LudgerTag V-Tag for N-glycan profiling and identification

Ludger

Highlights of the V-Tag System

V-Tag labeling

Allows glycan identification and quantitation using (U)HPLC. Provides data comparable to gold-standard glycoprofiling method based on 2-AB. V-Tag dye is 10 times more sensitive than 2-AB.

Sample Needed

V-Tag labeling is suitable for 10μg to 200μg of sample in a IgG type sample

Validated for GMP Labs

Validated to ICH Q2(R1) standards using IgG and Fetuin (Highly sialylated type sample)

Quick and Easy

PNGase F, labeling and clean up is completed within 2 hours for 30 IgG type samples.

Applications

This technology can be used for biopharma studies (e.g. IgG) and different biological samples (e.g. plasma)

Exoglycosidase digestion

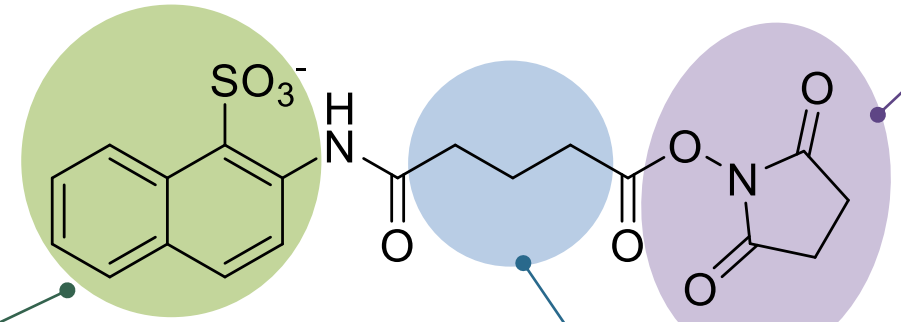
V-Tag labeled glycans are compatible with exoglycosidase digestion, including the removal of core alpha (1-6) fucose by Fucosidase from Bovine kidney (Fuc-BK)

Anatomy of V-Tag

The molecular aspects that make V-Tag work

Amine reactive succinimidyl ester

to react with the amino glycans



Fluorescent group for detection in UHPLC

λ_{ex} = 250nm, λ_{em} = 360nm

Simple, non-reactive alkyl chain to link the fluorescent moiety to the reactive succinimidyl ester

Components of the LT-VTAG-C30 Kit

1. Glycan release

Glycan release kit

LudgerZyme™ rPNGase F release kit
LZ-rPNGaseF-30

2. Labeling

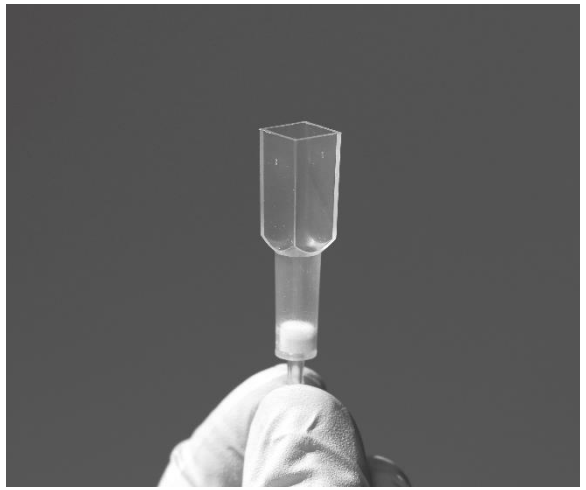
Reaction solvent

DMSO
LT-DMSO-02

Amine reactive fluorescent labeling reagent

V-Tag Labeling Dye
LT-VTAG-02

3. Clean up



HILIC resin cartridge

LudgerClean™ VSPE Cartridges
LC-VSPE-30

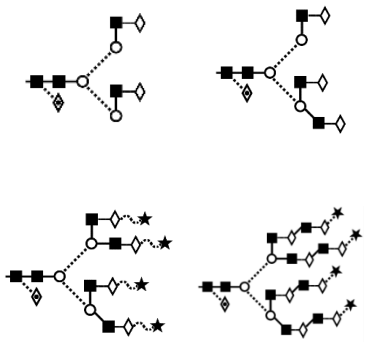
Solvents involved in HILIC clean-up

ammonium formate Buffer, pH 4.4
LC-N-BUFFX40-30

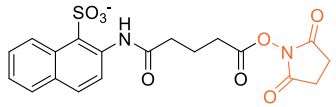
ammonium acetate Buffer
LC-AA-BUFF-30

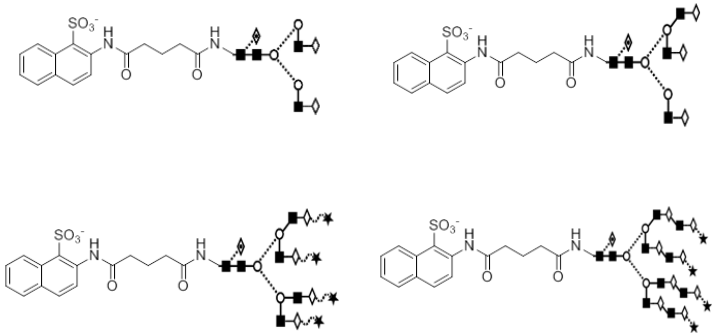
Workflow - LudgerTag V-Tag for N-glycan profiling and identification

Glycoprotein



N-glycans





V-Tag labeled glycans

Glycan release

1. Denaturation step (10min, 100°C)
2. PNGase F (10min, 37°C)

(Up to 3 hours for more complex samples)

Labeling

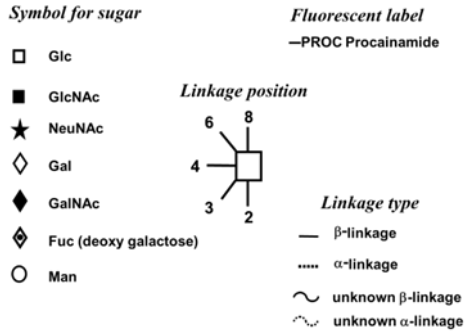
V-Tag Dye

(15min, 37°C)

