GENOVIS

SmartEnzymes™ for Better Biologics

Nature offers a vast source of enzymes, perfected through evolution to perform defined reactions.

At Genovis, we believe that enzymes with unique properties can be used as biological tools to support the research and development of complex biopharmaceuticals to help bring safe and effective medicines to patients in need.

Our task is to identify new enzymes and give them names.

We call them *SmartEnzymes*.

FabALACTICA®
Above hinge
digestion of human IgG1

FabDELLO™
Above hinge
digestion of human IgG1

FabRICATOR®
Below hinge
digestion of IgG

FabRICATOR® Z
Below hinge
digestion of mouse IgG

ANTIBODY DIGESTION

FucosEXO™
Hydrolysis of
α1-2,3,4 fucose

GalactEXO™
Hydrolysis of β1-3,4-
linked galactose

GalNAcEXO™
Hydrolysis of
α-linked GalNAcs

OglyZOR®
Hydrolysis of
core-1 O-glycans

GLYCAN PROFILING

GlycINATOR®
Hydrolysis of
IgG Fc glycans

IgGZERO®
Hydrolysis of
complex Fc glycans

GlyCLICK®
Site-specific
conjugation of native IgG

TransGLYCIT™
Transglycosylation
of IgG

ANTIBODY DEGLYCOSYLATION

ANTIBODY CONJUGATION

FabULOUS™
Above hinge
digestion of IgG

GingisKHAN®
Above hinge
digestion of human IgG1

GlySERIAS™
Hydrolysis of
flexible linkers

GingisREX®
Arginine-specific
protein digestion

PROTEOMICS

OmniGLYZOR™
Hydrolysis of N- and
Mucin-type O-glycans

OpeRATOR®
O-glycan-specific
protein digestion

PNGase F
Hydrolysis of
N-glycans

SialEXO®
Hydrolysis of
sialic acids

GlycOCATCH®
Enrichment of
O-glycoproteins

AFFINITY PURIFICATION

Anti-FabRICATOR®
Detection of the
FabRICATOR enzyme

RESEARCH ANTIBODIES

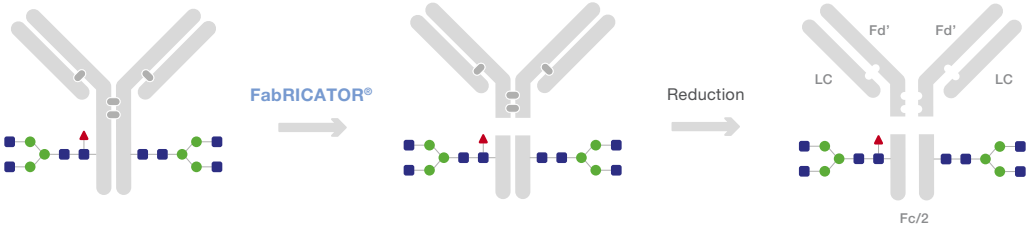
FabRICATOR®

Below Hinge Digestion of IgG

FabRICATOR (IdeS) is an IgG-specific cysteine protease that digests antibodies at a single amino acid site below the hinge, generating homogenous F(ab')2 and Fc fragments. The enzyme is widely used in characterization, quality control, stability testing, production monitoring and clone selection of antibody-based therapeutics such as mAbs, ADCs, biosimilars and Fc-fusion proteins.

- Human IgG1-4, IgG of some classes from monkey, rat, rabbit, sheep
- CPAPELLG / GPSVF
- 30 min reaction
- No reducing agents or co-factors needed

Antibody Subunit Workflow



The FabRICATOR enzyme digests antibodies in 30 minutes at 37°C to generate intact F(ab')2 and Fc subunits without the need for optimization and no risk of over-digestion, otherwise common using proteases such as papain, ficin or pepsin. For sample preparation in middle-level LC-MS workflows, the subunits can be reduced to generate 23-25 kDa fragments allowing increased mass resolution for accurate determination of critical quality attributes.

Product Formats

FabRICATOR®
Lyophilized enzyme for digestion of IgG

FragIT™
Immobilized enzyme for digestion of IgG in spin columns

FragIT™ kit
Immobilized enzyme and CaptureSelect™ Fc for purification of IgG subunits

FabRICATOR®-HPLC
Immobilized enzyme on HPLC-compatible resin for on-column digestion

FabRICATOR® MagIC
Immobilized enzyme on magnetic beads for automated workflows

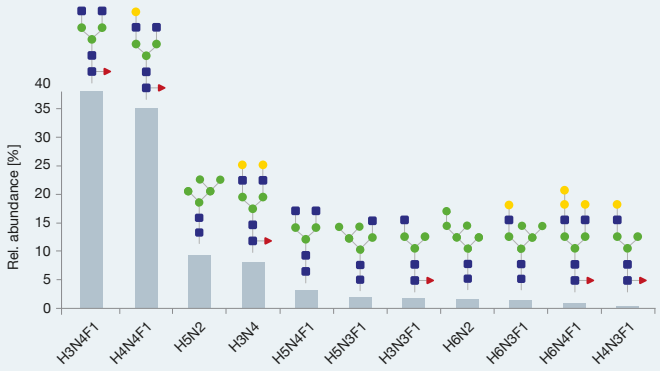
FabRICATOR® Validation kit
Three different batches for validation of FabRICATOR-based analytical methods

Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries.
Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

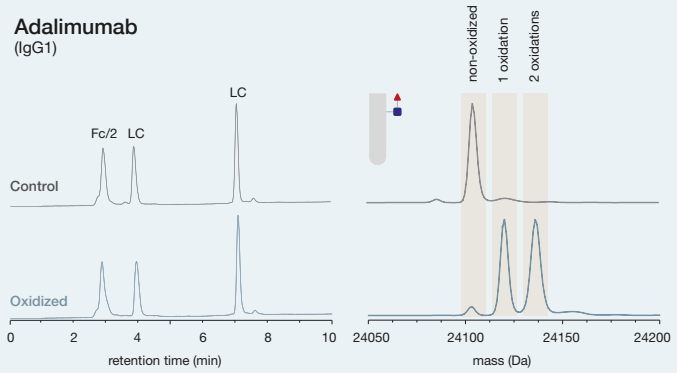
Determining Antibody Quality Attributes using FabRICATOR®

The homogenous fragments generated with FabRICATOR can be mono-isotopically resolved for in-depth mapping of attributes that may be unresolved at the intact level. This middle-level workflow can be used for amino acid sequence verification and oxidation analysis of quality attributes, including deamidation, pyroglutamination and glycan profiling. The relative abundance of eleven Fc glycans were quantified on a therapeutic antibody after FabRICATOR digestion for increased mass resolution, and no additional digestion or labeling were needed for similarity assessment.

Digestion of antibodies into homogenous subunits allows for middle-level analysis for rapid monitoring of changes that may occur during storage and formulation, such as methionine oxidation. A therapeutic antibody subjected to forced oxidation was site-specifically digested with FabRICATOR, deglycosylated by GlycINATOR and analyzed at the subunit level. Domain specific data was obtained in the analysis of oxidation events as shown in the deconvoluted mass spectra for the Fc/2 fragment. The analysis was performed in a high throughput format.



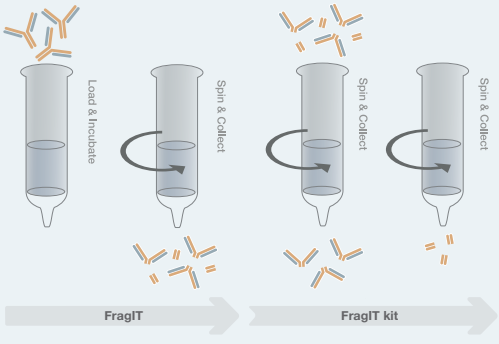
Determining antibody quality attributes. Relative abundance of glycans on cetuximab Fc/2 fragments, generated with FabRICATOR and analyzed with LC-MS at the subunit level by Ayoub and colleagues (Ayoub, 2013).



Quality control of antibody oxidation. Oxidation analysis of adalimumab showing UV (280 nm) chromatograms before (control) and after (oxidized) incubation with 0.25% H_2O_2. A deconvoluted mass spectrum of the deglycosylated Fc/2 fragments demonstrates the degree of oxidation.

Preparative Generation of F(ab')2 and Fc/2 Subunits with FragIT™ Kit

For applications that require fast and convenient digestion of antibodies or Fc-fusion proteins without residual enzyme in the preparation, FragIT spin columns that contain FabRICATOR immobilized on agarose beads can be used. With FragIT kit, the homogenous F(ab')2 and Fc fragments can also be separated using CaptureSelect™ affinity purification columns.

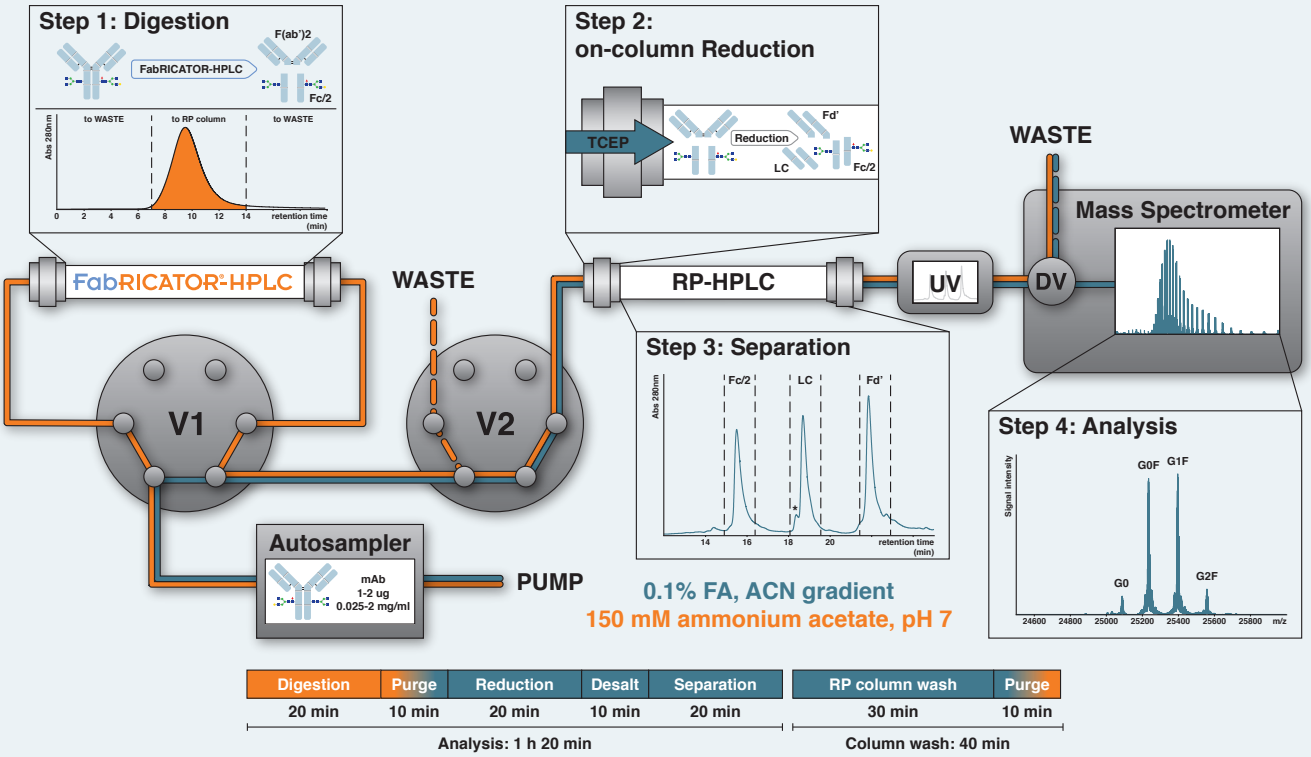


References

Ayoub, D. et al., 2013. *Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques.* mAbs, 5(5), pp.699–710.

On-column Digestion for Middle-Level Workflows with FabRICATOR®-HPLC

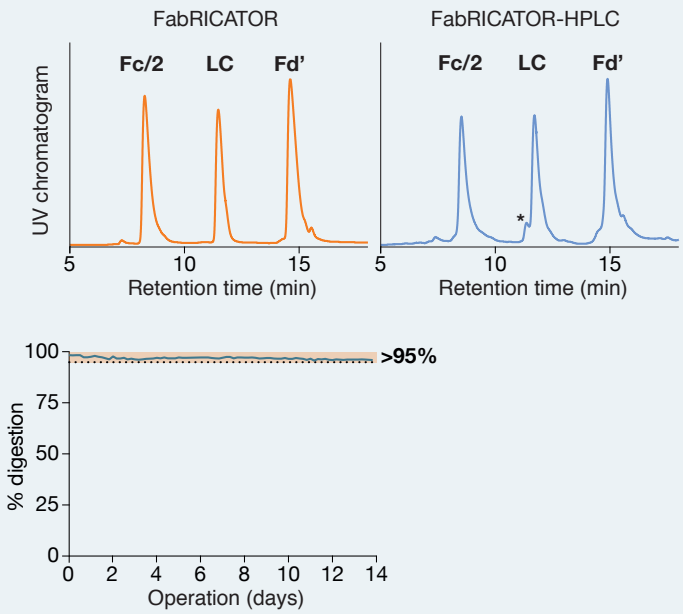
FabRICATOR-HPLC contains the FabRICATOR enzyme immobilized on an HPLC-compatible resin for consistent and reproducible on-column antibody digestion. The FabRICATOR-HPLC column can be used in a standard LC-MS routine analysis, but more advanced configurations are possible, for example with a 2D-LC setup. Ultimately, a bioreactor can be connected directly to the MS in an automated online middle-level workflow. This would significantly reduce operator time, sample handling errors and increase throughput.



FabRICATOR-HPLC in a middle-level workflow. Potential set-up for an automated middle-level workflow using FabRICATOR-HPLC that digests IgG to F(ab')2 and Fc, which is well suited for high resolution MS analysis.

Robust On-column Performance

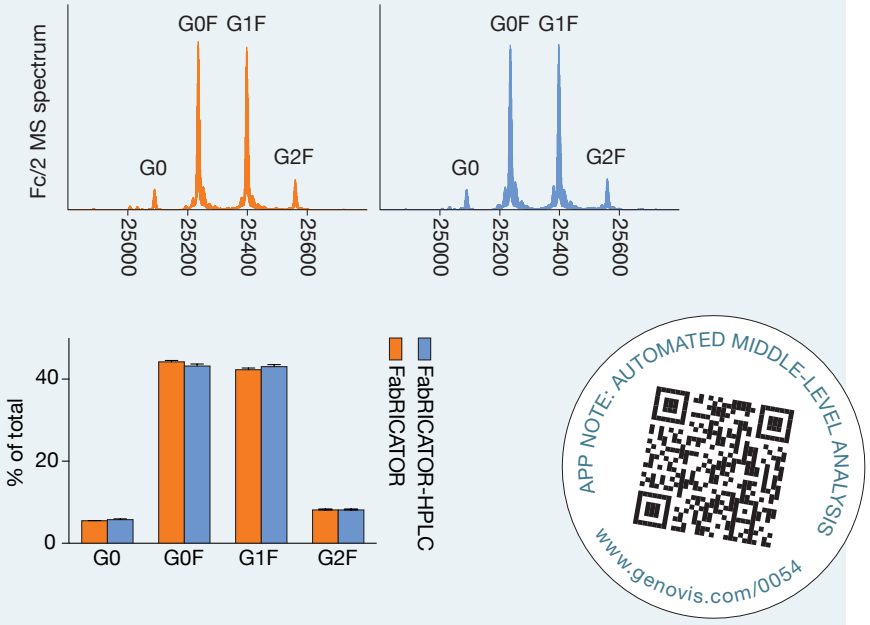
The on-column digestion efficiency was maintained by FabRICATOR-HPLC during continuous operation at 37°C for up to 14 days according to tests, where 2 µg trastuzumab was injected every 4 h and analyzed by LC-MS. The generated antibody fragments were reduced on-column and analyzed using an automated workflow with more than 95 % antibody digestion yield during the 14-day period.



Performance testing of FabRICATOR-HPLC. Comparison between digestion with standard in-solution FabRICATOR protocol (orange) and digestion using the automated FabRICATOR-HPLC workflow (blue), and quantification of the column's performance over a period of 14 days (bottom). The asterisk marks LC fragments that are not completely reduced with one intramolecular disulfide bridge intact.

Automated Antibody Fc Glycan Analysis

The performance and operational stability of the FabRICATOR-HPLC column are demonstrated here using analysis of trastuzumab Fc glycosylation. Automated middle-level analysis using FabRICATOR-HPLC yielded mass spectra virtually indistinguishable from the one obtained by a standard in-solution digestion workflow. The analytical results of the Fc glycosylation profiles were stable and reproducible during 14 days of continuous operation with standard deviations of less than 0.5 % for all glycoforms.



Glycan analysis using FabRICATOR-HPLC. Deconvoluted mass spectra of trastuzumab Fc/2 from in-solution FabRICATOR digestion (top, orange), FabRICATOR-HPLC (top, blue), and glycosylation profiles of trastuzumab (bottom, orange, n=10) or (bottom, blue, n=28).

FabRICATOR®-HPLC

Column hardware: PEEK/biocompatible

Column dimensions: 2.1 mmD × 50 mmL

Support resin: POROS® *

Typical flow rate: 0.025-0.05 mL/min

Maximum Pressure: 100 bar

Operating pH: 6.5 - 8.0

Operating temperature: 37° C

Storage conditions: +4-8° C (Do not freeze!)

Number of days of continuous operation: >10 **

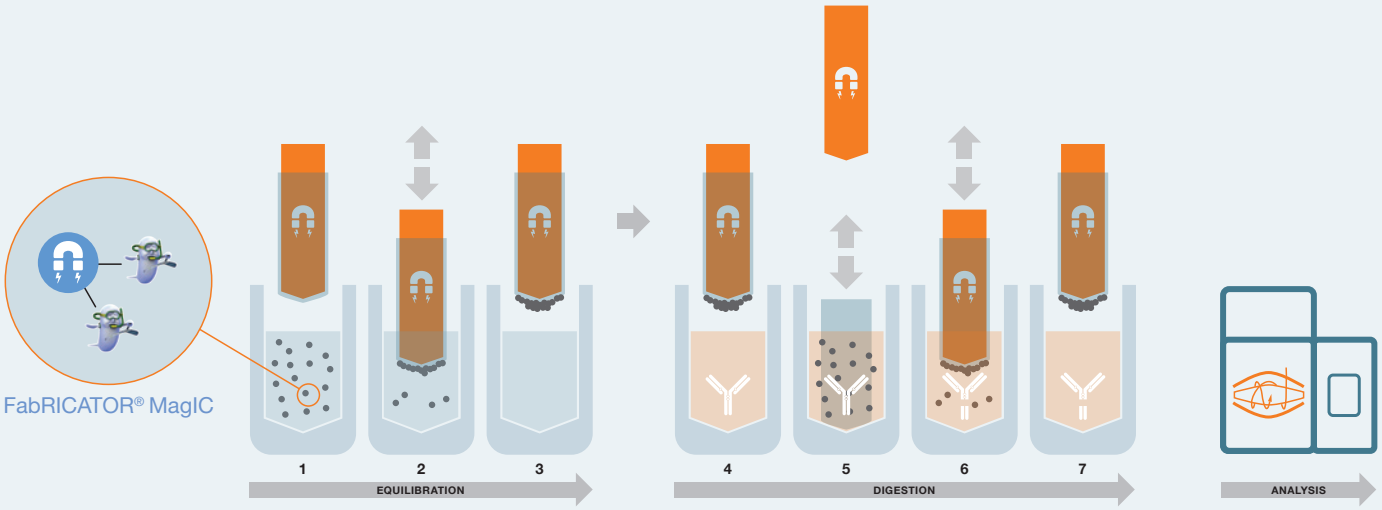
Injections per column: >200 **

Start material: Human IgG1-4, Fc-fusion proteins

* See legal and disclaimers. ** Depending on specific application

Parallel Subunit Generation of Antibodies for Automated Middle-level Workflows with FabRICATOR® MagIC

For automated processing of antibodies, FabRICATOR MagIC contains the FabRICATOR enzyme immobilized on magnetic agarose beads. The magnetic format enables parallel sample preparation and analysis of multiple antibodies in middle-level workflows. It can be used in both manual or automated setups for routine analysis of IgG-based biologics.



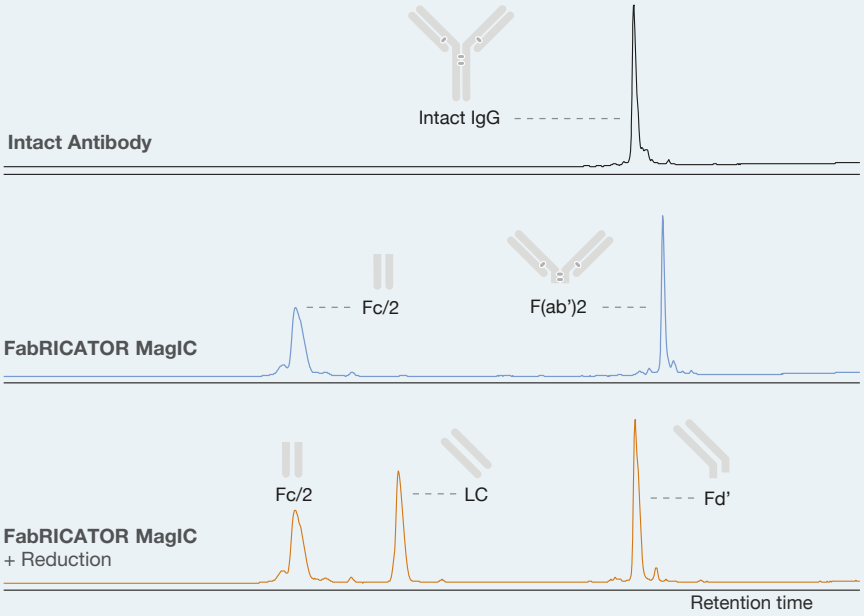
FabRICATOR MagIC workflow on Thermo Scientific KingFisher™ Purification System. IgG, FabRICATOR MagIC beads and PBS are dispensed into a 96-deepwell plate according to the KingFisher workflow. The beads are (1) transferred to wells containing PBS for (2) equilibration and (3) collection. The beads are (4) added to the samples and (5) incubated with mixing for 10-20 min at 37°C, prior to (6) collection and the pure F(ab')2 and Fc are left in the sample wells (7). The trademark KingFisher is the property of Thermo Fisher Scientific.

