosidase analysis of Human IgG N-glycans from a similar batch of GCP-IGG. *LudgerSepN2 chromatograms are shown.*

Peak No.	Structure	GU	Whole Pool % Area
1	F(6)A2	5.76	16.5
2	F(6)A2B A2[6]G(4)1 A2[6]BG(4)1	6.14 6.14 6.14	4.9
3	A2[3]G(4)1 A2[3]BG(4)1 F(6)A2[6]G(4)1	6.59 6.59 6.59	18.2
4	F(6)A2[3]G(4)1	6.70	7.2
5	F(6)A2[6]BG(4)1 F(6)A2[3]BG(4)1	6.85 6.85	4.4
6	A2G(4)2	7.08	2.1
7	F(6)A2G(4)2 F(6)A2BG(4)2	7.53 7.53	19.4
8	A2G(4)2S1	7.98	3.0
9	F(6)A2G(4)2S1	8.42	11.6
10	F(6)A2BG(4)2S1	8.66	3.3
11	A2G(4)2S2 A2BG(4)2S2	8.90 8.90	1.6
12	F(6)A2G(4)2S2	9.32	3.6
13	F(6)A2BG(4)2S2	9.47	3.3

Table 1: Summary of Human IgG N-glycans found in a similar batch of GCP-IGG. *See the end of this document for details of the glycan nomenclature used. GU – glucose units – a system of comparing glycans to a glucose homopolymer standard. Many common N-glycans have reported GU values. A combination of GU value, mass spectrometry and exoglycosidase digestion (Figure 3), can be used to unambiguously identify most N-glycans.*

Structure Abbreviations

All N-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core fucose, (6) after the F indicates that the fucose is α1-6 linked to the inner GlcNAc; Mx, number (x) of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β1-2 linked; A3, triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-4 to the α1-3 linked mannose; A3', triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-6 to the α1-6 linked mannose; A4, GlcNAcs linked as A3 with additional GlcNAc β1-6 linked to α1-6 mannose; B, bisecting GlcNAc linked β1-4 to β1-3 mannose; Gx, number (x) of linked galactose on antenna, (4) or (3) after the G indicates that the Gal is β1-4 or β1-3 linked; [3]G1 and [6]G1 indicates that the galactose is on the antenna of the α1-3 or α1-6 mannose; Sx, number (x) of sialic acids linked to galactose; the numbers 3 or 6 in parentheses after S indicate whether the sialic acid is in an α2-3 or α2-6 linkage.

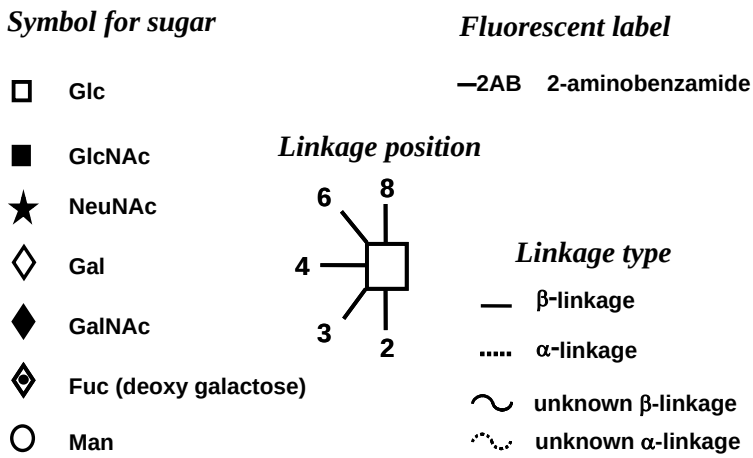

Figure 4. Symbols used to depict glycan structures.

Warranties and Liabilities

Ludger warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse Ludger will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and Ludger makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.
Ludger shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

Address

Ludger Ltd, Culham Science Centre, Oxford OX14 3EB United Kingdom

Tel: +44 1865 408 554
Fax: +44 870 163 4620
Email: info@ludger.com
www.ludger.com