Clean up

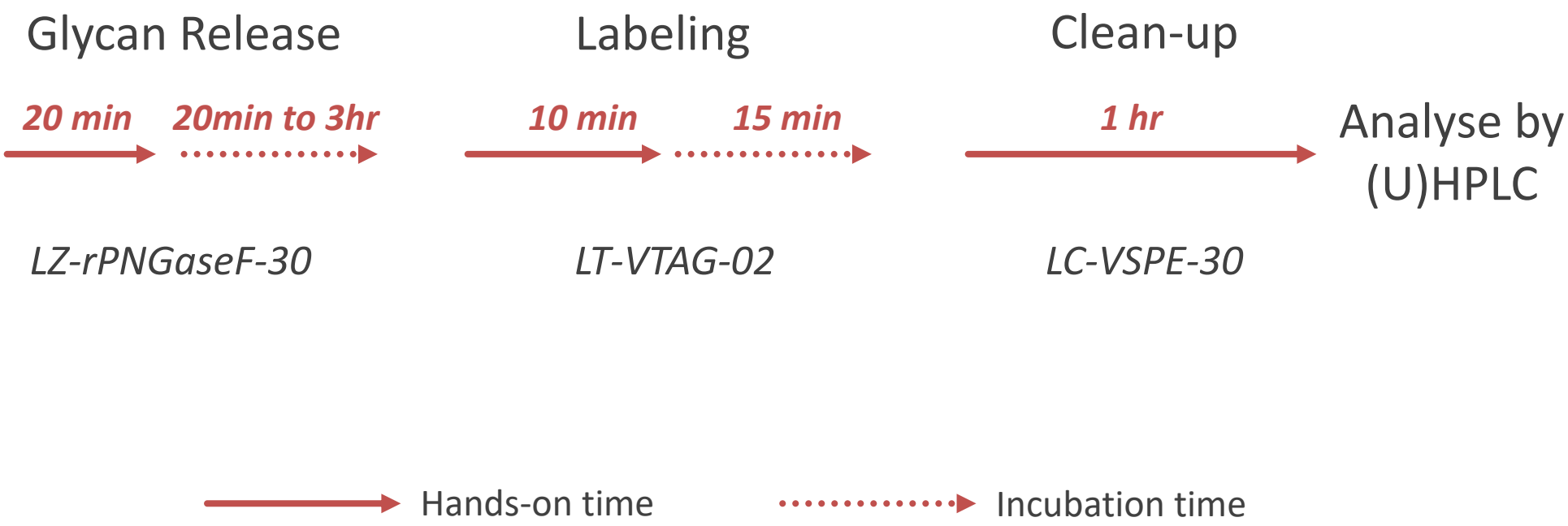
SPE clean up (1h)

Labels the amino group of the released glycans



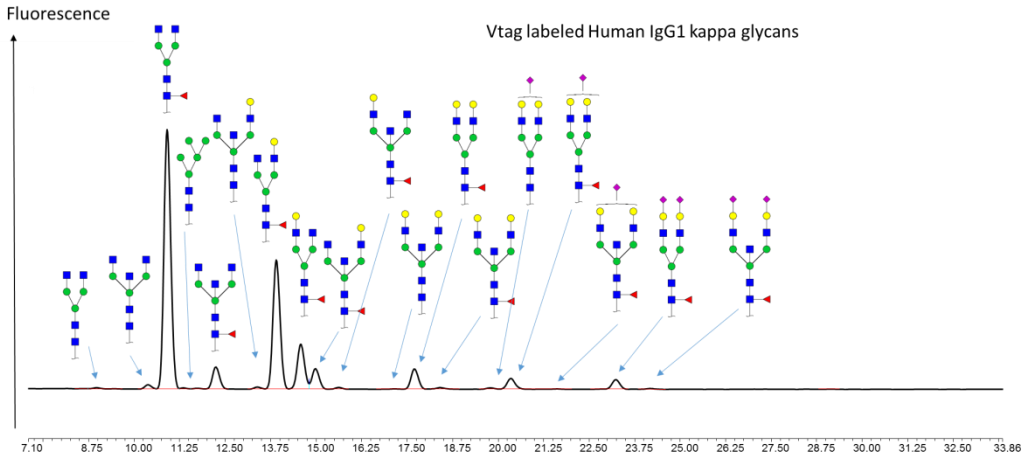
Sample preparation within a day-N-glycans