Automated Middle-Level Workflow

FabRICATOR MagIC generates intact F(ab')2 and Fc subunits and the disulfide bonds can easily be reduced using 5 mM TCEP in a one-pot reaction.

The generated Fc/2, LC and Fd' fragments allow detailed analysis and monitoring using middle-level workflow, and results in a better resolution compared to intact level approaches.

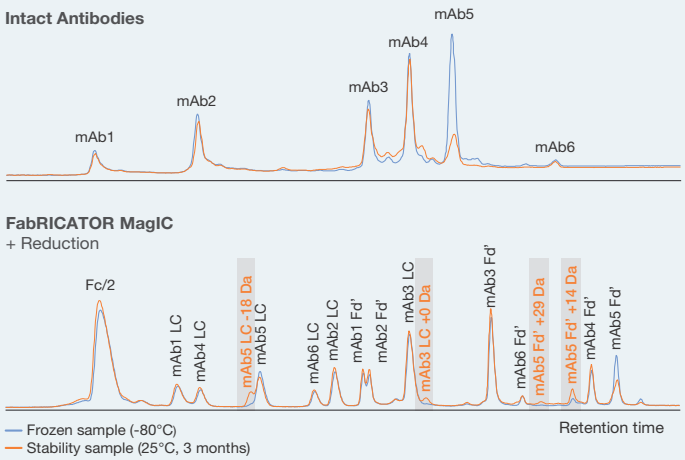
Reversed-phase LC-MS analysis of the resulting fragments from an antibody processed with FabRICATOR MagIC demonstrates the complete digestion.



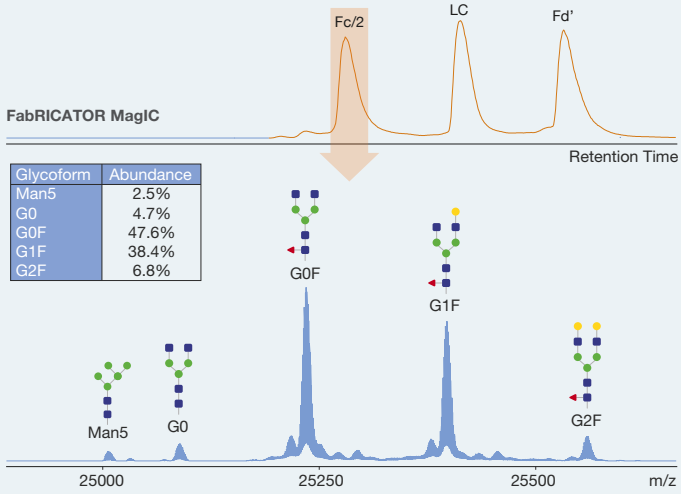
Generation of antibody subunits with FabRICATOR MagIC. Analysis of a monoclonal antibody by RPLC-MS at the intact level (top), after FabRICATOR MagIC digestion (middle) and after treatment with FabRICATOR MagIC and 5 mM TCEP (bottom). Data was obtained in collaboration with Symphogen A/S (Ballerup, Denmark).

Fast Automated Monitoring of CQAs

Using FabRICATOR MagIC for automated digestion of multiple mAbs, different CQAs such as oxidation can be analyzed. A mix of six different mAbs subjected to forced degradation was analyzed by LC-MS at the intact or the subunit level. The results show the ability to quickly detect and monitor such modifications in a complex mixture of mAbs using FabRICATOR MagIC. A detailed Fc-glycan profiling was also performed on a mAb by middle-level analysis after FabRICATOR MagIC digestion and reduction with 5 mM TCEP.



Oxidation studies using FabRICATOR MagIC. Six mAbs were analyzed for tryptophan oxidation during forced degradation studies by RPLC-MS at the intact level (top panel) and after FabRICATOR MagIC with 5 mM TCEP (bottom panel). New peaks detectable by the subunit analysis are highlighted. Data was obtained in collaboration with Symphogen A/S (Ballerup, Denmark).



Glycan analysis using FabRICATOR MagIC. Analysis of Fc glycosylation by middle-level LC-MS using FabRICATOR MagIC (top) and quantification of glycoforms from a deconvoluted mass spectrum of the Fc/2 fragment (bottom).

FabRICATOR®

Lyophilized enzyme for digestion of IgG.

PRODUCT	DESCRIPTION	ID
FabRICATOR, 2000 units	Digests 2 mg IgG	A0-FR1-020
FabRICATOR, 5000 units	Digests 5 mg IgG	A0-FR1-050
FabRICATOR, 5 x 5000 units	Digests 5 × 5 mg IgG	A0-FR1-250
FabRICATOR, 96 × 100 units	Digests 96 × 100 µg IgG	A0-FR1-096
FabRICATOR, 8 × 100 units	Digests 8 × 100 µg IgG	A0-FR1-008
FabRICATOR LE (low endotoxin), 2000 units	Digests 2 mg IgG	A0-FR8-020
FabRICATOR LE (low endotoxin), 5000 units	Digests 5 mg IgG	A0-FR8-050

FabRICATOR® Validation kit

Three different batches of the FabRICATOR enzyme for validation of FabRICATOR-based analytical methods.

PRODUCT	DESCRIPTION	ID
FabRICATOR, 3 x 2000 units	Digests 3 × 2 mg IgG	A0-FR4-060

FragIT™

Immobilized FabRICATOR enzyme for digestion of IgG in spin columns.

PRODUCT	DESCRIPTION	ID
FragIT, Microspin 2 × 0.5 mg	Digests 2 × 0.5 mg IgG	A0-FR6-010
FragIT, Microspin 5 × 0.5 mg	Digests 5 × 0.5 mg IgG	A0-FR6-025
FragIT, Microspin 10 × 0.5 mg	Digests 10 × 0.5 mg IgG	A0-FR6-050
FragIT, Midispin 1-10 mg	Digests 1-10 mg IgG	A0-FR6-100
FragIT, Maxispin 10-100 mg	Digests 10-100 mg IgG	A0-FR6-1000

FragIT™ kit

Immobilized FabRICATOR enzyme and CaptureSelect™ Fc affinity spin columns for digestion and purification of IgG subunits.

PRODUCT	DESCRIPTION	ID
FragIT kit, Microspin 0.5 mg	Digests and purifies 0.5 mg IgG	A2-FR2-005
FragIT kit, Microspin 5 × 0.5 mg	Digests and purifies 5 × 0.5 mg IgG	A2-FR2-025
FragIT kit, Microspin 10 × 0.5 mg	Digests and purifies 10 × 0.5 mg IgG	A2-FR2-050
FragIT kit, Midispin 10 mg	Digests and purifies 10 mg IgG	A2-FR2-100
FragIT kit, Maxispin 100 mg	Digests and purifies 100 mg IgG	A2-FR2-1000

FabRICATOR®-HPLC

Immobilized FabRICATOR enzyme on HPLC-compatible resin for on-column digestion.

PRODUCT	DESCRIPTION	ID
FabRICATOR-HPLC	Number of injections per column: >200	A0-FRC-050

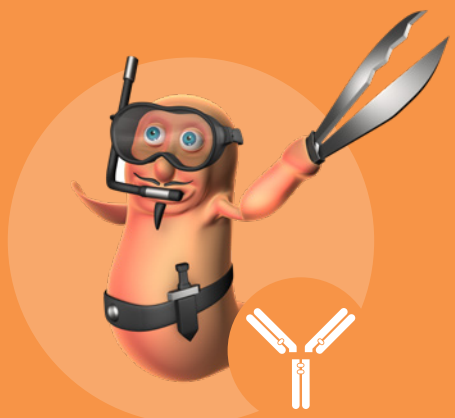
FabRICATOR® MagIC

Immobilized FabRICATOR enzyme on magnetic beads for automated workflows.

PRODUCT	DESCRIPTION	ID
FabRICATOR MagIC, 2 ml	Digests up to 24 × 200 µg IgG	A0-FRM-024
FabRICATOR MagIC, 4 × 2 ml	Digests up to 96 × 200 µg IgG	A0-FRM-096

FabALACTICA®

Above Hinge Digestion of Human IgG1



FabALACTICA (IgdE) is a cysteine protease that digests human IgG1 at a specific site above the hinge, generating intact and homogenous Fab and Fc fragments. The enzyme facilitates the characterization of bi- and multispecific antibodies and analyses of intact and paired Fc glycosylation. It also simplifies the study of monovalent binding, higher order structures, disulfide scrambling and antibodies with mutated hinge regions.

- Human IgG1
- KSCDKT / HTCPPC
- O/N reaction
- No reducing agents or co-factors needed

Antibody Subunit Workflow

The use of proteases with high specificity for IgG has allowed for subunit profiling of antibody-based therapeutics and studies of key quality attributes using LC-MS.

The FabALACTICA enzyme specifically digests human IgG1 at a single site above the hinge. The generated fragments can be used for a broad range of applications, including antibody characterization and NMR studies for higher order structures.

Product Formats

FabALACTICA®
Lyophilized enzyme for digestion of human IgG1

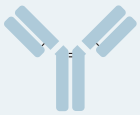
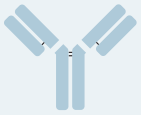
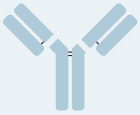
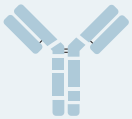
Immobilized FabALACTICA®
Immobilized enzyme for digestion of human IgG1 in spin columns

FabALACTICA® Fab kit
Immobilized enzyme and CaptureSelect™ Fc for purification of human IgG1 subunits

Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries.
Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Comparison of Enzymatic Strategies for Obtaining Fab Fragments

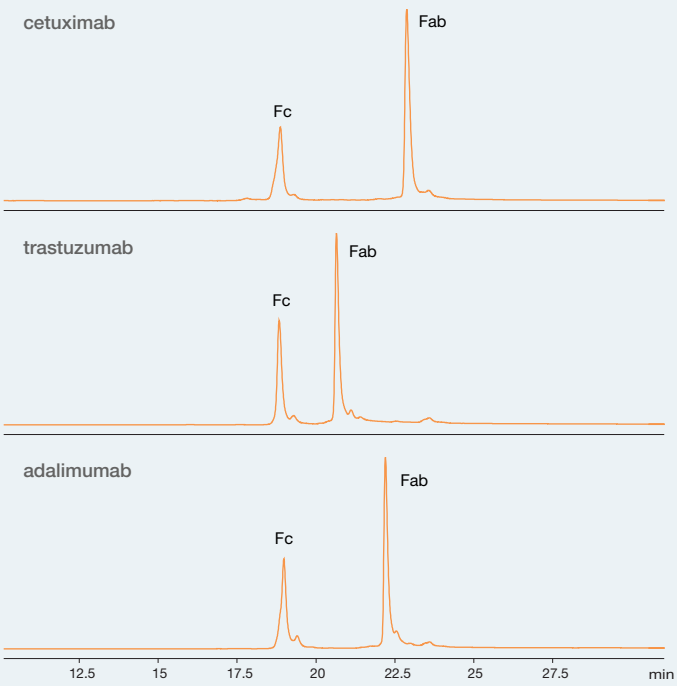
The SmartEnzymes FabALACTICA and GingisKHAN digests IgG at a single digestion site and generate homogenous Fab and Fc fragments from human IgG1. Traditional enzymes such as papain and Lys-C can also be used to generate Fab fragments, but the unspecific enzymatic activities need careful optimization to minimize over-digestion.

Enzyme	FabALACTICA	GingisKHAN	Papain	Lys-C
Digestion site				
Specificity	IgG-specific/ One digestion site	One digestion site	Unspecific/ Several digestion sites	Unspecific/ Several digestion sites
Selectivity	Human IgG1	Human IgG1	Several proteins	Several proteins
Reducing conditions	No	Yes, 2 mM cysteine	Yes	No
Reaction time	O/N (16-18 h)	1 h	1-24 h	1-24 h

Generation of Intact Fab and Fc Fragments

In order to demonstrate the activity of FabALACTICA, the three therapeutic monoclonal antibodies cetuximab, trastuzumab and adalimumab were digested and the resulting fragments were analyzed using RP-HPLC.

The defined peaks display the homogenous fragment generation and the robust enzymatic performance.

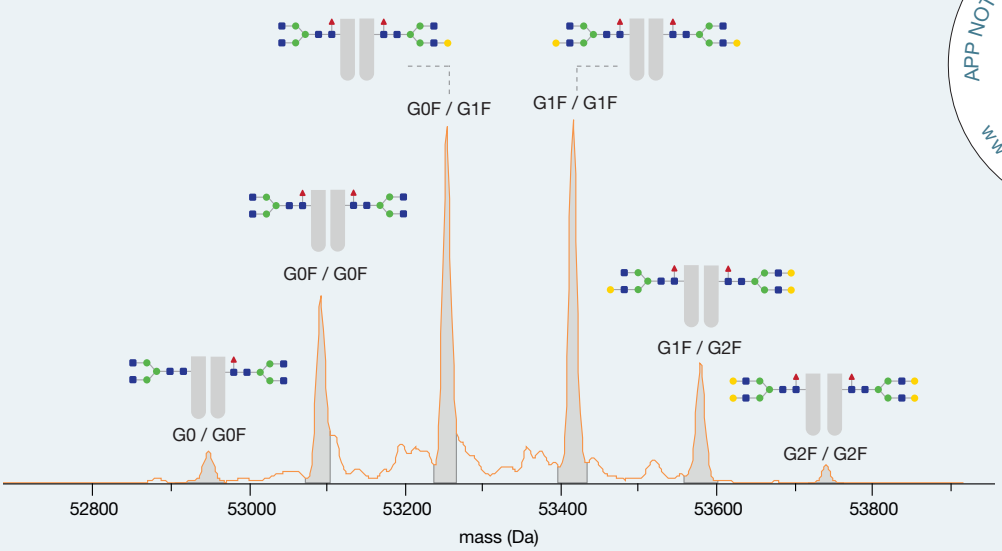


Separation of intact Fab and Fc fragments by RP-HPLC after FabALACTICA digestion. The digestion was performed O/N at 37°C.

Paired Glycan Analysis using FabALACTICA® and LC-MS

Fc glycosylation is a critical quality attribute of monoclonal antibodies that can affect their stability, solubility, pharmacodynamic-, and pharmacokinetic properties. This post-translational modification requires close monitoring during both development and manufacturing of a therapeutic antibody. Here, trastuzumab was digested using FabALACTICA, and the resulting intact Fc fragment (~53 kDa) enabled simultaneous characterization of the two conserved Fc glycosylation sites and their pairing with high mass accuracy.

APP NOTE: PAIRED GLYCAN ANALYSIS OF MABS
www.genovis.com/0046

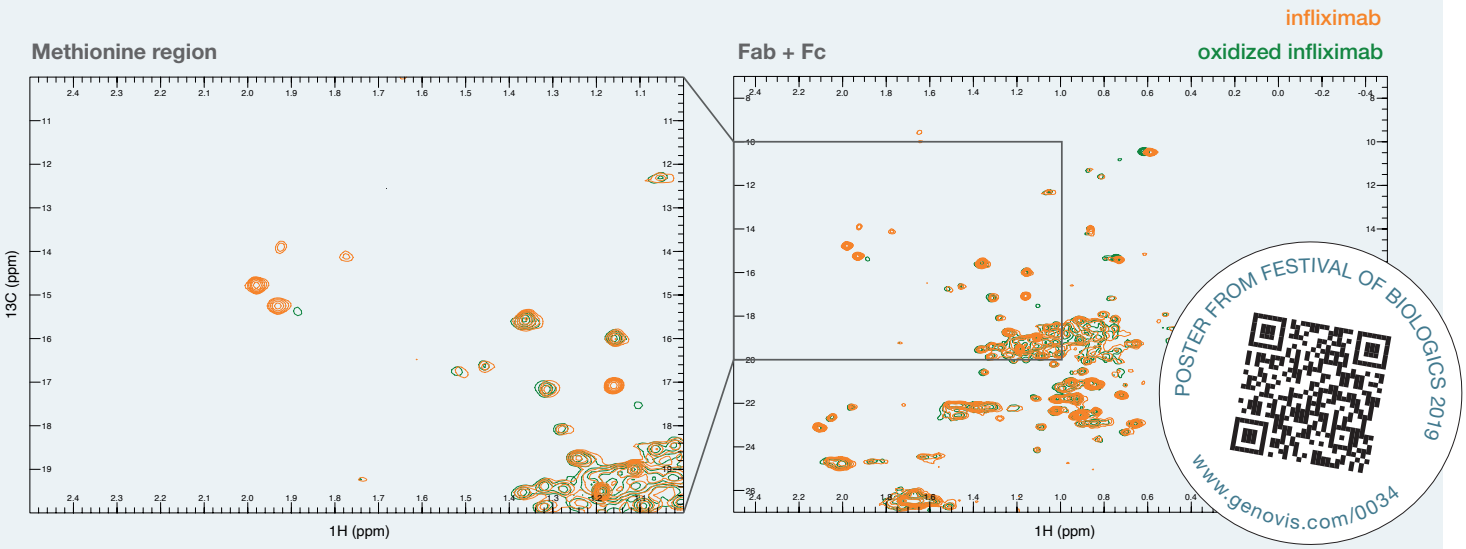


Paired glycan analysis of trastuzumab Fc fragments. Trastuzumab was digested using FabALACTICA O/N at 37°C, and intact Fc and Fab fragments were studied using LC-MS.

2D-NMR Spectroscopy Analysis using Immobilized FabALACTICA®

Critical quality attributes such as aggregation and unwanted inherent heterogeneity can be assessed by measuring the higher order structure (HOS) of the protein. Two-dimensional nuclear magnetic resonance spectroscopy (2D-NMR) allows for the precise atomic-level comparison of HOS for IgG-based biopharmaceuticals. Here, infliximab was first treated with H_2O_2 to induce oxidation before it was digested using Immobilized FabALACTICA.

The NMR spectra of the oxidized sample displayed an altered local environment for the methionines due to oxidation in both Fc and Fab fragments, while the higher order structure remained largely unaffected.

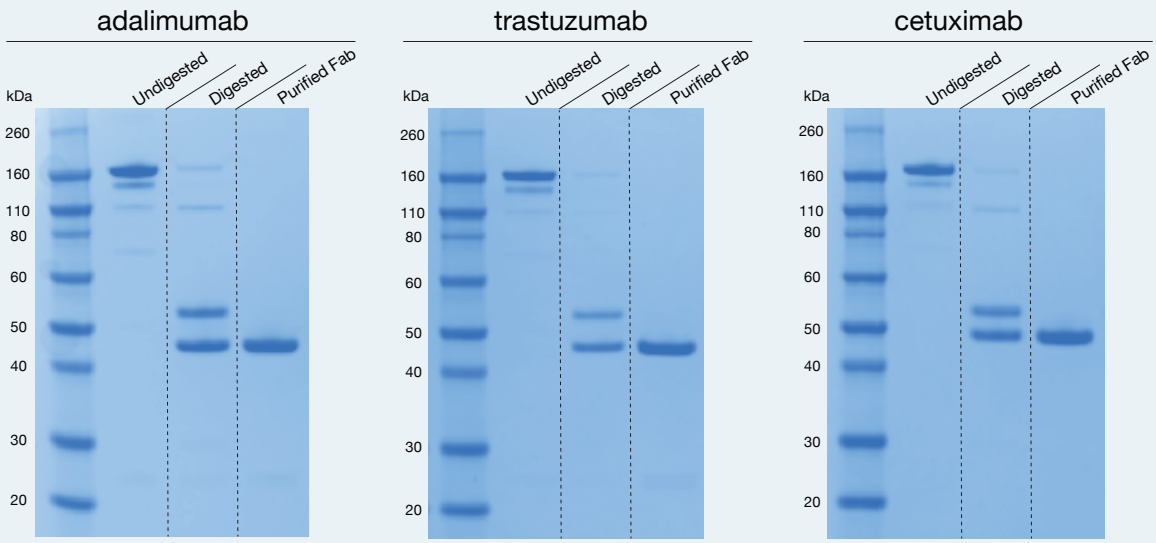


POSTER FROM FESTIVAL OF BIOLOGICS 2019
www.genovis.com/0034

2D-NMR analysis following FabALACTICA digestion to evaluate the impact of oxidation on infliximab. 1H-13C methyl HSQC of the combined Fab and Fc fragments of infliximab. Originator (orange) and oxidized originator sample (green). The spectra were acquired at 310 K using an 800 mHz Bruker Avance at the Swedish NMR Center. Data was obtained in collaboration with SARomics Biostructures (Lund, Sweden).

Preparation of Pure Fabs using FabALACTICA® Fab Kit

The FabALACTICA Fab kit consists of spin columns of Immobilized FabALACTICA for antibody digestion and spin columns of CaptureSelect™ Fc resin for affinity binding of the Fc fragments. Here, the therapeutic antibodies adalimumab, trastuzumab and cetuximab were incubated with Immobilized FabALACTICA before the Fc fragments were captured in the CaptureSelect™ Fc spin columns. The Fabs were easily collected by centrifugation, and the resulting Fab preparation was homogenous and pure.



Preparation of pure Fabs from three monoclonal antibodies using the FabALACTICA Fab kit. Adalimumab, trastuzumab and cetuximab digested by Immobilized FabALACTICA. Pure Fab fragments were obtained in a high yield from all three mAbs using the FabALACTICA Fab kit.

FabALACTICA®

Lyophilized enzyme for digestion of human IgG1.

PRODUCT	DESCRIPTION	ID
FabALACTICA, 2000 units	Digests 2 mg human IgG1	A0-AG1-020

Immobilized FabALACTICA®

Immobilized enzyme for digestion of human IgG1 in spin columns.

PRODUCT	DESCRIPTION	ID
Immobilized FabALACTICA, Microspin 2 × 0.5 mg	Digests 2 × 0.5 mg human IgG1	A0-AG6-010
Immobilized FabALACTICA, Microspin 10 × 0.5 mg	Digests 10 × 0.5 mg human IgG1	A0-AG6-050
Immobilized FabALACTICA, Midispin 5-10 mg	Digests 5-10 mg human IgG1	A0-AG6-100
Immobilized FabALACTICA, Maxispin 10-100 mg	Digests 10-100 mg human IgG1	A0-AG6-1000

FabALACTICA® Fab kit

Immobilized enzyme and CaptureSelect™ Fc affinity spin columns for generation and purification of human IgG1 subunits.

PRODUCT	DESCRIPTION	ID
FabALACTICA Fab kit, Microspin 0.5 mg	Digests and purifies 0.5 mg human IgG1	A2-AFK-005
FabALACTICA Fab kit, Microspin 5 × 0.5 mg	Digests and purifies 5 × 0.5 mg human IgG1	A2-AFK-025
FabALACTICA Fab kit, Midispin 10 mg	Digests and purifies 10 mg human IgG1	A2-AFK-100
FabALACTICA Fab kit, Maxispin 100 mg	Digests and purifies 100 mg human IgG1	A2-AFK-1000

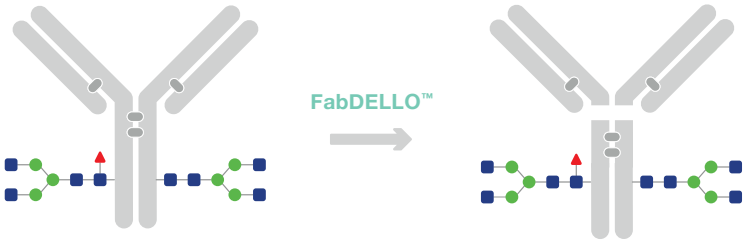
FabDELLO™

Above Hinge Digestion of Human IgG1

FabDELLO is a protease that specifically digests human IgG1 at a single site above the hinge, generating intact Fab and Fc fragments within two hours with no need for reducing conditions. The enzyme is active on antibodies with mutated hinge regions, such as the LALA mutation, and enables middle-level LC-MS analysis for characterization of critical quality attributes on antibodies with mutated hinges.

- Human IgG1
- KSCDK / THTCPPCP
- 2 hour reaction
- Requires presence of calcium ions. 200 mM $CaCl_2$ is included.

Antibody Subunit Workflow



FabDELLO digests human IgG1 at a single site above the hinge (...KSCDK / THTCPPCP...), generating intact Fab and Fc fragments. A second digestion site may appear on the Fc if the N-glycans are removed. FabDELLO is active under native conditions and requires the presence of calcium ions. Optimal activity is obtained at 37°C and pH 7-8.5.

Product Formats

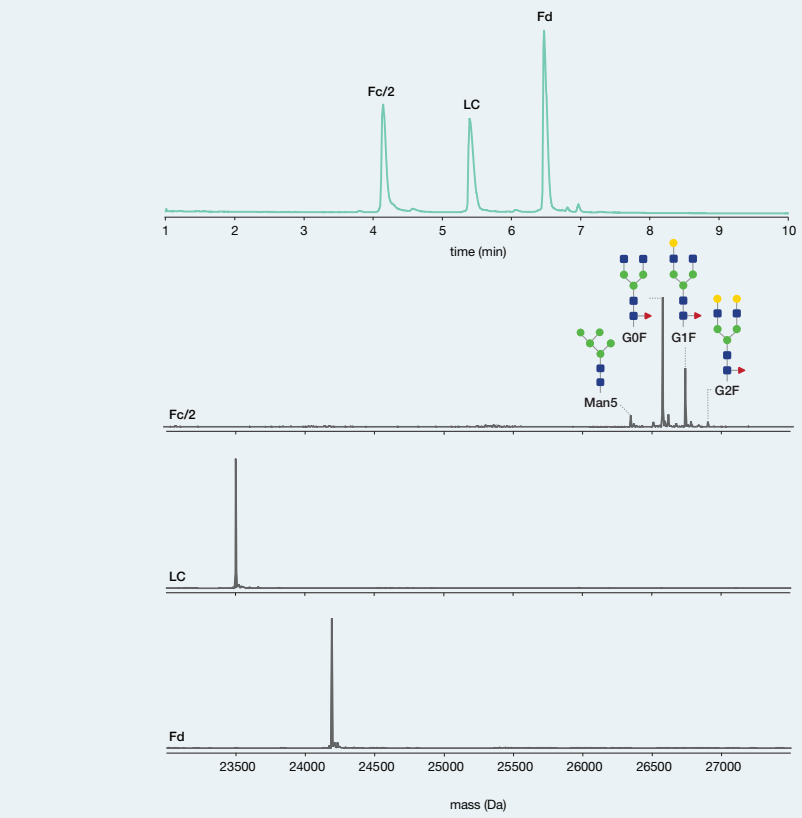
FabDELLO™
Lyophilized enzyme for digestion of human IgG1

FabDELLO

PRODUCT	DESCRIPTION	ID
FabDELLO, 8 × 100 units	Digests 8 × 100 µg human IgG1	B1-BD1-008
FabDELLO, 96 × 100 units	Digests 96 × 100 µg human IgG1	B1-BD1-096

Middle-level LC-MS Analysis of Mutated Antibodies

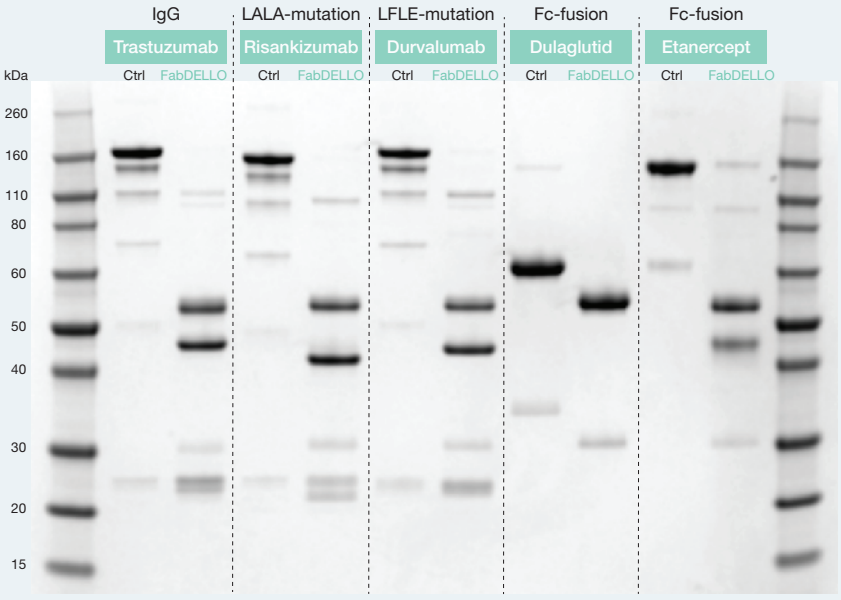
Mutated hinge regions are common modifications of therapeutic antibody candidates. The hinge mutations may affect the antibody digestion efficiency of enzymes with high substrate specificity. FabDELLO acts specifically at an exposed lysine residue above the hinge and enables middle-level LC-MS analysis of antibodies with mutated hinge regions. Here, the commercially available IgG1 risankizumab, with a LALA mutation in the lower hinge region, was digested with FabDELLO and reduced before middle-level LC-MS analysis. The defined peaks observed after digestion with FabDELLO show homogenous fragment generation and robust performance of the enzyme.



Middle-level LC-MS analysis of risankizumab after digestion with FabDELLO. The digested material was subjected to 20 mM DTT for 1 h at 37°C to reduce the cysteine bridges. The sample was analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1 × 50 mm). UV 280 chromatogram showing Fc/2, LC and Fd, and deconvoluted Fc/2, LC and Fd are shown.

Complete Digestion of Mutated Antibodies and Fc-fusion Proteins

FabDELLO digests human IgG1 at a single site above the hinge and generates intact Fab and Fc fragments. To demonstrate the specific activity of FabDELLO, a selection of IgG1 and Fc-fusion proteins were digested and analyzed by non-reduced SDS-PAGE. A complete digestion was achieved for all substrates, including IgG1 with the otherwise difficult LALA and LFLE mutations. The precise and effective performance of the FabDELLO enzyme on this wide range of substrates validates its use for the development of biopharmaceuticals.



SDS-PAGE analysis under non-reducing conditions of commercial antibodies and Fc-fusion proteins digested with FabDELLO.

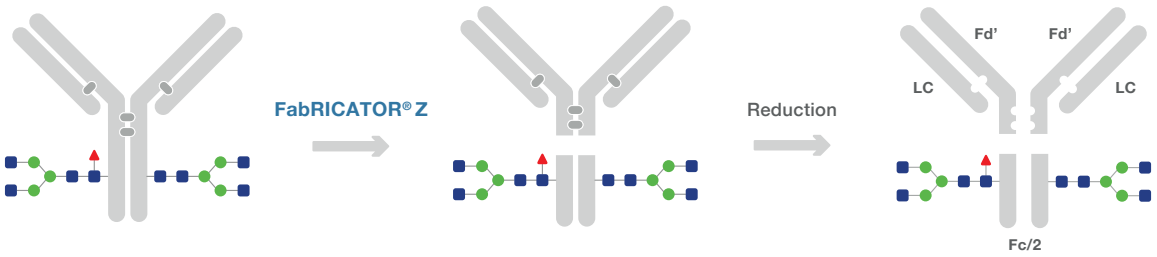
FabRICATOR®Z

Below Hinge Digestion of Mouse IgG

FabRICATOR Z (IdeZ) is a cysteine protease that digests mouse IgG2a and IgG3 at a specific site below the hinge, generating a homogenous pool of F(ab')2 and Fc fragments after a two-hour incubation.

- Mouse IgG2a and IgG3, human IgG1-4, IgG of some classes from monkey, rabbit and sheep
- CPAPNLLG / GPSVF
- 2 h reaction
- No need for reducing agents or co-factors

Antibody Digestion Workflow



FabRICATOR Z digests mouse IgG2a and IgG3 at a specific site below the hinge region, generating intact F(ab')2 and Fc fragments. Some mouse IgG2a that FabRICATOR fails to digest, are readily digested by FabRICATOR Z, but longer incubation times may be required. There is no risk of over-digestion because of the high specificity of the enzyme.

Product Formats

FabRICATOR® Z
Lyophilized enzyme for digestion of mouse IgG2a and IgG3

FragIT™ Z
Immobilized enzyme for digestion of mouse IgG2a and IgG3 in spin columns

FragIT™ Z kit
Immobilized enzyme and CaptureSelect™ Fc for purification of IgG subunits

Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries.
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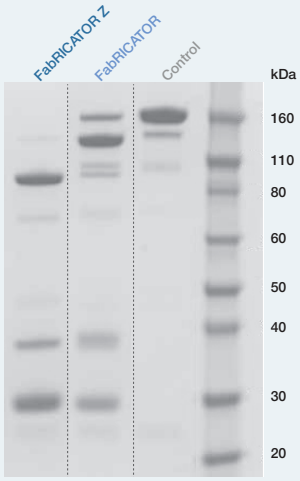
Digestion of Mouse IgG2a using FabRICATOR® Z

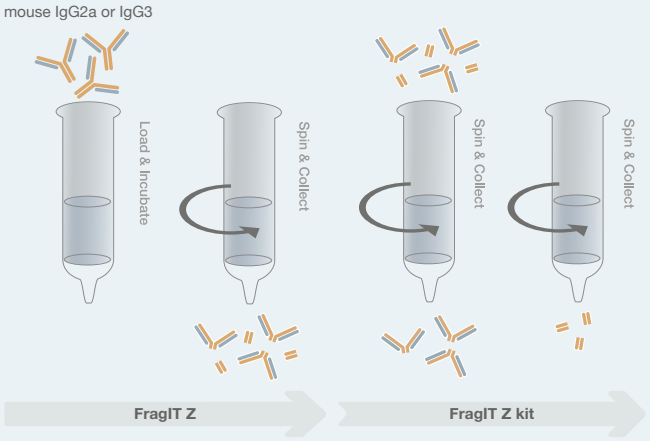
FabRICATOR Z (IdeZ) and FabRICATOR (IdeS) were here used to digest mouse IgG2a. After 2 hours of incubation, FabRICATOR Z (IdeZ) was found to readily digest mouse IgG2a, whereas FabRICATOR only digested a small amount of the antibody.



Digestion of mouse IgG2a using FabRICATOR Z and FabRICATOR. The F(ab')2 fragment is detected at ~110 kDa and the Fc fragments at ~30 kDa. The enzyme is detected at ~37 kDa.

Preparative Generation of Mouse IgG2a and IgG3 Subunits with FragIT™ Z Kit

For applications that require fast and easy digestion of mouse IgG2a without residual enzyme in the preparation, the FragIT Z spin columns containing FabRICATOR Z immobilized on agarose beads can be used. With FragIT Z kit, the homogenous F(ab')2 and Fc fragments can be separated with affinity purification columns for preparation of pure antibody fragments.



Schematic overview of FragIT Z and FragIT Z kit. Generation and purification of F(ab')2 and Fc fragments from mouse IgG2a and IgG3 using FragIT Z and FragIT Z kit.

FabRICATOR® Z

PRODUCT	DESCRIPTION	ID
FabRICATOR Z, 2000 units	Digests 2 mg IgG	A0-FRZ-020

FragIT™ Z

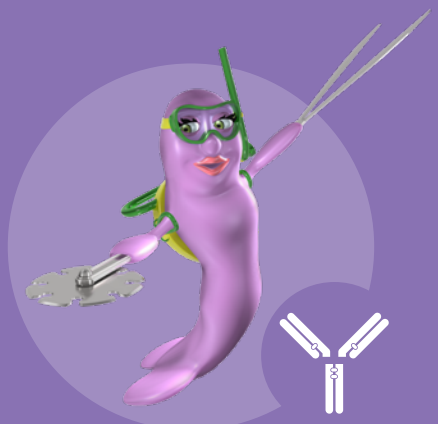
PRODUCT	DESCRIPTION	ID
FragIT Z, Microspin 2 × 0.5 mg	Digests 2 × 0.5 mg IgG	A0-FZ6-010
FragIT Z, Microspin 5 × 0.5 mg	Digests 5 × 0.5 mg IgG	A0-FZ6-025
FragIT Z, Microspin 10 × 0.5 mg	Digests 10 × 0.5 mg IgG	A0-FZ6-050

FragIT™ Z kit

PRODUCT	DESCRIPTION	ID
FragIT Z kit, Microspin 0.5 mg	Digests and purifies 0.5 mg IgG	A2-FZ2-005
FragIT Z kit, Microspin 5 × 0.5 mg	Digests and purifies 5 × 0.5 mg IgG	A2-FZ2-025

FabULOUS™

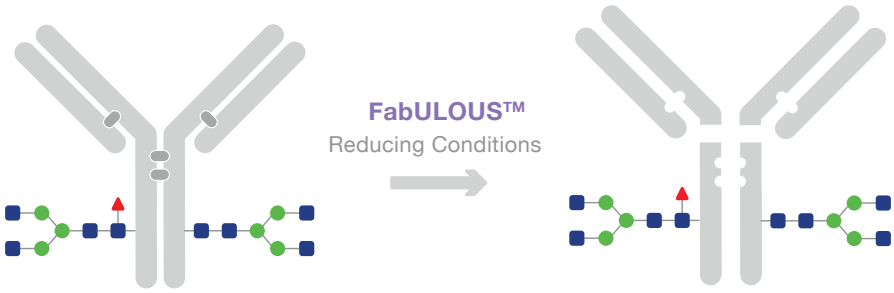
Above Hinge Digestion of IgG



FabULOUS (SpeB) is a cysteine protease that digests in the hinge region of IgG from several different species and subclasses, generating a homogenous pool of Fab fragments. The prepared Fab fragments can for example be used in affinity studies, studies of Fab glycosylation and structural studies.

- Human IgG and IgG from mouse, rat, goat, sheep and rabbit.
- KTHT / CPPCPAP (human IgG1)
- 60 min reaction
- Requires reducing conditions

Antibody Digestion Workflow



FabULOUS digests IgG and generates Fab and Fc fragments. The primary digestion site on human IgG1 is between the amino acids T225 and C226. The FabULOUS enzyme digests IgG from several species and subclasses, including mouse (such as mouse IgG1), rat, goat, sheep and rabbit. The enzyme requires reducing conditions for activity on IgG, and if strongly reducing conditions are used, it is likely that the interchain disulfide bonds will be reduced. The reaction is performed at neutral pH under reducing conditions and the digestion is complete after incubation at 37°C for 1 hour.

Product Formats

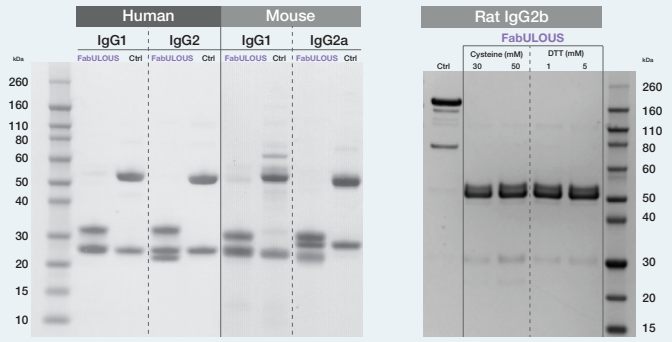
FabULOUS™
Lyophilized enzyme for digestion of IgG

FabULOUS™ Fab kit
Lyophilized enzyme and CaptureSelect™ LC-kappa (mur) for purification of mouse IgG subunits

Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries.
Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Generating Fab and Fc Fragments using FabULOUS™

The preparation of intact antibody fragments with enzymes can be challenging for many different species and subclasses of IgG due to unspecific enzymatic activity. FabULOUS is a valuable research tool that can generate intact Fab fragments from several species of IgG, including mouse and human. For some species of IgG, FabULOUS is active under mild reducing conditions that allows for the generation of intact Fab fragments without reduction of interchain disulfides. Intact Fab fragments can also be achieved if a buffer exchange is performed after digestion at stronger reducing conditions. This enables reformation of the disulfide bonds in the Fab.

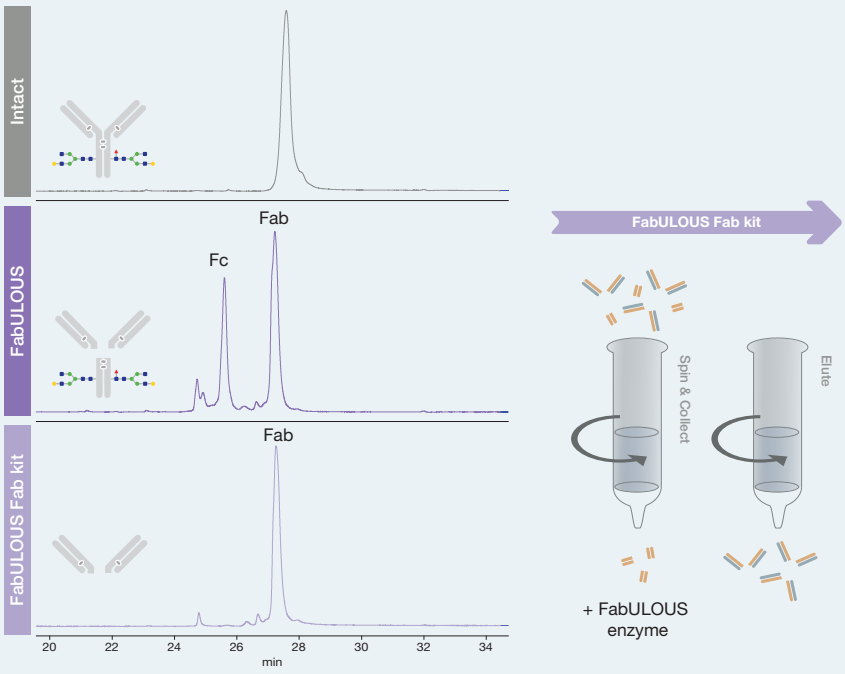


POSTER FROM CASSS 2013
www.genovis.com/0004

Generating Fab and Fc Fragments using FabULOUS. Left: SDS-PAGE analysis of human IgG1 and IgG2 and mouse IgG1 and IgG2a digested in the hinge region using FabULOUS. The generated fragments were analyzed with SDS-PAGE under reducing conditions. Right: SDS-PAGE analysis of rat IgG2b digested in the hinge region using FabULOUS at different reducing conditions. The generated fragments were buffer exchanged and analyzed with SDS-PAGE under non-reducing conditions.

Generating and Purifying Fab Fragments from Mouse IgG using FabULOUS™ Fab Kit

The FabULOUS Fab kit is designed to generate and purify Fab fragments from mouse IgG. The kit consists of the lyophilized FabULOUS enzyme for antibody digestion and CaptureSelect™ LC-Kappa (mur) affinity spin columns for easy purification of the prepared Fab fragments from mouse IgG. The antibody is digested within 60 minutes using the lyophilized FabULOUS enzyme, and subsequently the prepared Fab fragments bind to the affinity spin columns and are easily eluted. The prepared Fab fragments from mouse IgG1 using FabULOUS Fab kit are shown here.



Generation and purification of Fab fragments from mouse IgG1. The top panel shows the intact monoclonal mouse IgG1 antibody, the middle panel shows the analysis of the fragments after FabULOUS digestion, and the bottom panel shows the eluted Fab fragments from the CaptureSelect™ LC-Kappa (mur) column.