Timing for processing of 30 samples

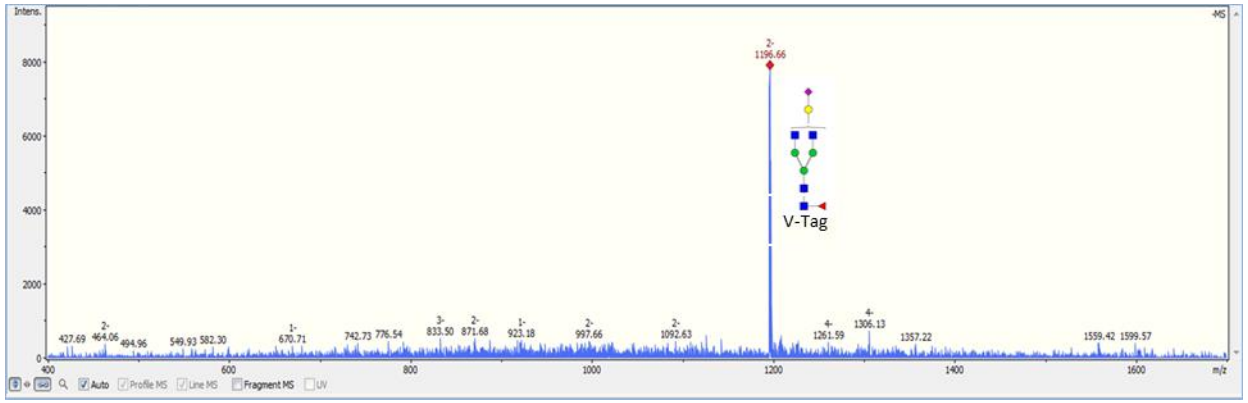


Typical data

(U)HPLC profile



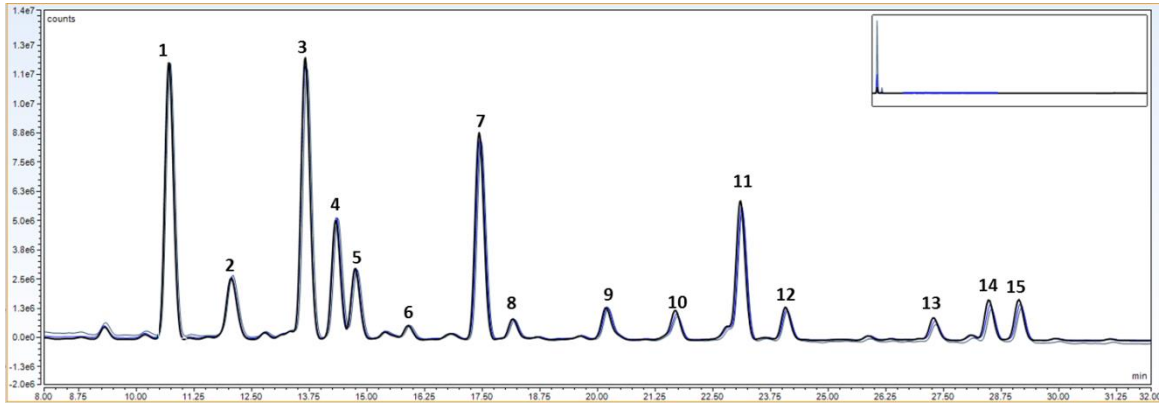
ESI-MS profile



Repeatability

V-Tag System Has Been Validated to ICH Q2(R1) Level

IgG type samples



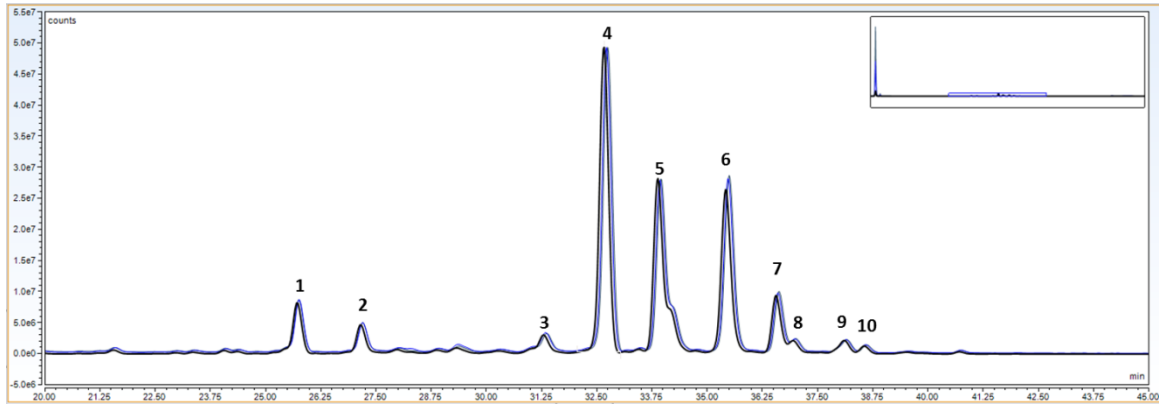
Overlaid IgG V-Tag labeled glycan profiles of nine UPLC analyses at 3 different concentrations (25µg, 50µg and 200µg)

Peak Number	Relative Area %		
	Av.	Std. Dev	CV
1	19.07	0.58	3.05
2	5.45	0.14	2.58
3	19.52	0.33	1.67
4	8.51	0.23	2.76
5	4.99	0.05	0.97
6	1.06	0.09	8.04
7	14.37	0.17	1.20
8	1.68	0.11	6.44
9	2.80	0.11	3.82
10	2.29	0.18	7.77
11	10.72	0.23	2.16
12	2.39	0.14	5.66
13	1.60	0.12	7.35
14	2.72	0.14	5.28
15	2.83	0.12	4.41

Average relative % area, SD and CVs for V-Tag labelled IgG glycans

Validation study shows repeatability for relative % areas

Highly sialylated type sample



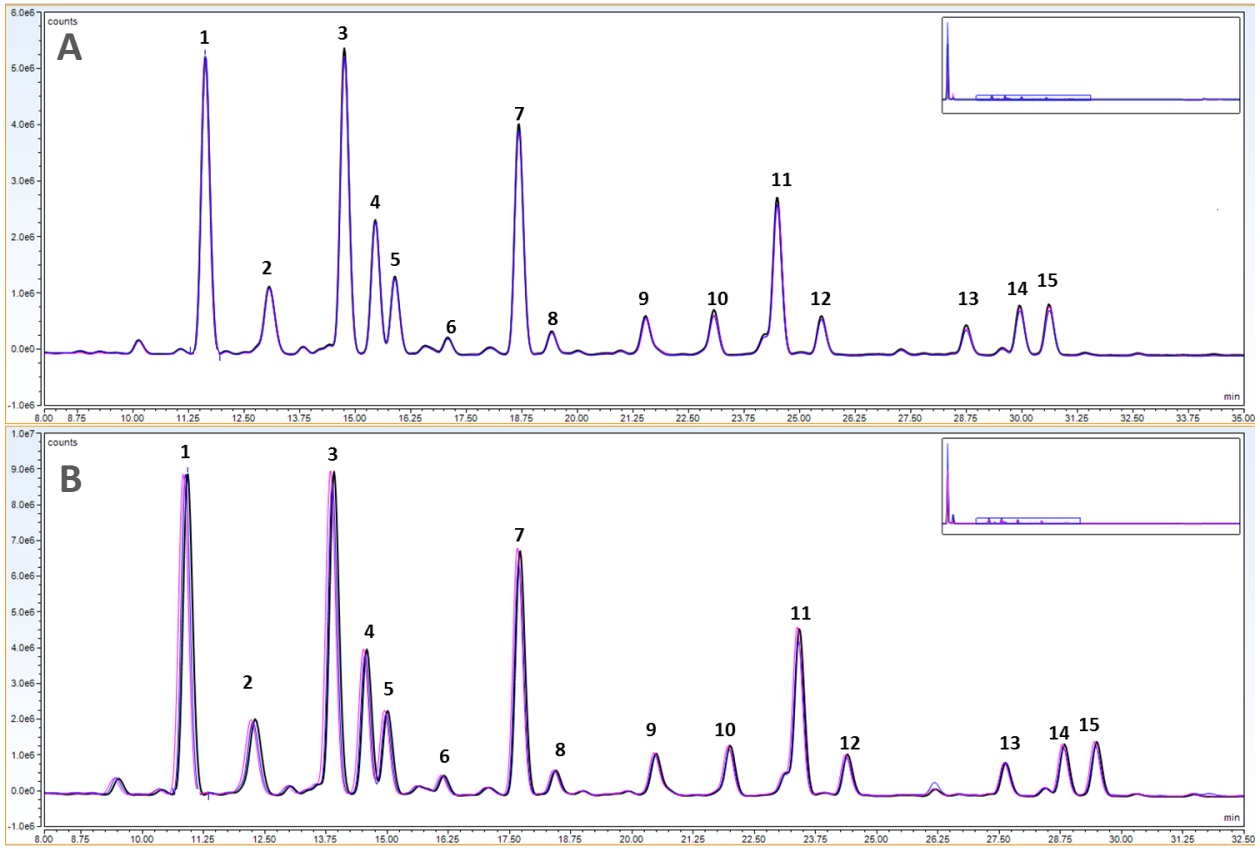
Overlaid Fetuin V-Tag labeled glycan profiles of nine UPLC analyses at 3 different concentrations (25µg, 50µg and 200µg)