FabULOUS™

PRODUCT	DESCRIPTION	ID
FabULOUS, 2000 units	Digests 2 mg IgG	A0-PU1-020

FabULOUS™ Fab kit

PRODUCT	DESCRIPTION	ID
FabULOUS Fab kit, mouse	Digests and purifies 2 mg mouse IgG	A1-PFK-020

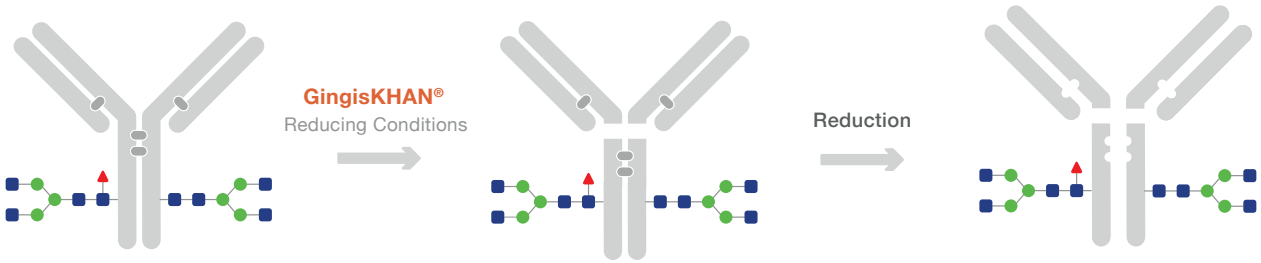
GingisKHAN®

Above Hinge Digestion of Human IgG1

GingisKHAN (Kgp) is a cysteine protease that digests human IgG1 at a specific site above the hinge, generating intact and homogenous Fab and Fc fragments. The enzyme is used to characterize antibody-based biotherapeutics using LC-MS, to study Fc glycan analysis, bispecific antibodies, affinity and avidity effects and to identify post-translational modifications.

- Human IgG1
- KSCDK / THTCPPCP
- 60 min reaction
- Requires mild reducing conditions. 2 mM cysteine is included

Antibody Subunit Workflow



GingisKHAN is a cysteine protease that digests human IgG1 at a specific site above the hinge, generating intact Fab and Fc fragments. A second digestion site on the Fc may appear if the N-glycans are removed. GingisKHAN requires mild reducing conditions to be active, and optimal activity is obtained at 37°C and pH 8.

Product Formats

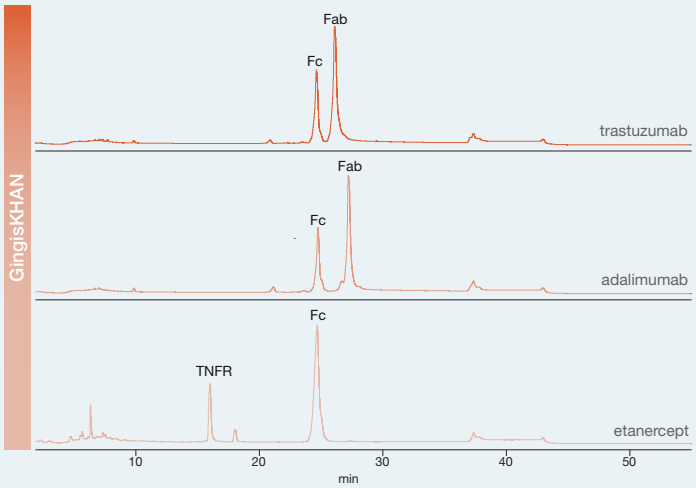
GingisKHAN®
Lyophilized enzyme for digestion of human IgG1

GingisKHAN® Fab kit
Lyophilized enzyme and CaptureSelect™ CH1 for purification of human IgG1 subunits

Thermo Scientific™ CaptureSelect™ resin from Thermo Fisher Scientific Inc. and its subsidiaries.
Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Digestion of Monoclonal Antibodies and Fc-Fusion Proteins using GingisKHAN®

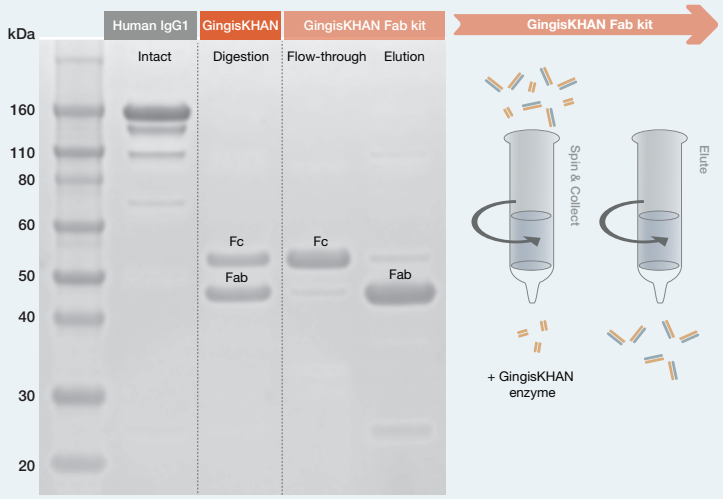
The specific digestion of Fc-fusion proteins or antibodies with mutated hinges into smaller subunits can be challenging. The GingisKHAN enzyme digests human IgG1 at a single exposed lysine site above the hinge under reducing conditions. The required reducing condition is mild enough to preserve the intra- and interchain disulphide bridges, resulting in intact Fab and Fc fragments. Here, a selection of monoclonal human IgG1 antibodies and a Fc-fusion protein were incubated with GingisKHAN for 1 hour at 37°C and analyzed using HPLC. The resulting fragments displayed the efficient digestion of GingisKHAN for the generation of intact Fab fragments from trastuzumab and adalimumab and the separation of the Fc from the fused TNFR domain of etanercept.



Specific digestion of human IgG1 using GingisKHAN. LC separation of Fab and Fc fragments from a selection of therapeutic antibodies (trastuzumab and adalimumab) and a Fc-fusion protein (etanercept) after GingisKHAN digestion.

Pure Human IgG1 Fab Fragments using GingisKHAN® Fab Kit

Pure and intact human IgG1 Fab fragments can be generated and purified using the GingisKHAN Fab kit. Here, trastuzumab Fab fragments were generated after GingisKHAN digestion. The fragments were affinity purified using the CaptureSelect™ human CH1 spin column. The enzyme together with the trastuzumab Fc was yielded in the flow-through, and the Fab was easily eluted by lowering the pH.



SDS-PAGE analysis under non-reducing conditions of the human IgG1 trastuzumab digested by GingisKHAN. After digestion, the Fab fragments were captured in the affinity spin column and could easily be eluted.

GingisKHAN®

PRODUCT	DESCRIPTION	ID
GingisKHAN, 2000 units	Digests 2 mg human IgG1	B0-GKH-020

GingisKHAN® Fab kit

PRODUCT	DESCRIPTION	ID
GingisKHAN Fab kit	Digests and purifies 2 mg human IgG1	B0-GFK-020

GlySERIAS™

Hydrolysis of Flexible Linkers

GlySERIAS is a unique enzyme that digests flexible glycine-rich fusion protein linkers such as Gly_4Ser and Gly_xSer_y (GS) and polyglycine (G) linkers. The enzyme enables separation of the individual domains of multi-functional fusion proteins to facilitate characterization. Middle-level analysis of fusion proteins serves to both reduce the overall sample complexity and allow for domain-specific identification and monitoring of post-translational modifications.

- Fusion proteins with flexible GS or G linkers
- Flexible linkers consisting of repetitive sequences of Gly or Gly and Ser residues
- 1 hour reaction (optimization for individual proteins may be required)
- No reducing agents or co-factors needed

Digestion Workflow

GlySERIAS is a unique enzyme for digestion of fusion proteins designed with flexible linkers consisting of repetitive sequences of glycine, or glycine and serine, residues. The enzyme is active under native reaction conditions and enables middle-level characterization of complex fusion proteins. The linker regions are digested at several sites simultaneously, leading to separation of the previously linked components. Optimal activity occurs at 37°C, pH 7.6 and the length of the incubation can be varied depending on the desired end-products. A shorter incubation time will allow for a more complete coverage of the linker sequence whereas a longer incubation time will result in more homogeneous subunits.

Product Formats

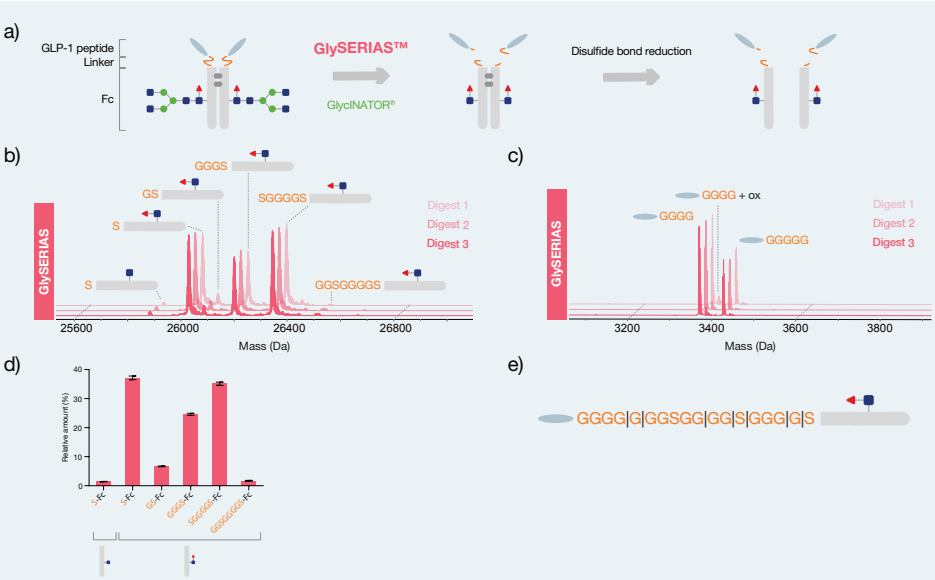
GlySERIAS™
Lyophilized enzyme for digestion of fusion proteins

GlySERIAS™

PRODUCT	DESCRIPTION	ID
GlySERIAS, 2000 units	Digests 2 mg fusion protein	A0-GS1-020

Digestion of a GS-linked Fusion Protein

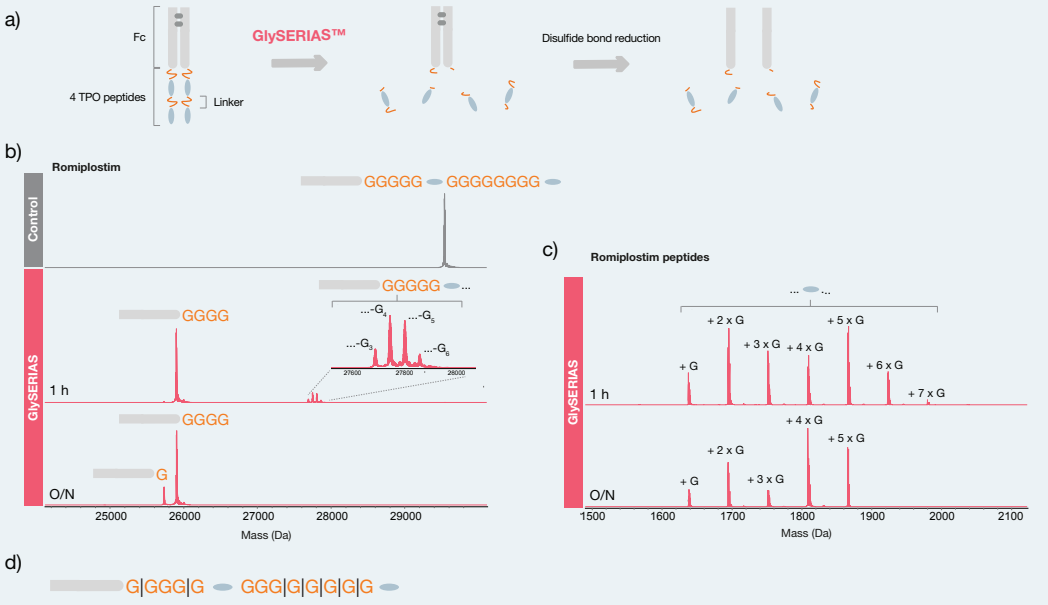
The fusion protein dulaglutide consists of two glucagon-like peptide-1 (GLP-1) molecules linked to an Fc region of human IgG4 via flexible GS linkers. To study the peptides and Fc region separately and thereby be able to identify specific PTMs, dulaglutide was digested using GlySERIAS. Analysis of the sample by reversed-phase LC-MS showed that the peptides were completely removed from the Fc region upon linker digestion using GlySERIAS. In addition, an oxidation modification on the GLP-1 peptide was identified. Despite GlySERIAS digesting at several sites simultaneously, triplicate digests showed repeatable results in the relative amount of the different Fc/2 variants obtained.



Digestion of dulaglutide using GlySERIAS. The flexible GS linker of dulaglutide was digested with GlySERIAS for 1 hour at 37°C under native conditions, and the Fc glycans were concurrently hydrolyzed using GlycINATOR to reduce sample complexity. To stop the GlySERIAS reaction, 1 mM $ZnCl_2$ was added. The interchain disulfide bonds were reduced with 20 mM DTT for 30 minutes at 37°C. The digest was performed in triplicate. **a)** Illustration of the sample preparation workflow. The samples were analyzed by reversed-phase LC-MS. **b)** Deconvoluted mass spectrum of the Fc/2 subunit. **c)** Deconvoluted mass spectrum of the GLP-1 peptide. **d)** Relative amount of the identified Fc/2 variants, displaying the mean value between the triplicate digests and error bars representing the standard deviation. The digestion products were separated by reversed-phase chromatography (BioResolve™ RP mAb Polyphenyl, 450 Å, 2.7 µm 2.1 × 100 mm, Waters™) and analyzed with ESI-QTOF MS (Bruker Impact II). **e)** Schematic image of the flexible linker, connecting the GLP-1 peptide to the Fc/2 subunit, and the identified digestion sites.

Digestion of a Polyglycine-linked Fusion Protein

Romiplostim consists of four identical thrombopoietin (TPO) receptor binding peptides and one human IgG1 Fc region, linked together by flexible polyglycine sequences. For a more detailed analysis of the individual domains, this protein was digested using GlySERIAS and reduced with DTT. Here we show that GlySERIAS is able to digest polyglycine-linked fusion proteins for domain-specific analysis, even for proteins containing several linked domains.



Digestion of romiplostim using GlySERIAS. The polyglycine linkers of romiplostim were digested with GlySERIAS for 1 hour and overnight at 37°C under native conditions. **a)** Illustration of the sample preparation workflow. The samples were analyzed by reversed-phase LC-MS. **b)** Deconvoluted mass spectrum of the Fc/2 subunit. **c)** Deconvoluted mass spectrum of the TPO receptor binding peptide. The glycine residues can be present at both ends of the peptide. The digestion products were separated by reversed-phase chromatography (BioResolve™ RP mAb Polyphenyl, 450 Å, 2.7 µm 2.1 × 100 mm, Waters™) and analyzed with ESI-QTOF MS (Bruker Impact II). **d)** Schematic image of the two flexible linkers, connecting first the Fc/2 subunit to one TPO receptor binding peptide and then to the second TPO receptor binding peptide, and the identified digestion sites.

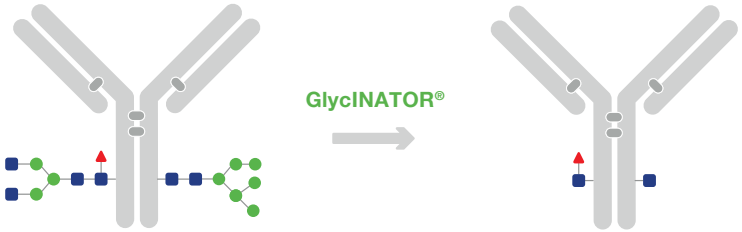
GlycINATOR®

Hydrolysis of IgG Fc glycans

GlycINATOR (EndoS2) is an IgG-specific endoglycosidase that hydrolyzes all glycoforms present at the Fc glycosylation site. The enzyme acts on native IgG and leaves the core GlcNAc with or without fucose. The enzyme is used to reduce the complexity of antibody-based therapeutics to study core afucosylation, and as an initial step in the GlyCLICK and TransGLYCIT platforms.

- Human IgG1-4, Fc-fusion proteins, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- Hydrolyzes the β1,4-linkage between the two innermost GlcNAc residues
- 30 min reaction
- Requires native IgG fold

Antibody Deglycosylation Workflow



The GlycINATOR enzyme requires a native fold of the Fc in order to trim the N-glycan. It hydrolyzes the β1,4-linkage between the two innermost GlcNAc residues of the Fc glycan, leaving the core GlcNAc intact on the Fc. The Fc glycosylation site is conserved among many species, and GlycINATOR deglycosylates antibodies from several species and subclasses. It effectively hydrolyzes all IgG glycoforms, including complex, high-mannose, hybrid and bisected glycans.

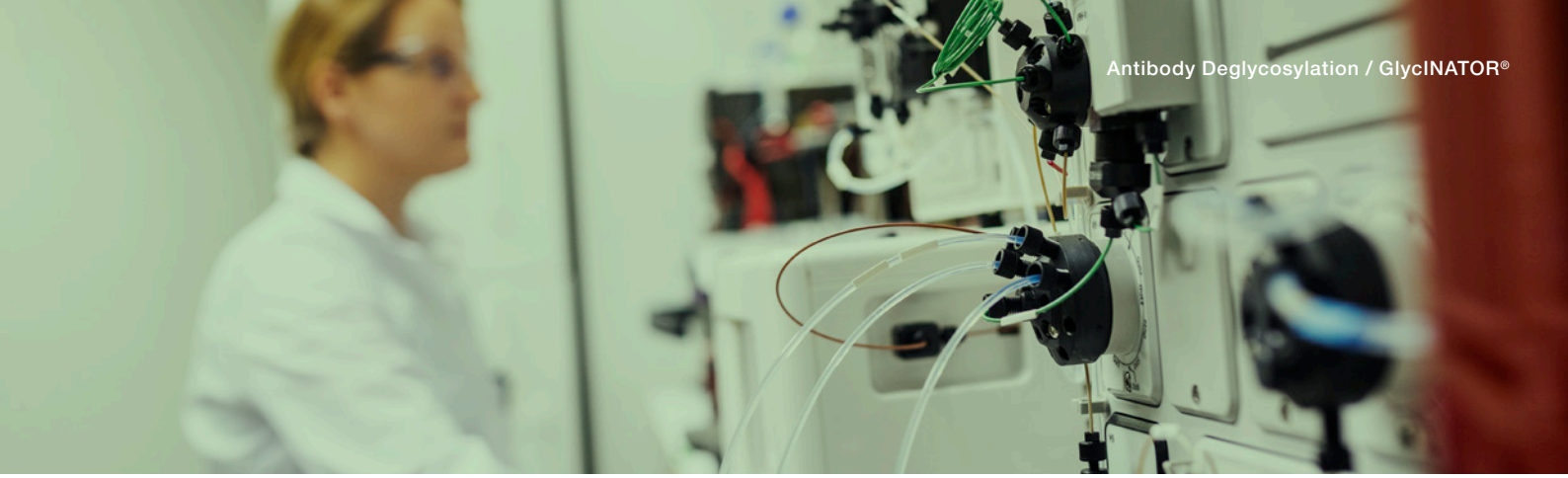
Product Formats

GlycINATOR®
Lyophilized enzyme for deglycosylation of IgG

Immobilized GlycINATOR®
Immobilized enzyme for deglycosylation of IgG in spin columns

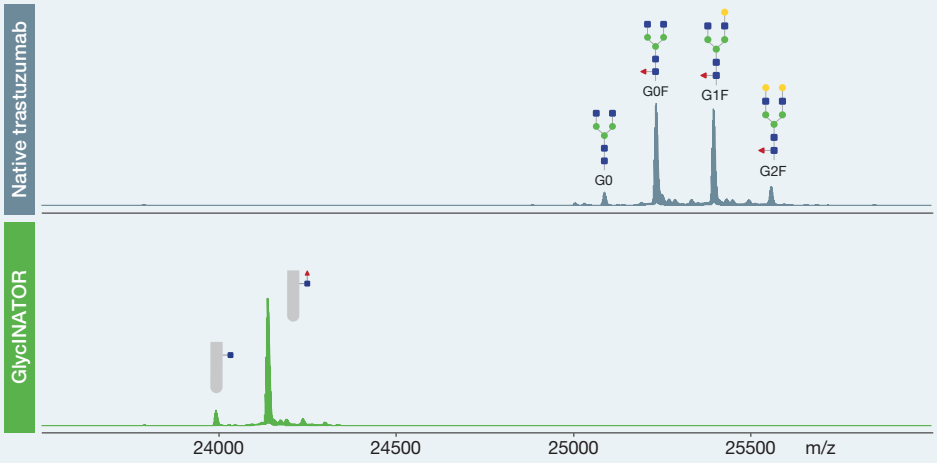
GlycINATOR®

PRODUCT	DESCRIPTION	ID
GlycINATOR, 2000 units	Deglycosylates 2 mg IgG	A0-GL1-020
GlycINATOR LE (low endotoxin), 2000 units	Deglycosylates 2 mg IgG	A0-GL8-020



Hydrolysis of Fc glycans using GlycINATOR®

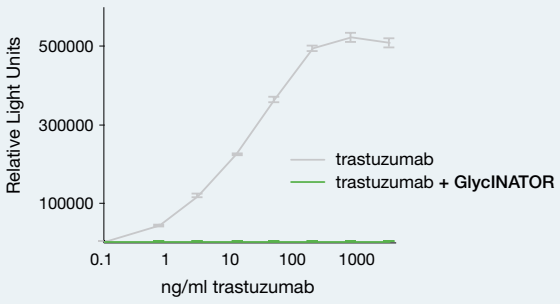
To characterize the activity of GlycINATOR on a therapeutic antibody, trastuzumab was incubated with GlycINATOR for 30 min at 37°C. The antibody was then digested using the FabRICATOR (IdeS) enzyme to generate F(ab')2 and Fc/2 fragments suitable for LC-MS analysis. The MS results demonstrate hydrolysis of the glycan structure after the core GlcNAc with or without the core fucose when the antibody is incubated with GlycINATOR.



LC-MS characterization of Fc glycan hydrolysis by GlycINATOR. Native and GlycINATOR-treated trastuzumab was digested with FabRICATOR to generate Fc/2 fragments before LC-MS analysis.

Antibodies Deglycosylated with GlycINATOR® do Not Elicit ADCC

Afucosylation of the Fc N-glycans found on IgG can impact the binding to Fc receptors and thus the antibody-dependent cellular cytotoxicity (ADCC). Here, the ability of native and deglycosylated trastuzumab to elicit ADCC was analyzed using the cell based iLite® ADCC assay. The native antibody showed an expected increase in signal when more antibody was added to the assay. However, the antibody deglycosylated by GlycINATOR showed no signal even at larger doses, indicating an abolished ADCC activity after GlycINATOR treatment.



POSTER FROM AIS 2018
www.genovis.com/0033

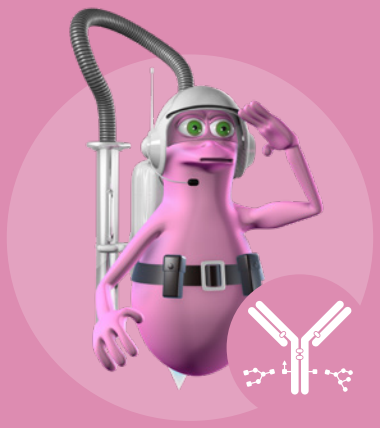
Study of ADCC by native and deglycosylated trastuzumab. The effect of trastuzumab was analyzed using iLite ADCC Effector (V) Assay Ready Cells (BM4001) and iLite ADCC Target HER2 (+) Assay Ready Cells (BM4011). Effector and Target cells were diluted and mixed with the antibody solutions. Incubation was performed at 37°C and 5% CO_2. Luminescence reading was performed after 6 h.

Immobilized GlycINATOR®

PRODUCT	DESCRIPTION	ID
Immobilized GlycINATOR, Microspin 2 × 0.5 mg	Deglycosylates 2 × 0.5 mg IgG	A0-GL6-010
Immobilized GlycINATOR, Microspin 5 × 0.5 mg	Deglycosylates 5 × 0.5 mg IgG	A0-GL6-025
Immobilized GlycINATOR, Microspin 10 × 0.5 mg	Deglycosylates 10 × 0.5 mg IgG	A0-GL6-050
Immobilized GlycINATOR, Midispin 1-10 mg	Deglycosylates 1-10 mg IgG	A0-GL6-100
Immobilized GlycINATOR, Maxispin 10-100 mg	Deglycosylates 10-100 mg IgG	A0-GL6-1000

IgGZERO®

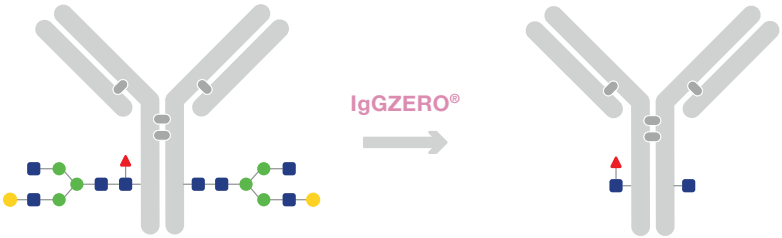
Hydrolysis of Complex Fc glycans



IgGZERO (EndoS) is an IgG-specific endoglycosidase hydrolyzing complex N-glycans at the Fc glycosylation sites. The enzyme deglycosylates IgG after the core GlcNAc and display limited activity on high-mannose and hybrid-type glycans. The enzyme is used to rapidly reduce sample complexity, to inactivate Fc-mediated effector functions in immunoassays and as a tool to improve imaging by reducing Fc interactions.

- Human IgG1-4, Fc-fusion proteins, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- Hydrolyzes the β1,4-linkage between the two innermost GlcNAc residues
- 30 min reaction
- Requires native IgG fold

Antibody Deglycosylation Workflow



The IgGZERO enzyme hydrolyzes N-glycans specifically at the Fc glycosylation site on IgG. In contrast to GlycINATOR, IgGZERO has a limited activity on high-mannose and hybrid-type glycoforms. The hydrolysis of complex glycans is fast and carried out under native and physiological conditions, since the enzyme requires native IgG fold for activity.

Product Formats

IgGZERO®
Lyophilized enzyme for deglycosylation of IgG

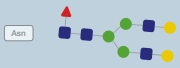
deGlycIT™
Immobilized enzyme for deglycosylation of IgG in spin columns

IgGZERO®

PRODUCT	DESCRIPTION	ID
IgGZERO, 1000 units	Deglycosylates 1 mg IgG	A0-IZ1-010
IgGZERO, 5000 units	Deglycosylates 5 mg IgG	A0-IZ1-050
IgGZERO LE (low endotoxin), 2000 units	Deglycosylates 2 mg IgG	A0-IZ8-020

Comparison of Glycosidases for N-glycans

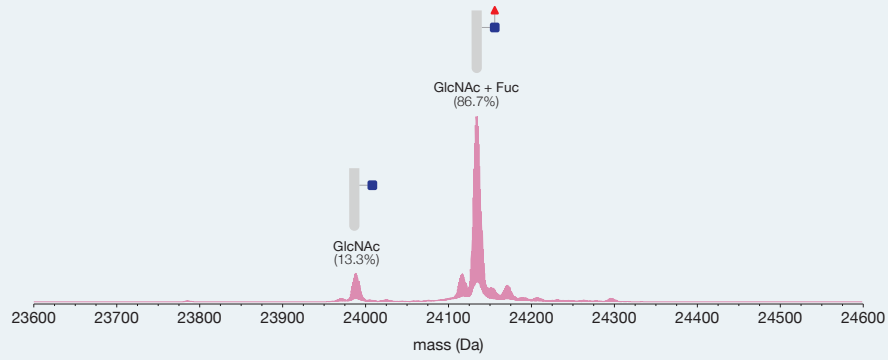
GlycINATOR, IgGZERO and EndoH are endoglycosidases acting between the core GlcNAc of the N-glycan, whereas PNGase F is a general amidase acting on the bond between the core GlcNAc and the peptide chain. GlycINATOR and IgGZERO are specific for the Fc glycans of IgG, and EndoH is a general endoglycosidase for high-manose type glycoforms. PNGase F acts broadly on all mammalian N-glycoforms but cannot hydrolyze glycans with α1,3 core fucosylation.

	GLYCINATOR	IGGZERO	PNGASE F	ENDO H
Digestion site				
Works on native IgG	Yes	Yes	Yes	Yes/No
pH optimum	7.4	7.4	7.5	5-6
Reaction time	30 min	30 min	15 min +	1-16 h
IgG specific	Yes*	Yes	No	No
Glycoform specificity	All Fc glycoforms	Complex Fc glycoforms, limited activity on high-mannose and hybrid-type glycans	All glycoforms except core α1,3 fucosylation	High-mannose and some hybrid-type glycans

* GlycINATOR has one more known substrate, α-1-acid glycoprotein.

Degree of Fucosylation in Clone Selection using IgGZERO®

Absence of a core fucose on the Fc N-glycan of a therapeutic antibody is associated with an increased antibody-dependent cellular cytotoxicity (ADCC), a desirable property for many therapeutic antibodies. Since IgGZERO rapidly reduces sample complexity by removing the heterogeneous Fc glycans and leaves only the potentially fucosylated, innermost GlcNAc residue, interpretation of MS data is greatly simplified.
A technique for studying the degree of fucosylation using IgGZERO has been developed by researchers at Institute Pasteur and LFB Biotechnologies. Briefly, the cell supernatant was concentrated, treated with FabRICATOR and IgGZERO, reduced, and then analyzed using LC-MS. This allowed for the easy determination of the fucosylation levels of the antibody and enhanced clone selection (Henninot et. al., 2015).



Determination of fucosylation levels using IgGZERO.

References

Henninot, A. et al. *Characterization of monoclonal antibodies by a fast and easy LC- MS ToF analysis on culture supernatant.* Anal Biochem 1–8 (2015).

deGlyIT™

PRODUCT	DESCRIPTION	ID
deGlycIT, Microspin 2 × 0.5 mg	Deglycosylates 2 × 0.5 mg IgG	A0-IZ6-010
deGlycIT, Microspin 5 × 0.5 mg	Deglycosylates 5 × 0.5 mg IgG	A0-IZ6-025
deGlycIT, Microspin 10 × 0.5 mg	Deglycosylates 10 × 0.5 mg IgG	A0-IZ6-050
deGlycIT, Midispin 1-10 mg	Deglycosylates 1-10 mg IgG	A0-IZ6-100
deGlycIT, Maxispin 10-100 mg	Deglycosylates 10-100 mg IgG	A0-IZ6-1000

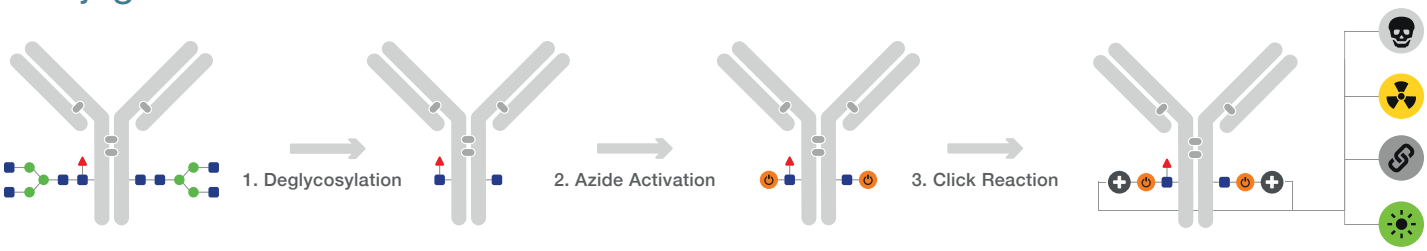
GlyCLICK®

Site-Specific Conjugation of Native IgG

GlyCLICK is a three-step conjugation technology for site-specific and quantitative conjugation of IgG from several species and subclasses. Specific Fc glycan hydrolysis allows for site-specific conjugation on the core GlcNAcs using robust click-chemistry and results in a degree of label (DOL) or antibody-drug ratio (DAR) of 2.0. The reliable performance ensures quantitative conjugation and intact immunoreactivity for sensitive applications, and the technology is available in a range of kit formats.

- Human IgG1-4, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- Conjugation occurs at the Fc N-glycan sites
- 2-3 day protocol
- Alexa Fluor®, biotin, DFO, MMAE, PNU, Azide activation

Conjugation Workflow



Deglycosylation: The Fc-specific endoglycosidase GlycINATOR (EndoS2) hydrolyzes the Fc glycans to the innermost GlcNAc moiety on several subclasses and species of IgG. The enzyme removes all glycoforms, including high-mannose, hybrid, complex, and bisecting type glycans.

Azide Activation: GalNAz is enzymatically attached to the exposed GlcNAc using ß-1,4-Galactosyltransferase (Y289L)[1] and UDP-GalNAz, to generate an azide-activated antibody that is reactive with a cyclooctyne-functionalized label of choice such as sDIBO or DBCO-reactive labels.

CLICK Reaction: The azide-activated antibody is conjugated with a cyclooctyne-functionalized label of choice in a bioorthogonal reaction through strain-promoted copper-free click chemistry (SPAAC) to form a covalent conjugation via a stable triazole.

Product Formats

GlyCLICK® Fluorophore
Site-specific conjugation of IgG with Alexa Fluor® 488, 555 or 647

GlyCLICK® Affinity
Site-specific conjugation of IgG with biotin

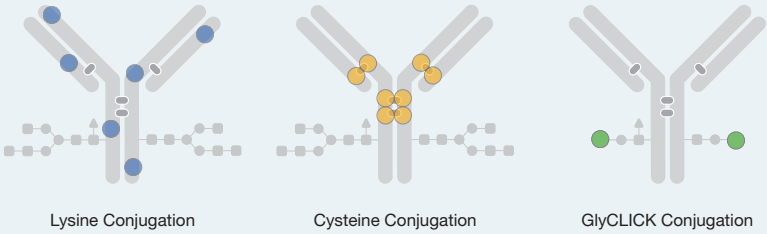
GlyCLICK® Chelator
Site-specific conjugation of IgG with DFO (desferrioxamine)

GlyCLICK® ADC
Site-specific conjugation of IgG with MMAE or PNU[2]

GlyCLICK® Azide Activation
Site-specific conjugation of IgG with any alkyne reactive click-chemistry

A New Generation of Antibody Conjugates using GlyCLICK®

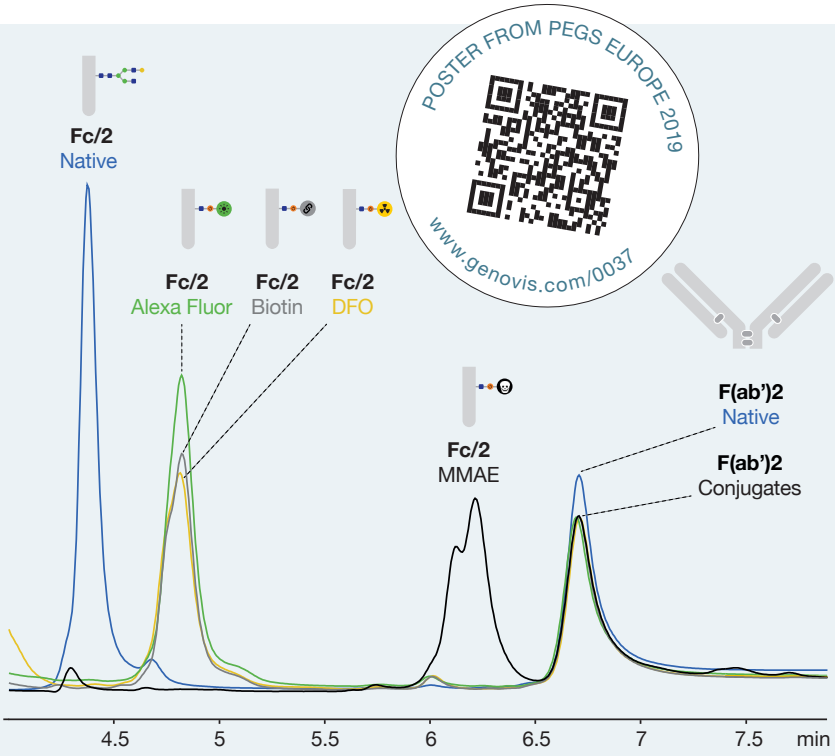
Conjugation is conventionally performed using random strategies by labeling at the available amines on lysine residues, or at the solvent accessible inter-chain cysteines. Although versatile, such methods offer limited control of the degree of labeling and site-occupancy and are thereby prone to influence stability, immunoreactivity and reproducibility of the final product. Site-specific strategies have become an attractive option to overcome these limitations, but often require genetic engineering to control and stabilize the process. Conjugation at the Fc glycan sites using GlyCLICK is not only site-specific, but also decreases the off-target effects of the IgG by disrupting the binding to Fc receptors (FcγRs) on immune cells.



Schematic representation of IgG and different conjugation technologies with degree of labeling. Lysine-based conjugation, DOL = varied; cysteine-based conjugation, DOL = 0-8; and GlyCLICK conjugation, DOL = 2.0.

Customized Labels for Versatile Applications using GlyCLICK®

The combination of enzymatic remodeling and copper-free click chemistry (SPAAC) enables GlyCLICK to site-specifically conjugate antibodies using any cyclooctyne-label of choice such as sDIBO or DBCO labels. To demonstrate the homogenous material obtained using GlyCLICK, panitumumab was conjugated with different labels and analyzed at the F(ab')2 and Fc/2 subunit level by RP-HPLC. The resulting separation shows shifts in retention detected only for the labeled Fc/2 subunits when compared to the native counterpart, demonstrating that GlyCLICK specifically conjugates at the Fc glycan sites.



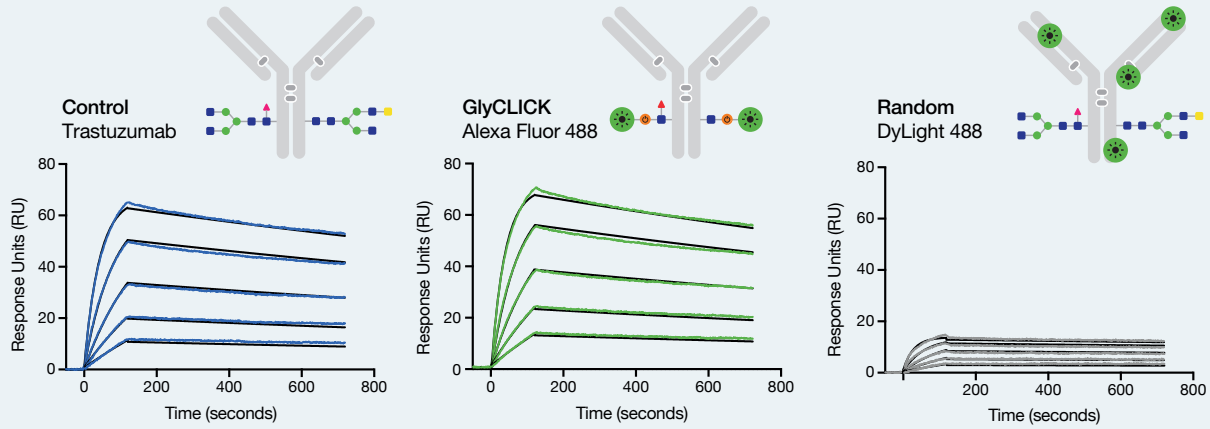
Analysis of Fc glycan conjugation using GlyCLICK. RP-HPLC analysis of unmodified panitumumab and panitumumab conjugated with desferrioxamine (DFO), Alexa Flour® 488, biotin or monomethyl auristatin E (MMAE) after digestion with FabRICATOR.

1. SiteClick™ is provided under an intellectual property license from Life Technologies Corporation. The trademark SiteClick™ is the property of Life Technologies Corporation.
2. Linker-toxin Payloads included in GlyCLICK® are provided under an intellectual property license from Glykos Finland ltd.

Reliable Antigen Detection with GlyCLICK®

Immunostaining using randomly labeled antibodies can influence the image quality through inadequate signals and unspecific binding due to excessive labeling or impaired immunoreactivity from labels resided in the antigen-binding Fab region.

To evaluate the immunoreactivity after labeling, trastuzumab was site-specifically conjugated to Alexa Fluor® 488 using GlyCLICK or by random labeling at the lysines using NHS-activated DyLight® 488. Surface plasmon resonance (SPR) response curves show that only 20% of the randomly labeled material retained binding capacity while the GlyCLICK conjugated antibodies displayed fully preserved immunoreactivity for sensitive and reliable detection.

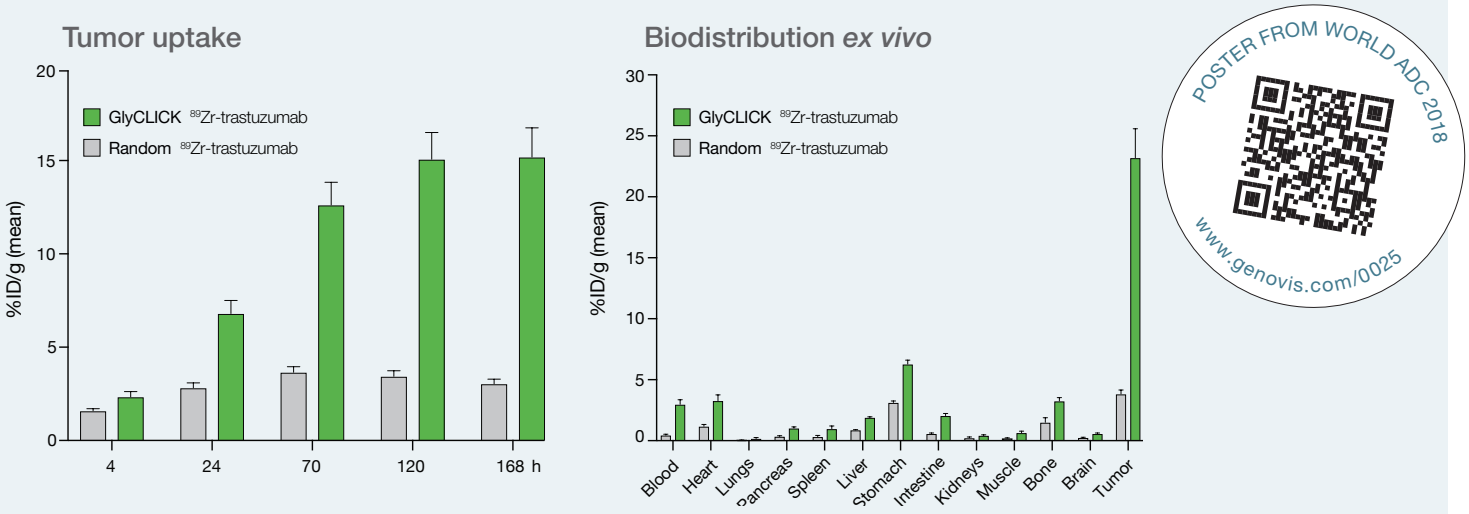


Affinity analysis of native and conjugated trastuzumab. Anti-human IgG (Fc) was used as the capturing molecule for trastuzumab. Native (control), Alexa Fluor® 488 (GlyCLICK, DOL=2), DyLight® 488 (random, DOL=10). HER2 was injected in a range to ensure sufficient curvature. All data were fitted against a 1:1 mathematical model.

In Vivo Performance of GlyCLICK® Conjugated Antibodies

Antibodies radiolabeled by using the site-specific GlyCLICK technology or produced by random conjugation at lysines were used in PET/CT imaging to evaluate their performance *in vivo*. Once chelated, the tracers were labeled with the standard radioisotope Zirconium-89 (^{89}Zr) before injected into tumor-bearing mice. Analysis of the injected mice was carried out using PET/CT imaging to measure the biodistribution and tumor uptake of the tracers.

The results demonstrate a superior tumor uptake and significantly longer circulation time of the site-specific GlyCLICK tracer, as compared to the randomly labeled tracer. The consistent number of labels attached per antibody using the GlyCLICK kit (DOL = 2) does not only improve performance *in vivo*, but also allows for better quantitation possibilities in PET-imaging experiments.