Peak Number	Relative Area %		
	Av.	Std. Dev	CV
1	5.82	0.12	2.03
2	3.28	0.09	2.73
3	2.95	0.15	5.08
4	33.48	0.39	1.16
5	23.50	0.27	1.13
6	20.14	0.52	2.56
7	6.68	0.11	1.63
8	1.55	0.05	3.51
9	1.77	0.03	1.63
10	0.83	0.04	4.20

Average relative % area, SD and CVs for V-Tag labeled Fetuin glycans

Intermediate Precision

IgG type sample

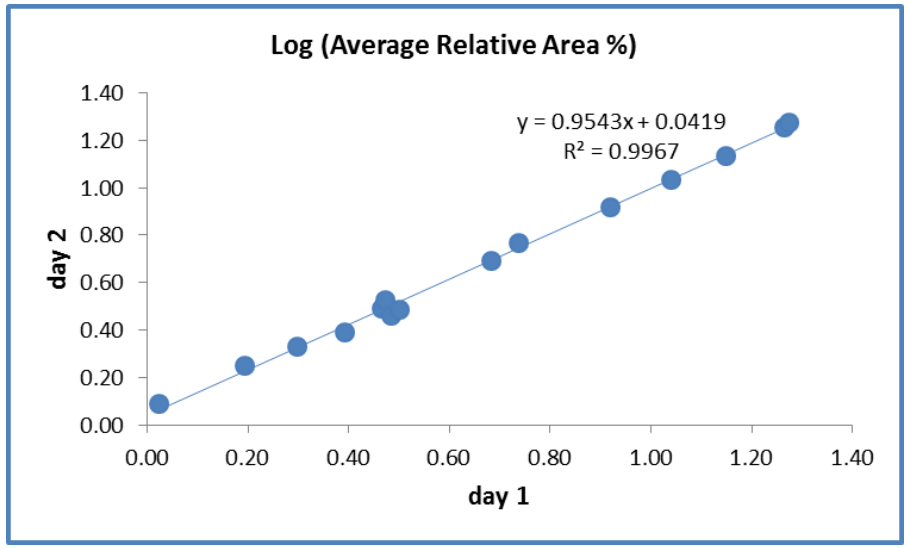


Overlaid IgG V-Tag labeled glycans profiles from day 1 (A) and day 2 (B) both run in triplicate (90µg of glycoprotein used)

The variation between samples when processed on two separated days

	Relative Area %					
	Day 1	Day 2	Day 1	Day 2		
Peak number	average	average	log Av	log Av	Ratio Av	% Variation
1	18.38	18.03	1.26	1.26	1.02	1.94
2	5.46	5.84	0.74	0.77	0.93	6.58
3	18.79	18.71	1.27	1.27	1.00	0.44
4	8.31	8.24	0.92	0.92	1.01	0.85
5	4.84	4.89	0.68	0.69	0.99	1.02
6	1.06	1.22	0.02	0.09	0.86	13.58
7	14.07	13.58	1.15	1.13	1.04	3.60
8	1.56	1.77	0.19	0.25	0.88	11.60
9	2.93	3.07	0.47	0.49	0.95	4.79
10	2.97	3.34	0.47	0.52	0.89	11.20
11	10.96	10.75	1.04	1.03	1.02	1.89
12	2.47	2.44	0.39	0.39	1.01	1.16
13	1.99	2.14	0.30	0.33	0.93	7.01
14	3.06	2.90	0.49	0.46	1.06	5.55
15	3.17	3.07	0.50	0.49	1.03	3.08

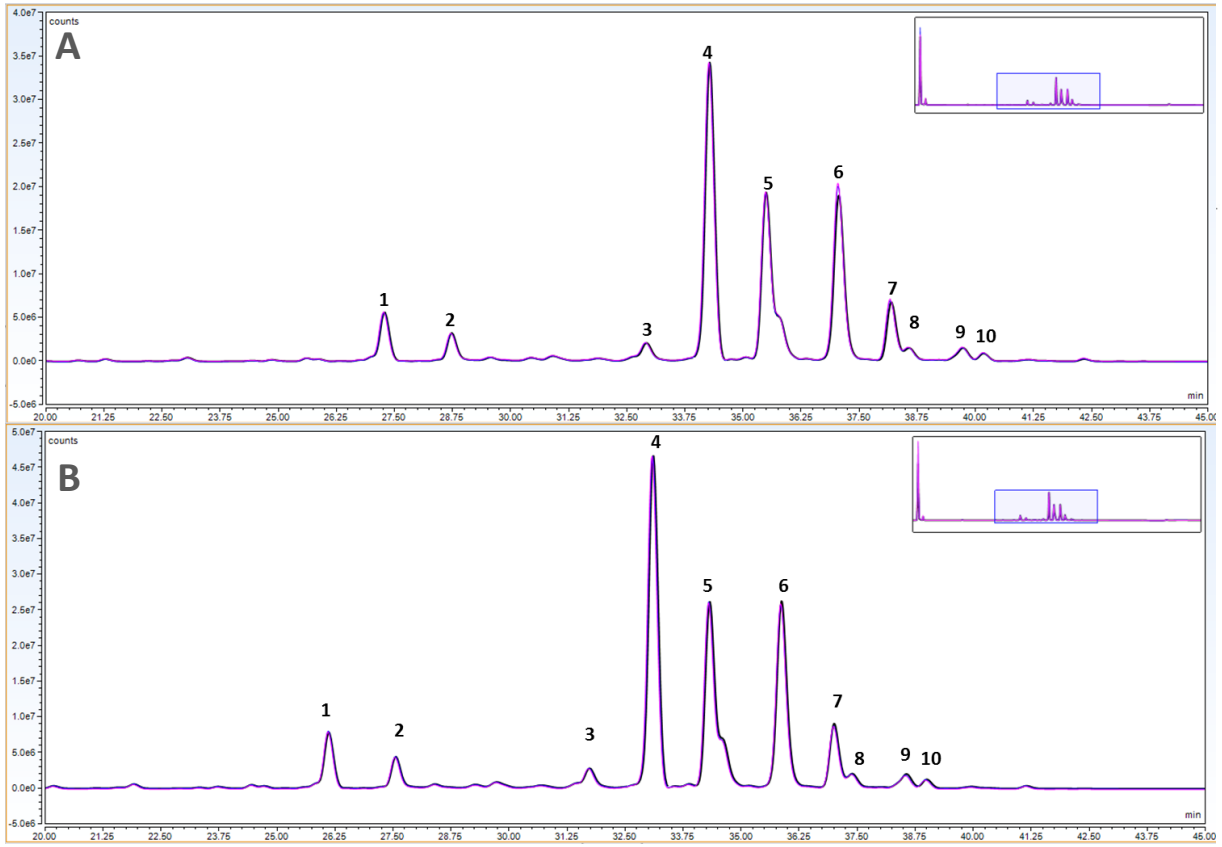
Average relative % area, SD and CVs for V-Tag labeled IgG glycans from day 1 and 2



IgG V-Tag average relative area % from day 1 compared to day 2.

Intermediate Precision

Highly sialylated type sample

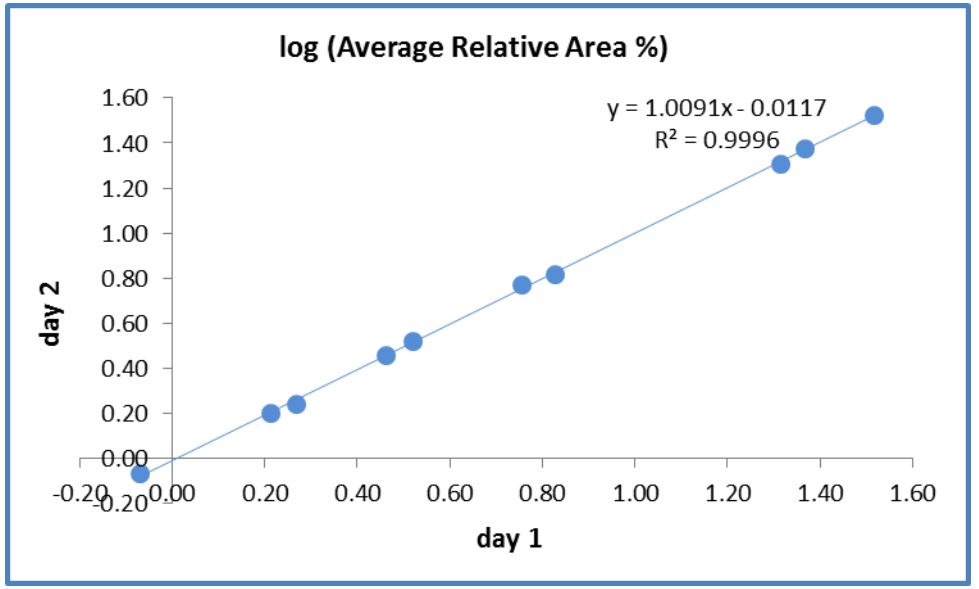


Overlaid Fetuin V-Tag labeled glycans profiles from day 1 (A) and day 2 (B) both run in triplicate (90µg of glycoprotein used)

The variation between samples when processed on two separated days

	Relative Area %					
	Day 1	Day 2	Day 1	Day 2		
Peak number	average	average	log Av	log Av	Ratio Av	% Variation
1	5.72	5.87	0.76	0.77	0.97	2.58
2	3.31	3.31	0.52	0.52	1.00	0.01
3	2.89	2.86	0.46	0.46	1.01	1.17
4	32.98	33.43	1.52	1.52	0.99	1.34
5	23.34	23.56	1.37	1.37	0.99	0.91
6	20.67	20.28	1.32	1.31	1.02	1.91
7	6.75	6.54	0.83	0.82	1.03	3.20
8	1.63	1.57	0.21	0.20	1.04	3.90
9	1.85	1.73	0.27	0.24	1.07	7.22
10	0.85	0.85	-0.07	-0.07	1.00	0.23

Average relative % area, SD and CVs for V-Tag labeled Fetuin glycans from day 1 and 2

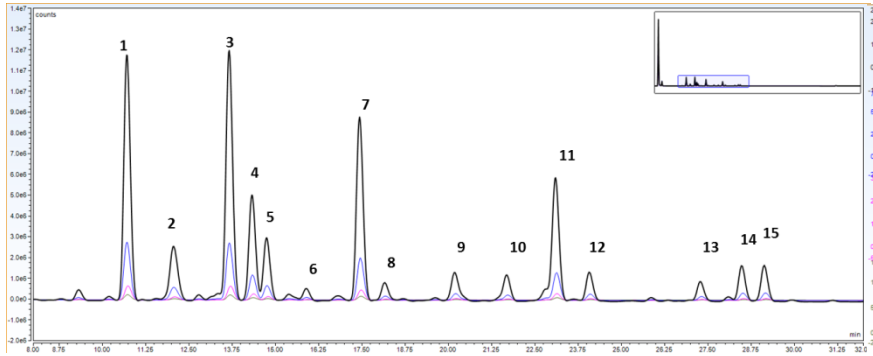


Fetuin V-Tag average relative area % from day 1 compared to day 2.