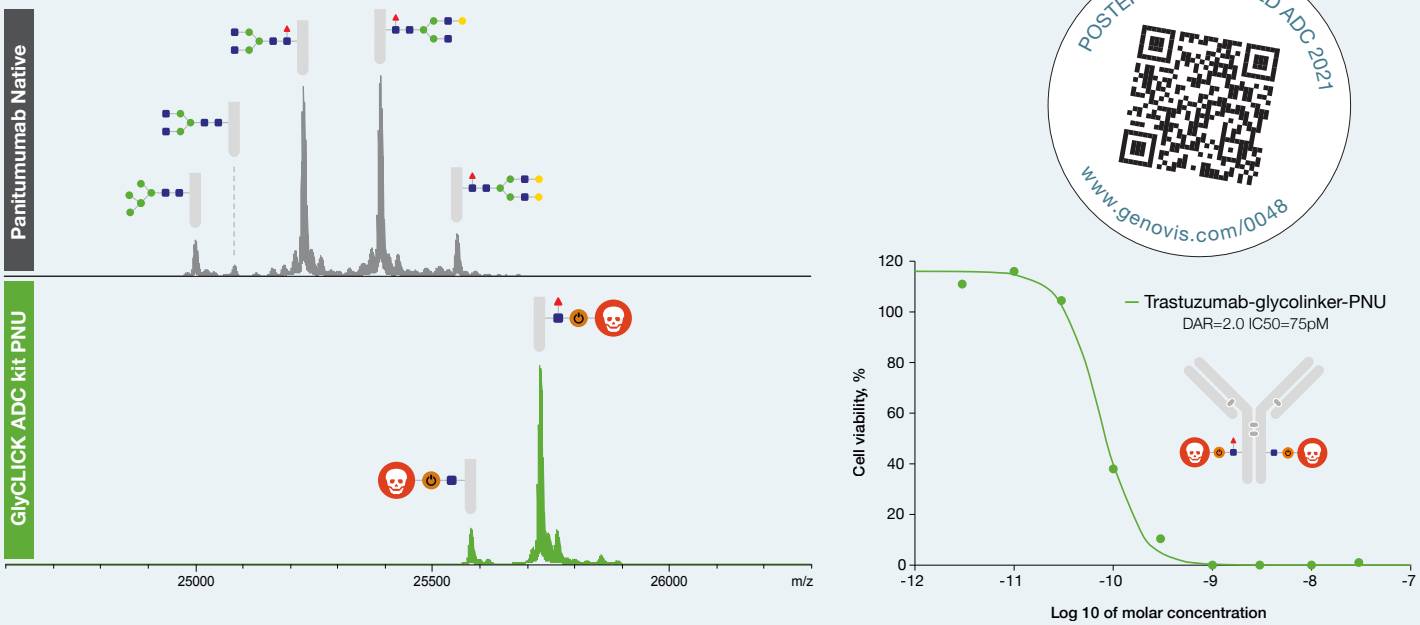


POSTER FROM WORLD ADC 2018
www.genovis.com/0025

Analysis of PET/CT imaging of SK-OV-3 tumor-bearing mice. Mean tumor uptake (left panel) over a time interval of 0 -168 hours post injection, and *ex vivo* biodistribution of ^{89}Zr-DFO-trastuzumab in major organs (right panel). Data was obtained in collaboration with Minerva Imaging (Copenhagen, Denmark).

Generation of Highly Potent ADCs using GlyCLICK® ADC Kit

The GlyCLICK ADC kits are designed to combine any native antibody with two-step cleavable linker-payloads carrying either the MMAE or PNU toxin. The site-specific GlyCLICK technology results in complete conjugation that incorporates linker-payloads to an antibody giving a DAR of 2.0. The kits generate homogenous ADCs with two-step cleavable linker-payloads for intracellular drug release, and produce conjugates that show a cytotoxic effect in the pM range, indicating functional and highly potent ADCs.



ADC generation using GlyCLICK. LC/MS analysis of panitumumab conjugated with the PNU linker-payload using GlyCLICK (left). The antibody was analyzed after FabRICATOR digestion to determine the native Fc/2 glycan profile and site-specific labeling after conjugation using GlyCLICK ADC kit PNU. *In vitro* cytotoxicity analysis of trastuzumab conjugated with the PNU linker-payload using GlyCLICK ADC kit PNU (right). HER2+ SK-BR-3 cells were incubated with the ADC and cell viability was measured using PrestoBlue after 5-6 days. Curves represent the average result from three replicates. Data was obtained in collaboration with Glykos Finland Oy (Helsinki, Finland).

GlyCLICK® Fluorophore

PRODUCT	DESCRIPTION	ID
GlyCLICK Alexa Fluor® 488	Conjugates 250 µg IgG	L1-F01-025
GlyCLICK Alexa Fluor® 488	Conjugates 1 × 2 mg IgG	L1-F01-200
GlyCLICK Alexa Fluor® 555	Conjugates 250 µg IgG	L1-F02-025
GlyCLICK Alexa Fluor® 555	Conjugates 1 × 2 mg IgG	L1-F02-200
GlyCLICK Alexa Fluor® 647	Conjugates 250 µg IgG	L1-F03-025
GlyCLICK Alexa Fluor® 647	Conjugates 1 × 2 mg IgG	L1-F03-200

GlyCLICK® Affinity

PRODUCT	DESCRIPTION	ID
GlyCLICK Biotin	Conjugates 250 µg IgG	L1-A01-025
GlyCLICK Biotin	Conjugates 1 × 2 mg IgG	L1-A01-200

GlyCLICK® Chelator

PRODUCT	DESCRIPTION	ID
GlyCLICK DFO	Conjugates 250 µg IgG	L1-C01-025
GlyCLICK DFO	Conjugates 1 × 2 mg IgG	L1-C01-200

GlyCLICK® ADC

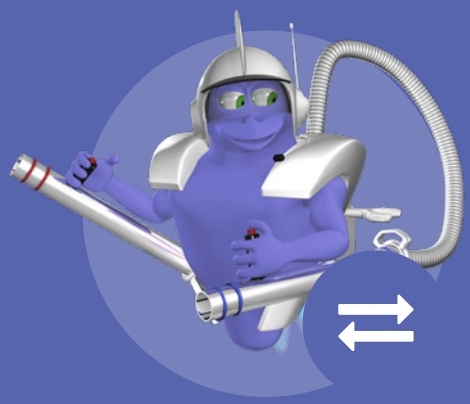
PRODUCT	DESCRIPTION	ID
GlyCLICK ADC kit, MMAE	Conjugates 1 × 2 mg IgG	L1-T02-200
GlyCLICK ADC kit, PNU	Conjugates 1 × 2 mg IgG	L1-T01-200

GlyCLICK® Azide Activation

PRODUCT	DESCRIPTION	ID
GlyCLICK Azide Activation	Activates 250 µg IgG	L1-AZ1-025
GlyCLICK Azide Activation	Activates 1 × 2 mg IgG	L1-AZ1-200
GlyCLICK Azide Activation	Activates 1 × 10 mg IgG	L1-AZ1-100

TransGLYCIT™

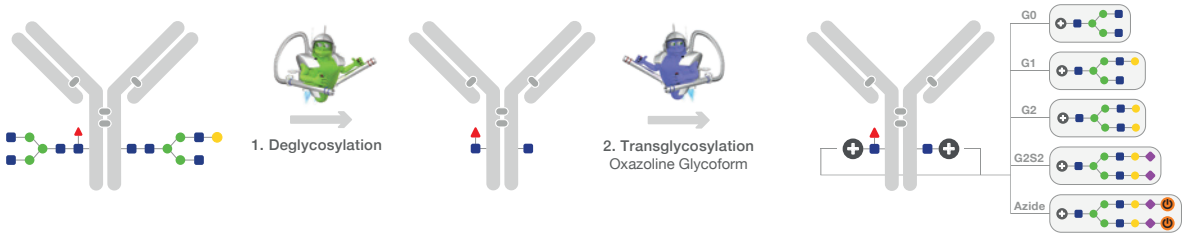
Transglycosylation of IgG



TransGLYCIT is a platform that enables efficient and site-specific human IgG glycan remodeling and conjugation. With TransGLYCIT, antibodies with defined and homogenous glycoforms or site-specific azide activation are generated using fast and robust enzymatic workflows. With the optional FucosEXO™16 enzyme in the glycan remodeling workflow, afucosylated antibodies carrying the Fc glycoform of choice (G0, G1, G2 or G2S2) can be obtained for comparison of antibodies with or without core fucose. With the azide activation workflow, antibodies are prepared for conjugation with any label of choice using click-chemistry.

- Human IgG1, IgG2 and IgG4
- Transglycosylation occurs at the Fc N-glycan sites
- 3-6 hour workflow
- G0, G1, G2, G2S2 or azide-activated glycoform is included

The Transglycosylation Workflow



Deglycosylation: The Fc N-glycans are trimmed to the core GlcNAc using the IgG-specific Immobilized GlycINATOR® (EndoS2) enzyme that hydrolyses all Fc glycoforms, including high-mannose, hybrid, complex and bisecting glycans. The degree of fucosylation is the same as on the original IgG.

Note that for the glycan remodeling products, it is possible to obtain afucosylated G0, G1, G2 or G2S2 glycoforms using TransGLYCIT Afucosylated, where the Immobilized FucosEXO™16 enzyme hydrolyzes the α1,6-linked core fucose prior to the transglycosylation step.

Transglycosylation: The engineered glycosynthase TransINATOR™ catalyzes the transglycosylation reaction between the oxazoline reactive G0, G1, G2, G2S2 or azide-activated glycoform and the core GlcNAc.

Product Formats

TransGLYCIT™
Glycan remodeling of human IgG with the G0, G1, G2 or G2S2 glycoform

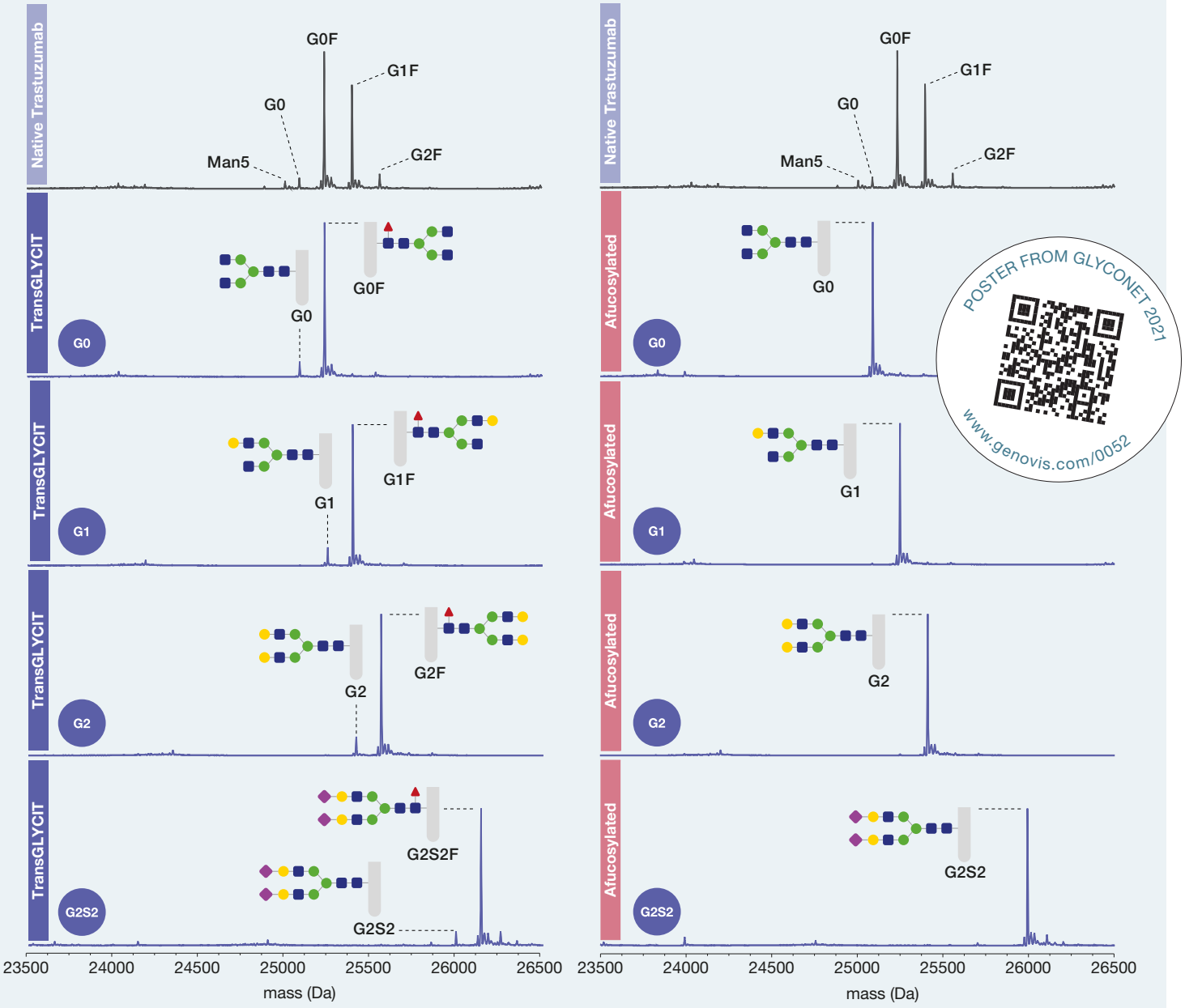
TransGLYCIT™ Azide Activation
Azide activation of human IgG1/IgG4 or IgG2

TransGLYCIT™ Afucosylated
Glycan remodeling of human IgG with the G0, G1, G2 or G2S2 glycoform and core afucosylation

Generating Agalactosylated, Galactosylated, or Sialylated Fc Glycoforms using TransGLYCIT™

Glycoengineering of the IgG Fc N-glycan profile is important for the development of next-generation therapeutic antibodies with enhanced or silenced effector functions. TransGLYCIT is used to generate antibodies carrying agalactosylated (G0), galactosylated (G1, G2) or sialylated (G2S2) glycan structures. With the option to generate glycan structures lacking the core fucose using the TransGLYCIT Afucosylated kit, antibodies that show increased binding to activating FcγIIIa receptors and thus an elevated ADCC response can be obtained for direct comparison between fucosylated and afucosylated antibodies.

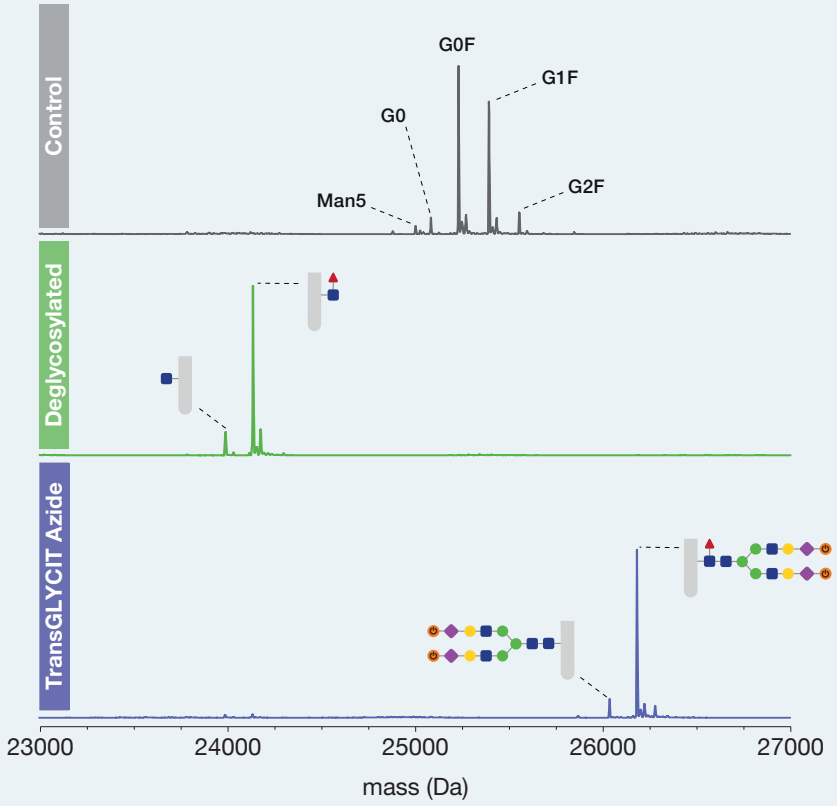
Here, the glycan profile of the therapeutic antibody trastuzumab was remodeled to carry either fucosylated or afucosylated G0, G1, G2 or G2S2 glycoforms. The resulting mass spectra show the heterogenous glycan profile of native trastuzumab and the mass shifts after complete transglycosylation of the antibody to generate G0, G1, G2 or G2S2 using TransGLYCIT. Homogenous afucosylated glycan profiles were generated using the TransGLYCIT Afucosylated kits.



Transglycosylation using TransGLYCIT. Deconvoluted mass spectra of the Fc/2 fragment of native trastuzumab (top) and after transglycosylation with TransGLYCIT (left panel) or TransGLYCIT Afucosylated (right panel). The mAb was digested with FabRICATOR and the subunits were analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1 × 50 mm).

Site-specific Azide Activation of Human IgG using TransGLYCIT™ Azide Activation

Site-specific Fc N-glycan conjugation is a powerful tool with applications ranging from biomedical research to the development of diagnostic methods and antibody-drug conjugates (ADCs). A reliable conjugation technology is crucial to obtain a homogenous and reproducible conjugate while preserving the stability and immunoreactivity of the final product. Using the TransGLYCIT Azide Activation technology, human IgG is azide-activated by transglycosylation within a few hours. Here, TransGLYCIT Azide Activation was used for site-specific azide activation of trastuzumab using the modified N-glycan oxazoline carrying two azide functionalities. The reaction generated an IgG with four azide-activated sites for subsequent conjugation, two sites on each Fc/2.



Azide activation of trastuzumab using the TransGLYCIT Azide Activation workflow. Deconvoluted mass spectra of the Fc/2 fragment of native trastuzumab (top), after deglycosylation by GlycINATOR (middle) and after transglycosylation by TransINATOR with the oxazoline glycoform azide as the substrate (bottom). The mAb was digested with FabRICATOR and the subunits were analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1×50mm).

TransGLYCIT™

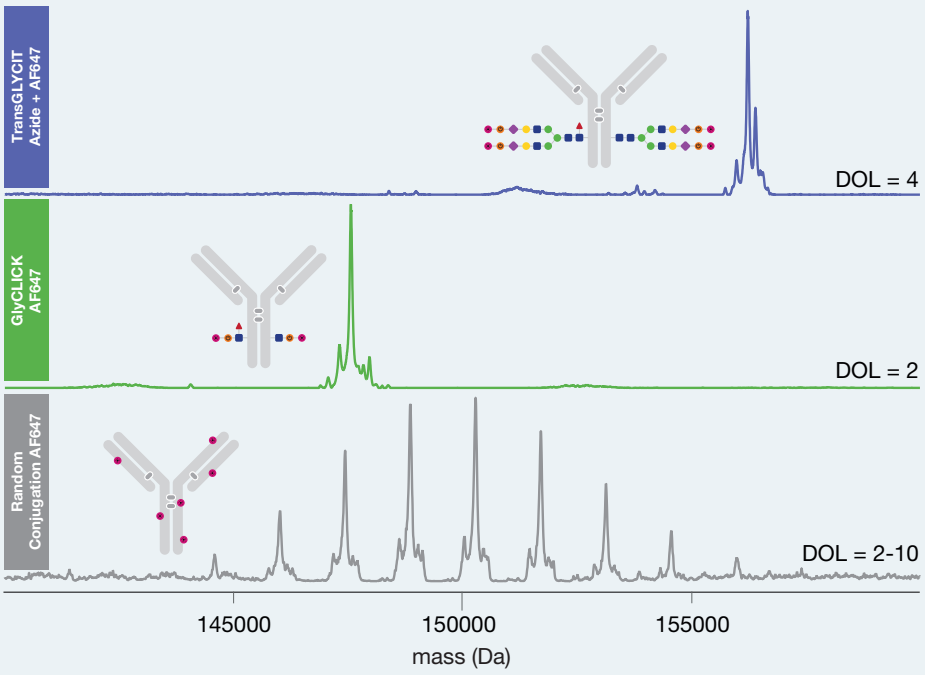
PRODUCT	DESCRIPTION	ID
TransGLYCIT G0, 1 mg	Generates 1 mg human IgG with the G0 glycoform	T1-G0F-010
TransGLYCIT G1, 1 mg	Generates 1 mg human IgG with the G1 glycoform	T1-G1F-010
TransGLYCIT G2, 1 mg	Generates 1 mg human IgG with the G2 glycoform	T1-G2F-010
TransGLYCIT G2S2, 1 mg	Generates 1 mg human IgG with the G2S2 glycoform	T1-S2F-010

TransGLYCIT™ Azide Activation

PRODUCT	DESCRIPTION	ID
TransGLYCIT Azide Activation, 100 µg	Activates 100 µg human IgG1 or IgG4	T1-AZ1-001
TransGLYCIT Azide Activation hIgG2, 100 µg	Activates 100 µg human IgG2	T1-AZ2-001

Comparison of Site-specific and Random Conjugation Technologies

The azide-activated Fc N-glycans on antibodies modified using the TransGLYCIT Azide Activation workflow can be used for site-specific conjugation with a label of choice via click chemistry, resulting in an antibody homogeneously labeled with four labels per molecule. Using site-specific conjugation instead of random conjugation technologies is favorable for preserving affinity and generating conjugates where quantitative properties are desired. To illustrate the impact of different labeling methods on the degree of labeling (DOL), we here analyzed trastuzumab conjugated with a fluorophore using either TransGLYCIT, GlyCLICK or random labeling by NHS-chemistry. The TransGLYCIT and GlyCLICK workflows resulted in homogeneously labeled samples with defined DOL 4 or DOL 2, respectively, whereas the random technique resulted in a heterogeneously labeled sample with a DOL varying from 2 to 10.



Conjugation of trastuzumab using site-specific and random technologies. Deconvoluted mass spectra of intact trastuzumab modified using TransGLYCIT Azide Activation followed by site-specific conjugation by strain-promoted azide alkyne click chemistry using DBCO-AF647 (DOL=4; top), conjugated using GlyCLICK AlexaFluor®647 (DOL=2; middle), and randomly labeled using NHS-activated AlexaFluor 647 resulting in heterogenous labeling (DOL=2-10; bottom). The randomly labeled sample was analyzed after deglycosylation by GlycINATOR. The intact masses of the trastuzumab conjugates were analyzed using a Bruker Impact II ESI-QTOF MS.