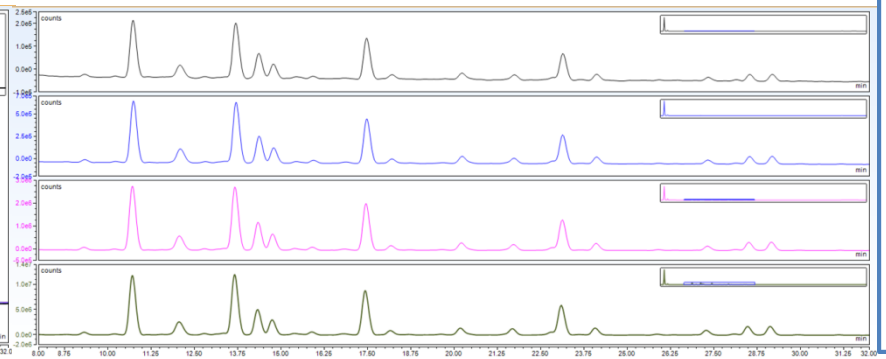
Linearity and Working range

V-Tag System Has Been Validated to ICH Q2(R1) Level

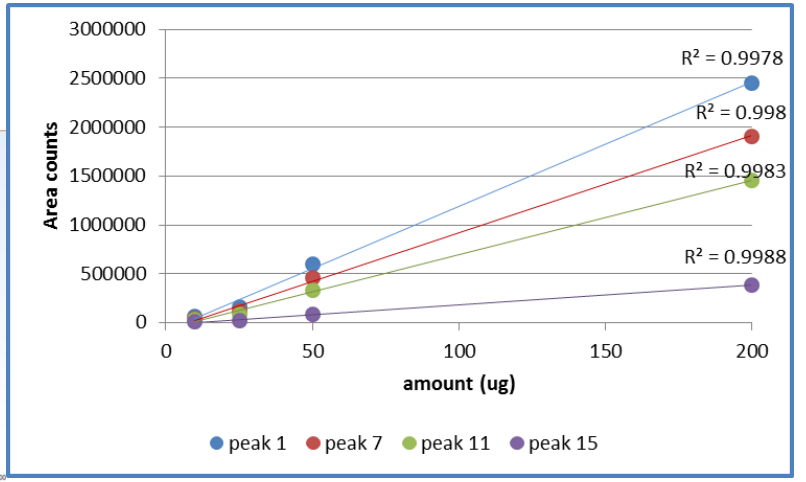
IgG type samples



Overlay plot of one replicate for each amount of IgG taken through labeling (10µg, 25µg, 50µg and 200µg)

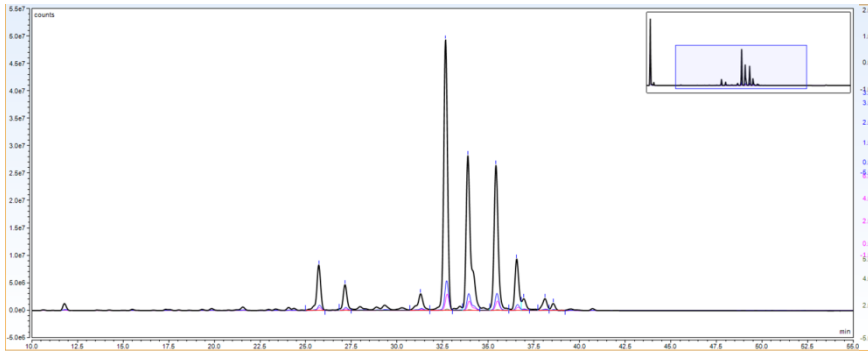


Stacked plot of one replicate for each amount of IgG taken through labeling.

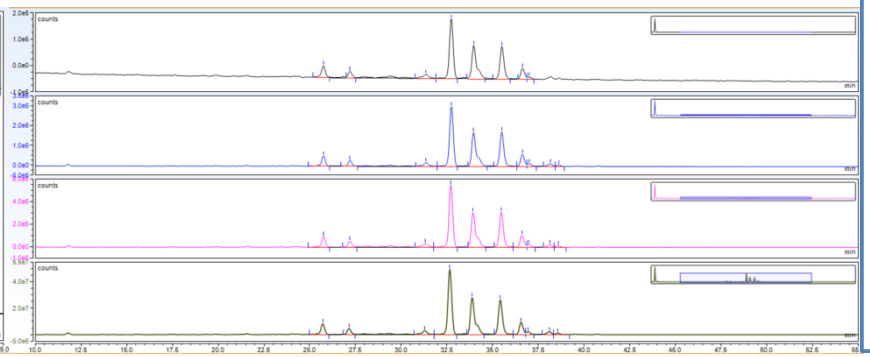


IgG area counts for peaks 1, 7, 11 and 15 versus the starting amount of IgG for each labeling.

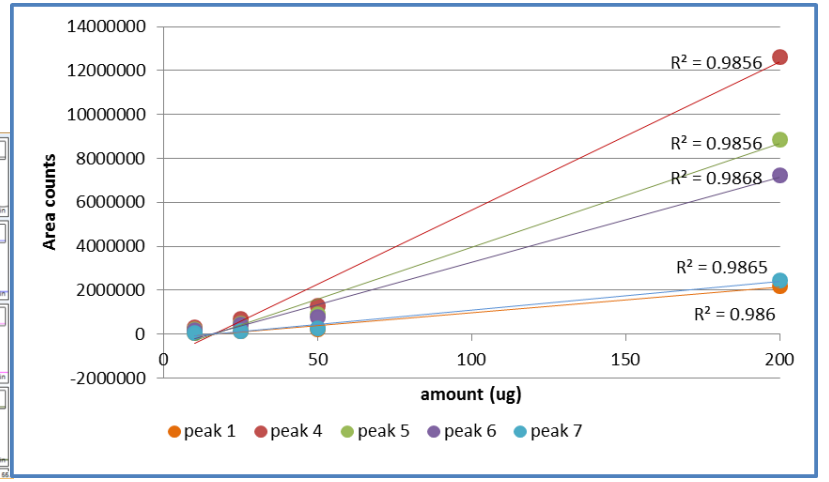
Highly sialylated type sample



Overlay plot of one replicate for each amount of Fetuin taken through labeling (10µg, 25µg, 50µg and 200µg)



Stacked plot of one replicate for each amount of Fetuin taken through labeling.

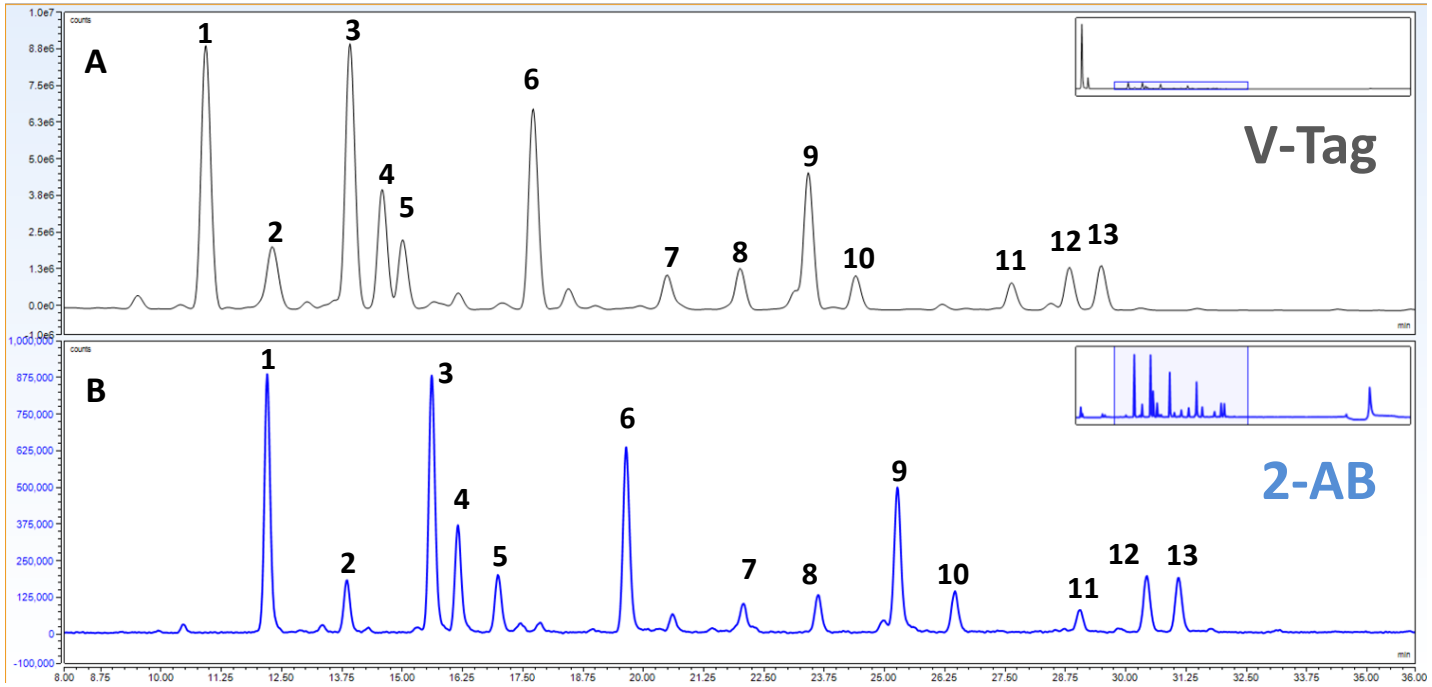


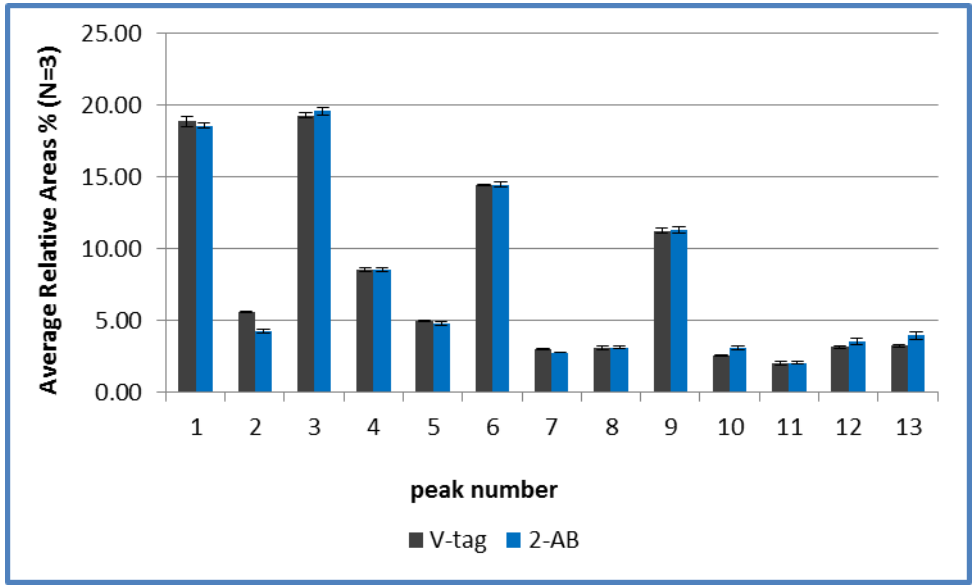
Fetuin area counts for peaks 1, 4, 5, 6 and 7 versus the starting amount of Fetuin for each labeling.