TransGLYCIT™ Afucosylated

PRODUCT	DESCRIPTION	ID
TransGLYCIT G0 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G0 glycoform	T1-G0A-010
TransGLYCIT G1 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G1 glycoform	T1-G1A-010
TransGLYCIT G2 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G2 glycoform	T1-G2A-010
TransGLYCIT G2S2 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G2S2 glycoform	T1-S2A-010

GingisREX®

Arginine-Specific Protein Digestion

GingisREX (RgpB) is an arginine-specific protease that digests proteins C-terminally of arginine residues, including arginine – proline linkages that are difficult to digest with other enzymes. The enzyme can be used in peptide mapping, *de novo* peptide sequencing and analysis of post-translational modifications.

- Digests any peptide or protein containing Arg. Specific for Arg-X motifs
- Digests C-terminally of Arg residues
- 60 min reaction
- Active in 6 M urea and 0.1% SDS

Protein Digestion Workflow

GingisREX is a cysteine protease that specifically digests peptide bonds C-terminally of arginine residues, including sites next to proline. Longer peptides are generated, as compared to trypsin digestion, which can be resolved and identified by high-resolution MS. This offers more options in sample preparation for bottom-up approaches to achieve alternative digestion profiles and increased sequence coverage. GingisREX does not have activity at lysines, as commonly observed using Arg-C. The enzyme is active at a broad pH range of 5.0 - 9.0, and requires cysteine but is inhibited by guanidine hydrochloride.

Product Formats

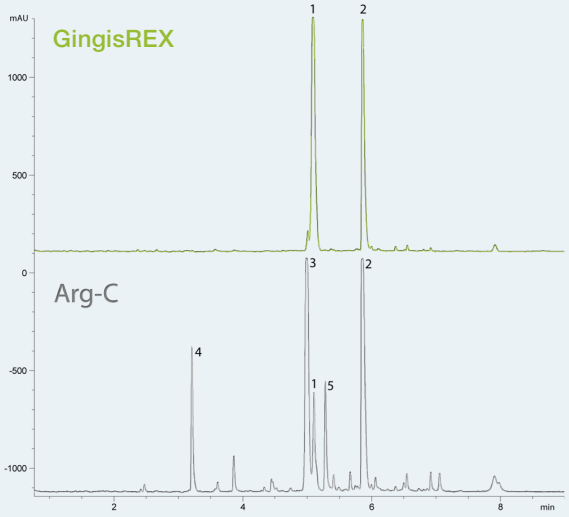
GingisREX®
Lyophilized enzyme for digestion
C-terminally of arginine residues

GingisREX®

PRODUCT	DESCRIPTION	ID
GingisREX, 5 µg enzyme	Enzyme:Protein ratio: 1:20 - 1:200	B0-GRX-005

Arginine-Specific Digestion using GingisREX®

Interpretation of bottom-up mass spectrometry data is often based on specific assumptions about the specificity of the protease used to digest the sample. Unspecific activities create uncertainties that make data interpretation difficult and increase both analysis time and the risk for misinterpretations. We compared the activities of GingisREX and Arg-C using oxidized insulin β-chain as a very simple model substrate. Oxidized insulin β-chain contains only one arginine as well as one lysine residue and should therefore only be digested at one single site by Arg-specific proteases. When analyzing the peptides generated by GingisREX and Arg-C by RP-HPLC and MS, GingisREX showed specific digestion at the arginine residues with no activity on the lysine residue and no other unspecific activities even after prolonged incubation over-night with a high enzyme to substrate ratio (1:5). In contrast, Arg-C displayed a primary activity at the arginine residue, but digestion could also be observed at the lysine residue as well as a number of other minor sites.



Digestion of oxidized β-chain of insulin with GingisREX and Arg-C. The digestion was performed O/N at 37°C, with an enzyme to substrate ratio of 1:20 (w/w), 20 mM cysteine in buffers at pH 7.4 (GingisREX) and pH 7.6 (Arg-C). The peptides were separated on RP-HPLC.

PEPTIDE NO.	AMINO ACID SEQUENCE
Intact protein	FVNQHLCGSHLVEALYLVCGERGFFYTPKA
1	GFFYTPKA
2	FVNQHLCGSHLVEALYLVCGER
3	GFFYTPK
4	FVNQHLCGSH
5	LVEALYLVCGER + Na

Sequences of oxidized insulin β-chain digested by GingisREX or Arg-C. Green indicates arginine residues and red indicates lysine residues.

Peptide Mapping of Therapeutic Antibodies using GingisREX®

On a large and complex sample, such as a therapeutic antibody, the digestion at arginine residues gives larger peptides and results in fewer peaks in the peptide map. Here, the GingisREX and Arg-C digestion profiles of trastuzumab are presented. The digestion by GingisREX at Arg sites resulted in fewer peaks and the peptide map was less complicated, a feature that is beneficial for data interpretation in mass fingerprint analysis.

Peptide maps of trastuzumab after digestion using GingisREX or Arg-C.

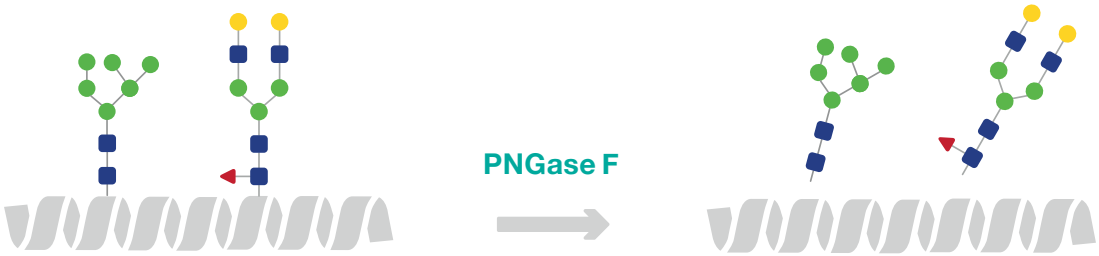
PNGase F

Hydrolysis of N-glycans

PNGase F (Peptide N-glycosidase F) is a glycoamidase hydrolyzing the amide bond between the polypeptide asparagine and the innermost GlcNAc of all mammalian asparagine-linked complex, hybrid, or high-mannose oligosaccharides. The enzyme is widely used for sample preparation prior to MS analysis – to reduce the protein heterogeneity and enable released glycan analysis – and to study the functional role of the N-glycan.

- N-linked glycans on glycoproteins
- Hydrolyzes the glycosidic bond between N-glycans and Asn
- 15 + min reaction time
- *Rapi*Gest™ included in the Immobilized PNGase F Denaturing kit

Deglycosylation Workflow



PNGase F hydrolyzes the glycosidic bond between the N-glycans and asparagine. During the reaction, the asparagine residue from which the glycan is removed is deamidated to aspartic acid. The released glycan is left intact and can be used for further analysis. PNGase F is active under both native and denaturing reaction conditions.

Product Formats

PNGase F
Lyophilized enzyme for deglycosylation of glycoproteins

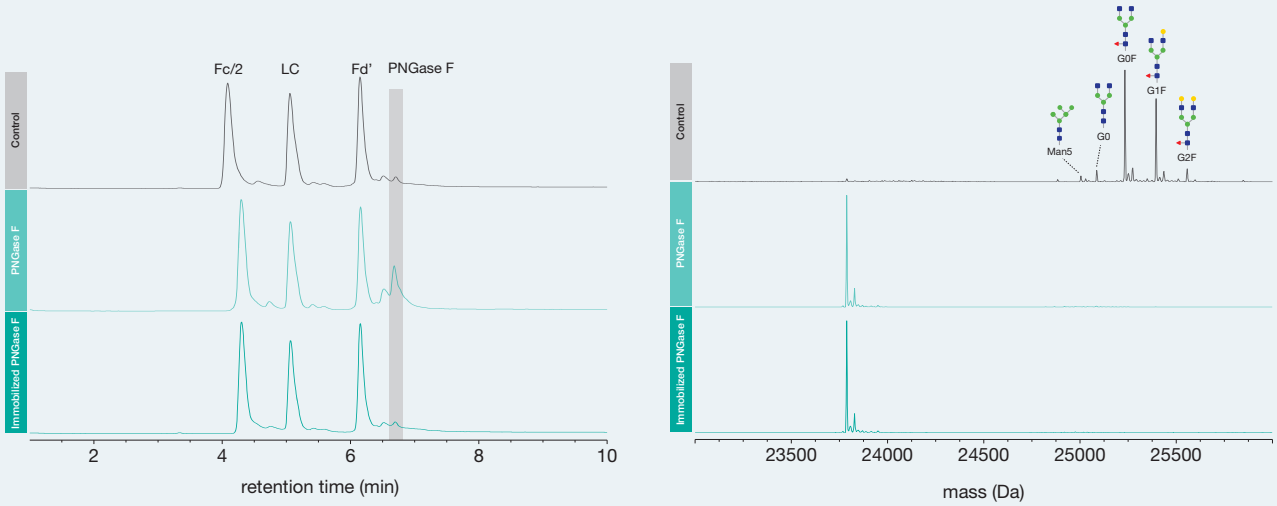
Immobilized PNGase F
Hydrolysis of N-glycans from glycoproteins in spin columns under native conditions

Immobilized PNGase F Denaturing
Hydrolysis of N-glycans from glycoproteins in spin columns under denaturing conditions

Removal of N-glycans under Native Conditions using PNGase F

Removing the Fc N-glycans with PNGase F under native conditions enables characterization of the free N-glycans as well as the function and structure of the deglycosylated antibody. Trastuzumab was used to demonstrate the efficient removal of N-glycans by PNGase F and Immobilized PNGase F under native conditions.

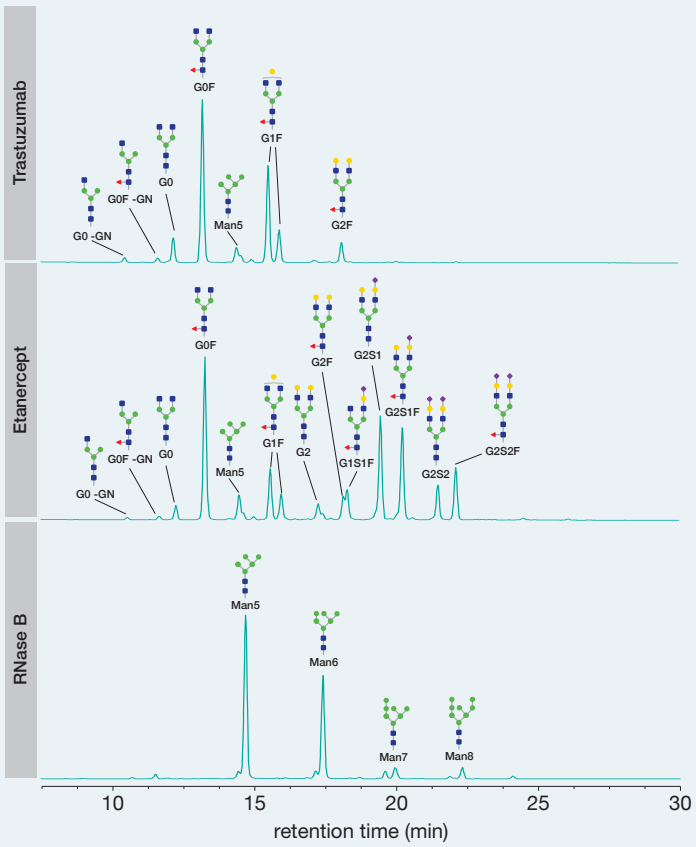
The mass shift demonstrates successful removal of the Fc N-glycans with no enzyme interfering in the analysis of the sample processed with Immobilized PNGase F.



Removal of N-glycans under native conditions. TIC chromatogram (left) and deconvoluted mass spectra of the Fc/2 fragment (right) of intact trastuzumab (top panel) and trastuzumab treated with Immobilized PNGase F (bottom panel) or PNGase F (middle panel) for 1 h at 37°C. The antibody samples were analyzed after digestion with FabRICATOR.

Released Glycan Analysis using PNGase F

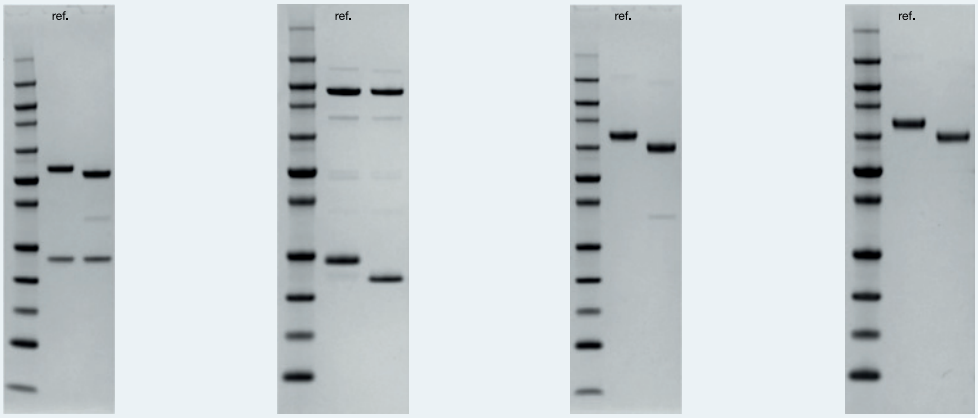
The N-glycosylation profile of a therapeutic protein is a critical quality attribute. It affects both safety and efficacy of the biopharmaceutical and therefore needs to be characterized and monitored during development and production. Here, the N-glycans of the therapeutic antibody trastuzumab, the Fc-fusion protein etanercept and the glycoprotein RNase B were analyzed using a released glycan approach. Trastuzumab and etanercept were deglycosylated with PNGase F under native conditions, while RNase B required denaturation and reduction to be fully deglycosylated. The resulting released glycans were labelled with *Rapi*Fluor-MS™ (Waters Corporation) and analyzed by HILIC UPLC-FLD-MS.



Released glycan analysis of glycoproteins. *Rapi*Fluor-MS™ labelled N-glycans released from trastuzumab (top), etanercept (middle) and RNase B (bottom) were analyzed by HILIC-FLD-MS using a Waters™ BioAccord™ system equipped with a Waters™ ACQUITY Premier Glycan BEH Amide column (130Å, 2.1 × 150mm). The fluorescence chromatograms are shown, and the glycan structures were annotated using a combination of glucose unit (GU) library search and MS confirmation.

N-glycan Removal using PNGase F

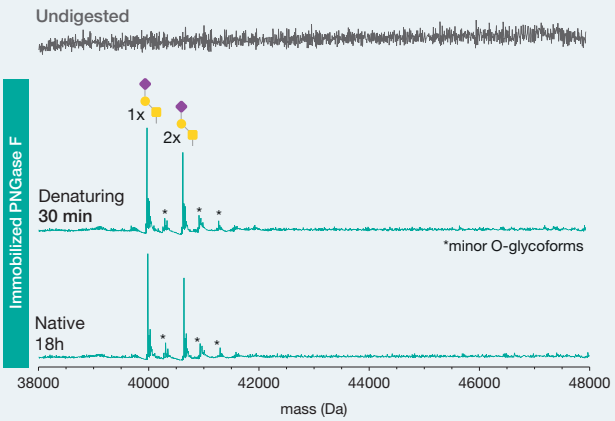
	TRASTUZUMAB		ETANERCEPT	
Protein type	IgG1		Fusion protein	
N-glycans	2		6	
O-glycans	–		26	
Product format	PNGase F	Immobilized PNGase F	PNGase F	Immobilized PNGase F
Reaction conditions	Native 37°C, 1 h	Native 37°C, 1 h	Native 37°C, 1 h	Native 37°C, 1 h
Preparation of samples for gel	Reduced	FabRICATOR digested	Reduced	Reduced



Key characteristics and SDS-PAGE assay of a selection of glycoproteins deglycosylated using PNGase F and Immobilized PNGase F. The table specifies important characteristics of the examined glycoproteins, the reaction conditions used for the deglycosylation and the sample preparations performed before SDS-PAGE analysis. The mass shifts on the gels show the successful removal of N-glycans after PNGase F or Immobilized PNGase F treatment from the various glycoprotein substrates.

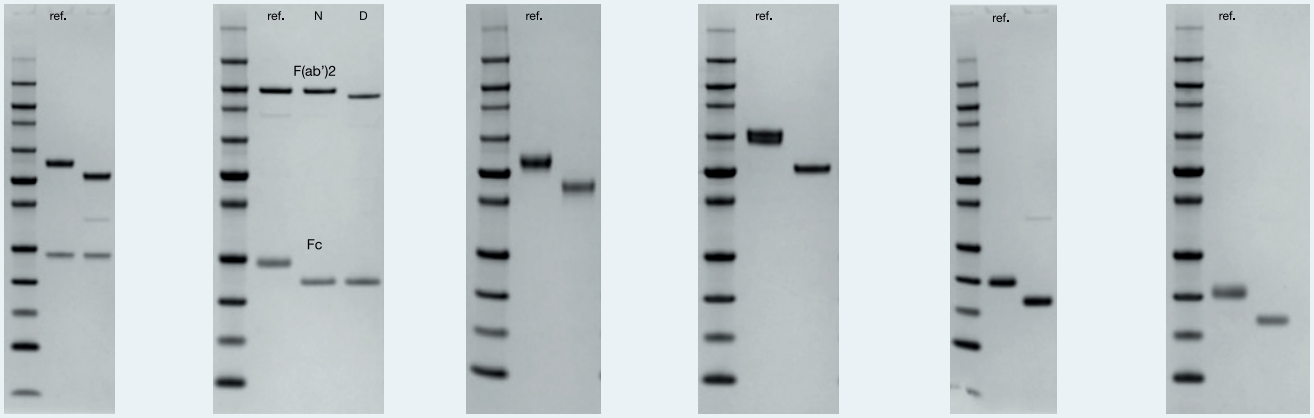
Rapid N-glycan Removal using Immobilized PNGase F Denaturing

Some glycosylation sites are poorly accessible for PNGase F and the deglycosylation reaction is slow or inhibited by steric hindrance. To demonstrate the increase in reaction speed under denaturing conditions, the fusion protein abatacept was processed with Immobilized PNGase F using either the native or the denaturing workflow. Native conditions allowed for removal of N-glycans without any additives or increased temperature but required overnight (18 h) incubation for complete deglycosylation. Removal of the N-glycans using denaturing conditions was complete within 30 minutes. As can be seen from the MS spectra, all N-glycans were removed while the O-glycans were left intact. The intact protein is complex to analyze, but after Immobilized PNGase F treatment, the complexity of the sample was greatly reduced.



Deconvoluted mass spectra of abatacept (top panel) treated with Immobilized PNGase F under denaturing (middle panel) or native conditions (bottom panel). The protein was incubated for 18 h with Immobilized PNGase F using native reaction conditions or 30 min using denaturing conditions. The deglycosylated protein samples were reduced and analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1 × 50 mm). The mass shifts show the complete removal of N-glycans, leaving only the O-glycoforms.

CETUXIMAB		ABATACEPT	AFLIBERCEPT	RNASE B (BOVINE PANCREAS)	
IgG1		Fusion protein	Fusion protein	Glycoprotein	
4		6	10	1	
–		8	–	–	
PNGase F	Immobilized PNGase F	Immobilized PNGase F	Immobilized PNGase F	PNGase F	Immobilized PNGase F
Denaturing, 50°C, 10 min	Native (N) 37°C, 1 h Denaturing (D) 50°C, 15 min	Denaturing 50°C, 30 min	Denaturing 50°C, 30 min	Denaturing and reducing, 50°C, 10 min	Denaturing and reducing, 50°C, 15 min
Reduced	FabRICATOR digested	Reduced	Reduced	None	None



PNGase F

PRODUCT	DESCRIPTION	ID
PNGase F, 1000 units	Deglycosylates 1 mg glycoproteins	G1-PF1-010
PNGase F, 5 × 1000 units	Deglycosylates 5 × 1 mg glycoproteins	G1-PF1-050

Immobilized PNGase F

PRODUCT	DESCRIPTION	ID
Immobilized PNGase F	5 × 0.2 mg microspin columns	G1-PF6-010
Immobilized PNGase F	10 × 0.2 mg microspin columns	G1-PF6-020

Immobilized PNGase F Denaturing

PRODUCT	DESCRIPTION	ID
Immobilized PNGase F Denaturing	5 × 0.2 mg microspin columns + 5 × 1 mg *Rapi*Gest™ SF	G2-PDK-010
Immobilized PNGase F Denaturing	10 × 0.2 mg microspin columns + 10 × 1 mg *Rapi*Gest™ SF	G2-PDK-020

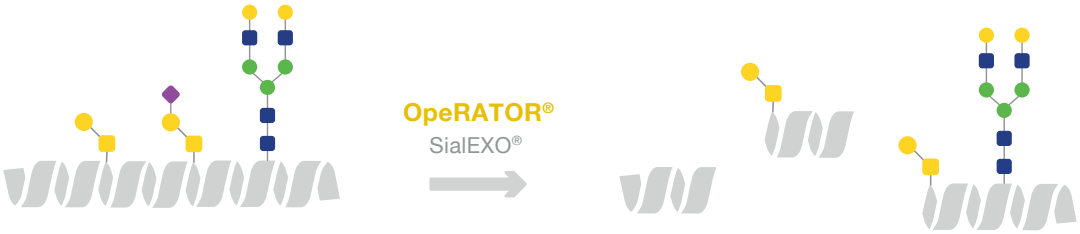
OpeRATOR®

O-glycan-specific Protein Digestion

OpeRATOR is an O-glycan-specific protease that digests proteins carrying mucin-type O-glycans N-terminally of the glycosylated Ser and Thr residues. Glycopeptides carrying O-glycans are generated, and the enzyme enables O-glycan profiling, O-glycopeptide mapping and site occupancy determination, as well as middle-level approaches using MS analysis.