Comparability of V-Tag and 2-AB

IgG type sample

Relative area comparison





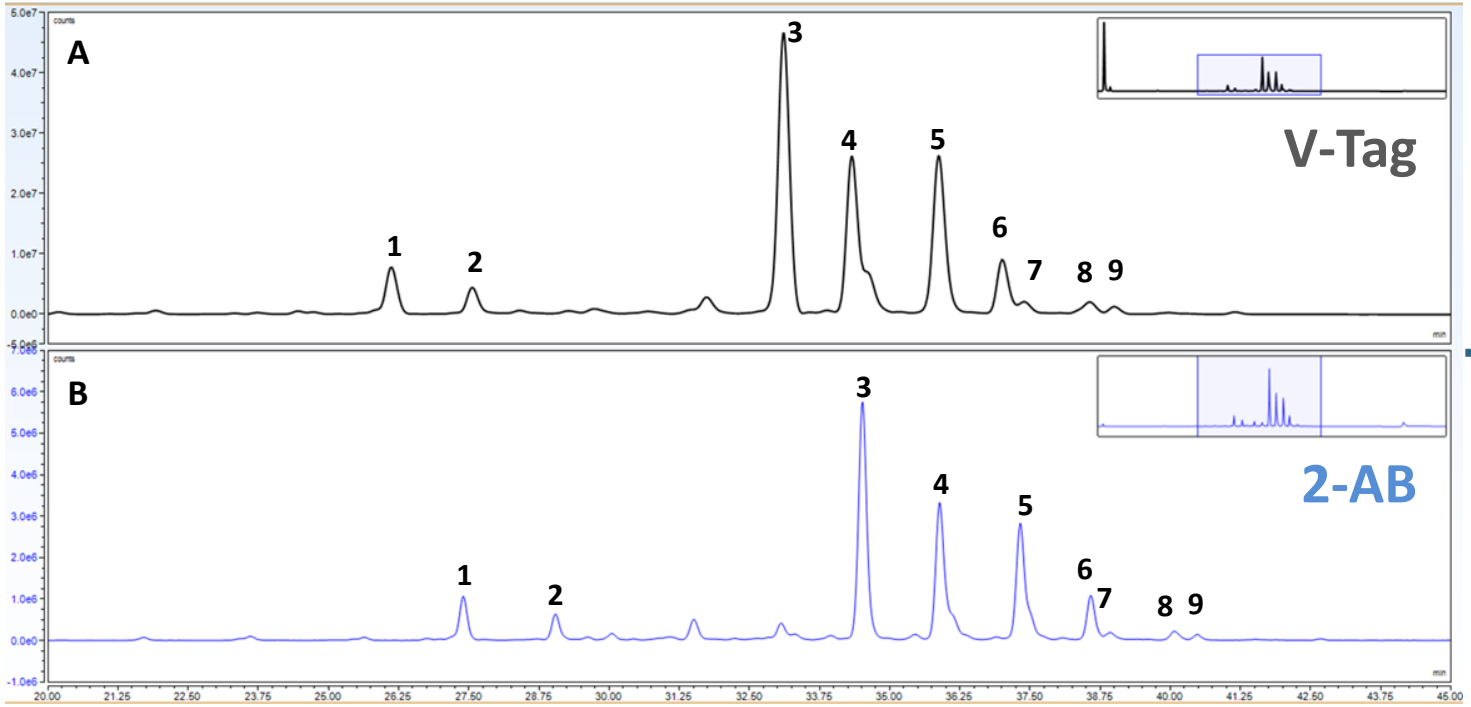
A) UHPLC chromatogram of V-Tag labeled glycans from IgG after using LT-VTAG-30.
B) UHPLC chromatogram of 2-AB labeled glycans from IgG after overnight PNGase F release, 1 hour labeling with 2AB and LudgerClean T1 purification.

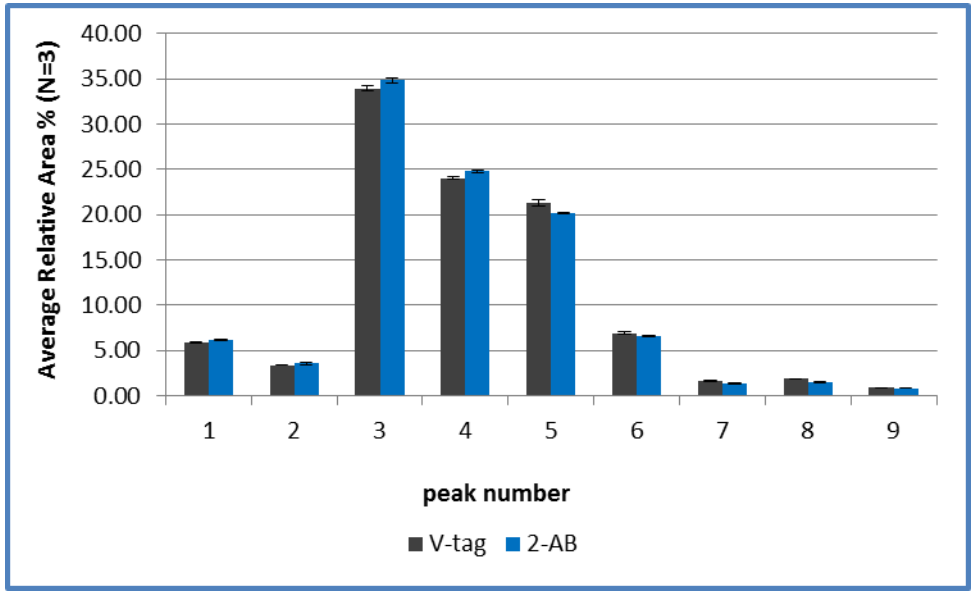
Comparable in quantitation and in reliability, using a HILIC column

Comparability of V-Tag and 2-AB

Highly sialylated type sample

Relative area comparison





A) UHPLC chromatogram of V-Tag labeled glycans from Fetuin after using LT-VTAG-30.
B) UHPLC chromatogram of 2-AB labeled glycans from Fetuin after overnight PNGase F release, 1 hour labeling with 2AB and LudgerClean T1 purification.

Comparable in quantitation and in reliability, using a HILIC column

Exoglycosidase digestion of V-Tag labeled N-glycans

UHPLC chromatogram of V-Tag labeled glycans from IgG after exoglycosidase digestion

Exoglycosidase digestion of V-Tag labeled N-glycans is possible, including the removal of core alpha (1-6) fucose by Fucosidase from Bovine kidney (Fuc-BK)

Und: undigested; Sial: Sialidase from Arthrobacter ureafaciens: specific for α2-3, 6, 8, 9 sialic acids [Ludger, E-S001]; Fuc (BK): fucosidase from Bovine kidney, specific for α1-6>2 fucose [Sigma, F-5884]; Fuc (EM): fucosidase from from Elizabethkingia miricola: specific for α1-6 fucose [E-F006]; Gal: β-galactosidase from Streptococcus pneumoniae: specific for β1-4 galactose [Ludger, E-BG-07]

Next Steps ...

If you have a question

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