- Native proteins with mucin-type O-glycosylation
- Digests N-terminally of O-glycosylated Ser and Thr residues
- 2h to O/N (16-18h) reaction
- Desialylation using SialEXO (included) increases performance

O-glycoprotein Digestion Workflow



With high specificity, OpeRATOR digests mucin-type O-glycoproteins and peptides N-terminally of the O-glycans, at serine or threonine residues. It will not digest glycoproteins at N-glycan sites. The enzyme is most active towards sites with asialylated core 1 O-glycans. It also digests sialylated core 1 and core 3, but to a much lower extent. OpeRATOR is compatible with SialEXO (a sialidase mix), which is included in the purchase.

Product Formats

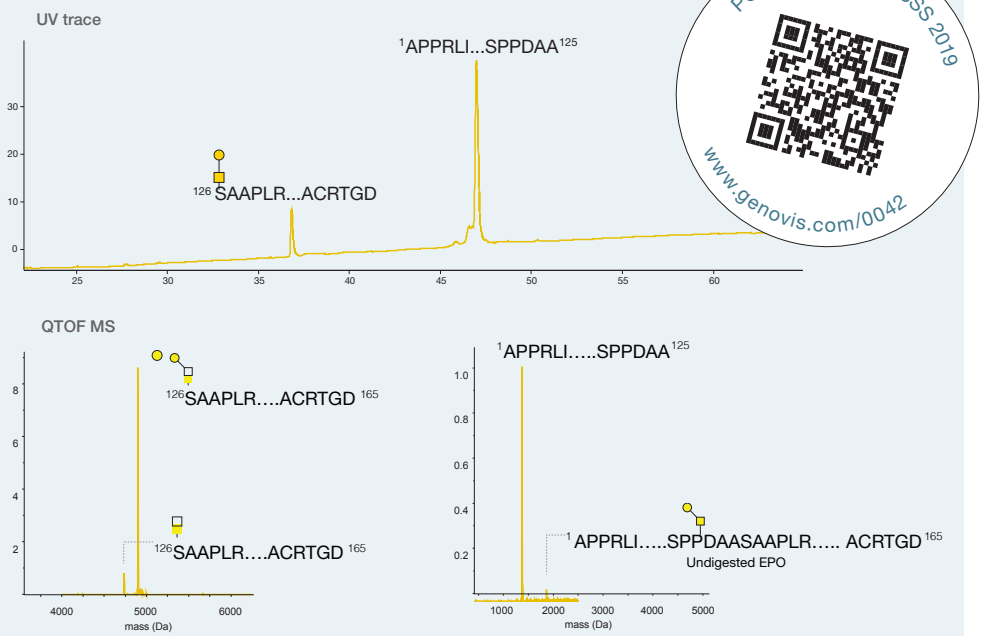
OpeRATOR®
Lyophilized enzyme for digestion of mucin-type O-glycoproteins and peptides

OpeRATOR®

PRODUCT	DESCRIPTION	ID
OpeRATOR, 2000 units	Digests 2 mg O-glycoprotein	G2-OP1-020

O-glycan Site-specific Digestion using OpeRATOR®

OpeRATOR can be used to map O-glycosylation sites at the middle-level. Indeed, for proteins carrying only one or a few O-glycans, the O-glycosylation site can be determined directly from identifying the site of OpeRATOR digestion. Erythropoietin (EPO) is a ~30 kDa glycoprotein with a single O-glycosylation site. After removal of the N-glycans by PNGaseF and desialylation using SialEXO, the native protein was digested N-terminally of the O-glycan site using OpeRATOR. The resulting protein fragments were analyzed by reversed-phase LC-MS.

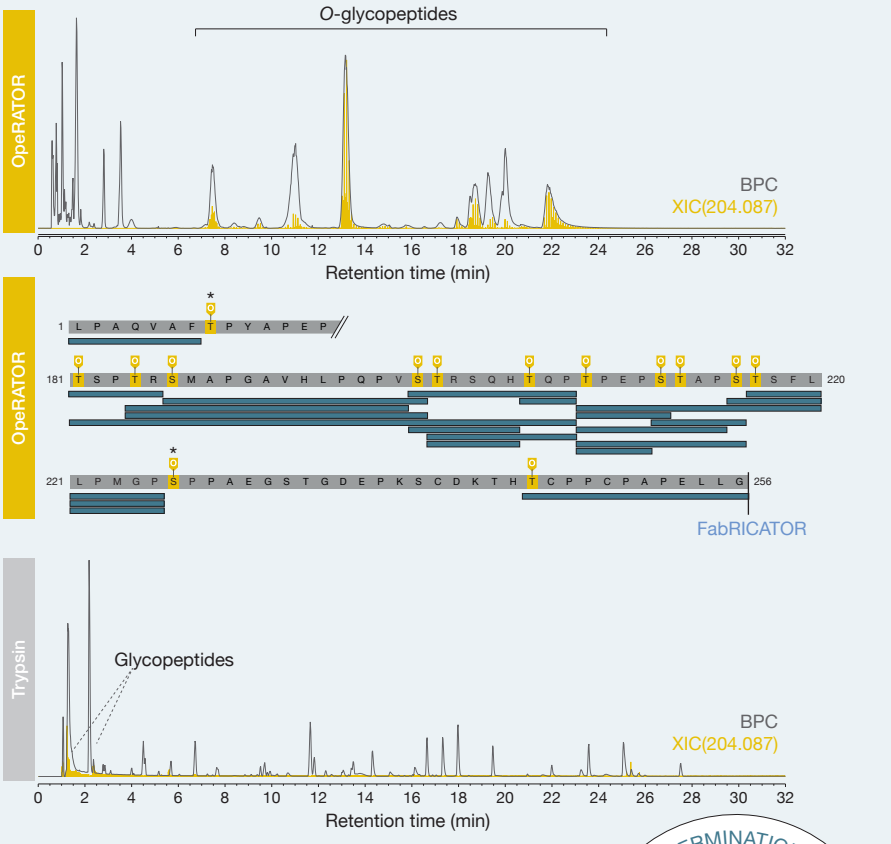


Specific digestion N-terminally of the O-glycosylation site. The reduced fragments were separated on a reversed-phase C4 column followed by ESI-QTOF MS detection. The EPO protein carrying one core 1 O-glycan was hydrolyzed at a single specific site N-terminally of the O-glycosylated serine using OpeRATOR.

POSTER FROM CASSS 2019
www.genovis.com/0042

A Complete Map of Etanercept O-glycan Sites using OpeRATOR®

Etanercept is an Fc-fusion protein with a highly O-glycosylated hinge region that is difficult to analyze by conventional assays. The protein was here incubated with OpeRATOR, and the resulting glycopeptides were analyzed using LC-MS/MS (top panel). Due to the heterogeneity in the O-glycan pattern of the protein and the OpeRATOR specificity for O-glycan structures, overlapping peptides were formed that made it possible to acquire a complete map of the O-glycan sites (middle panel). For comparison, a standard tryptic digest of the same material (bottom panel), shows only few and very large glycopeptides that were difficult to analyze.



In-depth analysis of the O-glycosylation sites of etanercept originator. HILIC-MS analysis (top panel) of etanercept peptides generated by digestion with OpeRATOR. The middle panel shows the peptide map of the O-glycosylation sites of etanercept based on the results from the OpeRATOR-based workflow. The identified O-glycosylation sites are marked in yellow. Two sites (marked with an asterisk) could only be inferred from the OpeRATOR digestion pattern without direct identification of a peptide containing the glycosylated amino acid. The bottom panel shows a RP-MS analysis of etanercept digested with trypsin. Data was obtained in collaboration with Thermo Fisher Scientific (UK).

APP NOTE: DETERMINATION OF O-GLYCAN SITES
www.genovis.com/0055

OglyZOR®

Hydrolysis of Core-1 O-glycans

OglyZOR is an endoglycosidase (O-Glycosidase) that specifically hydrolyzes core 1 O-glycan disaccharides on native glycoproteins. OglyZOR is used for removal of O-glycans for glycan analysis, confirmation of O-glycan presence and reduction of sample heterogeneity.

- O-glycans on glycoproteins
- Hydrolyzes Core 1 disaccharides
- 2-4 h reaction
- Requires removal of terminal sialic acids (SialEXO included)

O-glycoprotein Deglycosylation Workflow

OglyZOR is an endoglycosidase (endo-α-N-acetylgalactosaminidase) that hydrolyzes core 1 (Gal-β1-3-GalNAc), and to a limited extent core 3 (GlcNAc-β1-3-GalNAc) O-glycan disaccharides on native glycoproteins. The enzyme does usually not require denaturation of the substrate. Since the terminal sialic acids of the O-glycans need to be removed for OglyZOR activity, the sialidase mix SialEXO is included. Optimal activity is obtained at pH 6.5 to 7.5 and at 37°C.

Product Formats

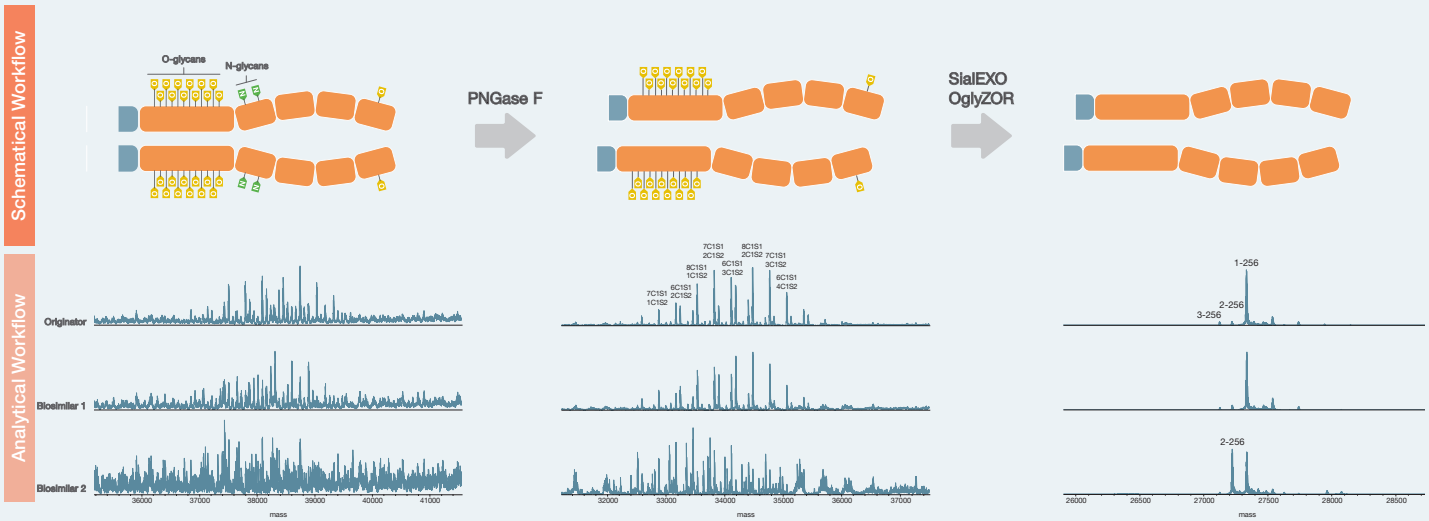
OglyZOR®
Lyophilized enzyme for hydrolysis of core 1 O-glycans on glycoproteins

OglyZOR®

PRODUCT	DESCRIPTION	ID
OglyZOR, 2000 units	Deglycosylates 2 mg glycoprotein	G2-OG1-020

Deglycosylation of Biopharmaceuticals using OglyZOR®

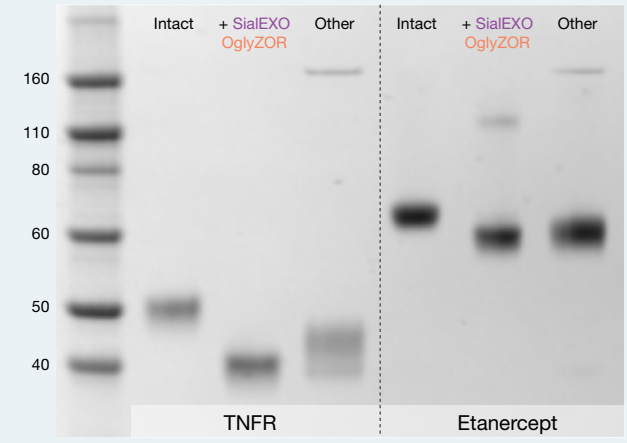
Etanercept is a highly glycosylated recombinant Fc-fusion protein and its analysis at the intact level is very challenging. Digestion with FabRICATOR (IdeS) allows for the two subunits (TNFR and Fc/2) to be analyzed separately in a middle-up LC-MS approach. While the untreated TNFR domain was too complex to be analyzed, the O-glycan composition could be resolved and compared after treatment with PNGase F. Complete glycan removal provided information of the primary structure of the TNFR part.



Deglycosylation of the TNFR subunit of etanercept from three commercially available preparations.
Deconvoluted mass spectra. Left panel: The TNFR subunit carrying both N- and O-glycans is too complex for all variants to be resolved. Middle panel: Treatment with PNGase F for removal of N-glycans enabled assessment of the O-glycan composition. C1S1 = core 1 monosialylated, C1S2 = core 1 disialylated. Right panel: Complete glycan removal (using OglyZOR, SialEXO, PNGase F) provided information of the primary structure of the TNFR part. N-terminal truncation, –L (2-256) and –LP (3-256) are visualized. A fraction of the deglycosylated subunits revealed mass shifts of +203 Da (HexNAc), indicating presence of incomplete core 1, not removed by OglyZOR.

Benchmarking of OglyZOR® Activity

The enzymatic activities of OglyZOR and SialEXO are here compared to other commercially available endoglycosidases and sialidases. When the sialic acids were removed, the OglyZOR enzyme efficiently hydrolyzed the O-glycans from TNFR and the intact etanercept molecule. OglyZOR efficiently hydrolyzes core 1 disaccharides on native glycoproteins, making the enzyme suitable for preparation of samples prior to LC-MS analysis for identification of other PTMs or confirmation of amino acid sequences.



Comparison of the enzymatic activities of OglyZOR and SialEXO to commercially available endoglycosidases and sialidases.
All incubations (4 h) were performed according to the manufacturer's instructions, and the samples were analyzed by SDS-PAGE.

SialEXO®

Hydrolysis of Sialic Acids

The SialEXO enzymes are sialidases for the removal and analysis of sialic acids. The enzymes are active on both N- and O-linked glycans present on native glycoproteins or released glycan structures. SialEXO is used to pretreat O-glycosylated proteins prior to digestion with OpeRATOR or OglyZOR. Other applications include reduction of sample complexity, charge variant analyses and exoglycosidase arrays.

- Sialic acids on N- and O-linked glycans
- α2-3, α2-6 and α2-8-linked sialic acids
- 30 min – 2 h reaction
- Requires no co-factors

Desialylation Workflow

The inherent negative charge of sialic acids might complicate analytical workflows and mask other important modifications. The removal of sialic acids therefore facilitates the study of underlying variants in the protein.

SialEXO hydrolyzes glycoproteins under native conditions and displays a high activity in a broad pH range, 6.5 to 9.

Product Formats

SialEXO®
Lyophilized enzyme mix for desialylation of glycoproteins

SialEXO® 23
Lyophilized enzyme for hydrolysis of α2-3-linked sialic acids

Immobilized SialEXO®
Immobilized enzymes for desialylation of glycoproteins in spin columns

SialEXO®

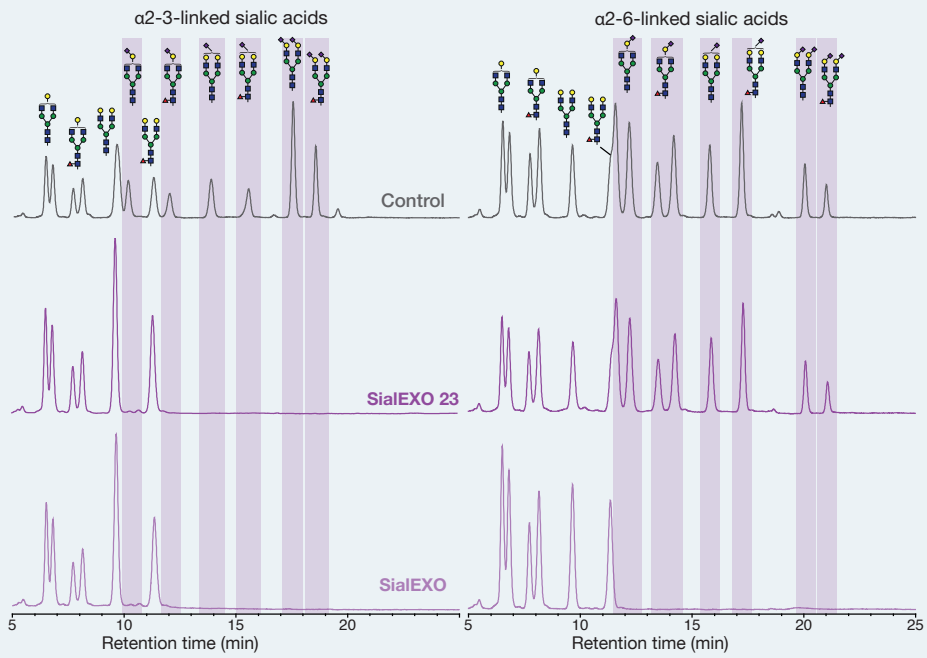
PRODUCT	DESCRIPTION	ID
SialEXO, 2000 units	Desialylates 2 mg glycoprotein	G1-SM1-020

SialEXO® 23

PRODUCT	DESCRIPTION	ID
SialEXO 23, 500 units	Desialylates 0.5 mg glycoprotein	G1-SD2-005

Analysis of Released Sialic Acids using SialEXO® and SialEXO® 23

Sialic acids present on biopharmaceuticals are primarily attached in α2-3 or α2-6-linkages. The broad acting sialidase mix SialEXO hydrolyzes all sialic acid linkages, including α2-3, α2-6, and α2-8. SialEXO 23 is an enzyme that display high specificity for α2-3-linked sialic acids. Here, hydrophilic interaction liquid chromatography (HILIC) was used to analyze two glycan libraries. One library contained released glycans with α2-3-linked sialic acids and the other contained α2-6-linked sialic acids. The shift in retention times clearly showed the release of α2-3 and α2-6-linked sialic acids from the glycans by SialEXO 23 and SialEXO respectively.

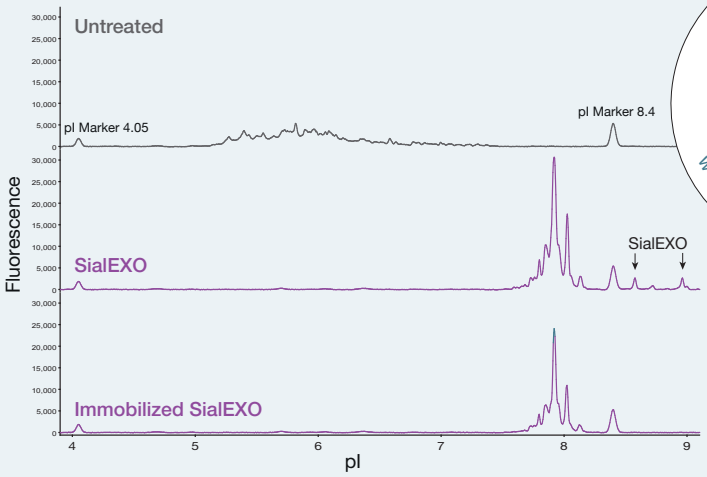


Analysis of released sialic acids. HILIC analysis of released glycans with α2-3-linked (left) or α2-6-linked (right) sialic acids after incubation with SialEXO 23 or SialEXO respectively. Sialylated glycan structures are shaded in purple.

Charge Variant Analysis using Immobilized SialEXO®

Capillary isoelectric focusing is commonly used to determine charge variants during characterization and quality control of biologics. Etanercept is a highly sialylated Fc-fusion protein which makes the analysis of its charge variants difficult.

Immobilized SialEXO microspin columns contain SialEXO covalently coupled to agarose beads for desialylation of native glycoproteins in 30 minutes without any residual enzyme in the final preparation. To study the underlying charge variants on etanercept, the protein was here desialylated using Immobilized SialEXO and analyzed by imaged capillary isoelectric focusing using Maurice from ProteinSimple. The desialylation removes the charge heterogeneity originating from the sialic acids and allows for the analysis of the underlying charge variants of the protein.



POSTER FROM AET 2019
www.genovis.com/0036

Unmasking charge variants. The analytical workflow improved the separation and enabled charge variant analysis of glycoproteins. Etanercept is difficult to analyze without first removing the sialic acids, but using Immobilized SialEXO, separated peaks were obtained. Data was obtained in collaboration with ProteinSimple (San Jose, CA, USA).

Immobilized SialEXO®

PRODUCT	DESCRIPTION	ID
Immobilized SialEXO, Microspin 2 × 0.5 mg	Desialylates 2 × 0.5 mg glycoprotein	G1-SM6-010
Immobilized SialEXO, Microspin 5 × 0.5 mg	Desialylates 5 × 0.5 mg glycoprotein	G1-SM6-025
Immobilized SialEXO, Microspin 10 × 0.5 mg	Desialylates 10 × 0.5 mg glycoprotein	G1-SM6-050

FucosEXO™

Hydrolysis of α1-2,3,4 Fucose

FucosEXO is a mix of α-fucosidases for efficient hydrolysis of α1-2, α1-3 and α1-4-linked fucose residues on native N- and O-glycosylated proteins or free oligosaccharides. It is a valuable tool for glycan structure analysis on N- and O-glycoproteins and in exoglycosidase arrays on oligosaccharides.

- Fucose on N- and O-glycosylated proteins
- Hydrolyzes α1-2,3,4-linked fucose
- 1-2 h reaction
- No need for co-factors

Defucosylation Workflow

FucosEXO hydrolyzes fucose on glycoproteins under native conditions and displays high activity in a pH ranging from 6 to 8. Since the enzymatic activity is not dependent on any co-factors or additives, the enzyme is compatible with most sample preparations, including for LC-MS analysis. Depending on the nature of the substrate, FucosEXO hydrolyzes fucose on glycoproteins within 1 to 2 hours, while longer incubation may be required for very complex samples.

Product Formats

FucosEXO™
Lyophilized enzyme for defucosylation of glycoproteins

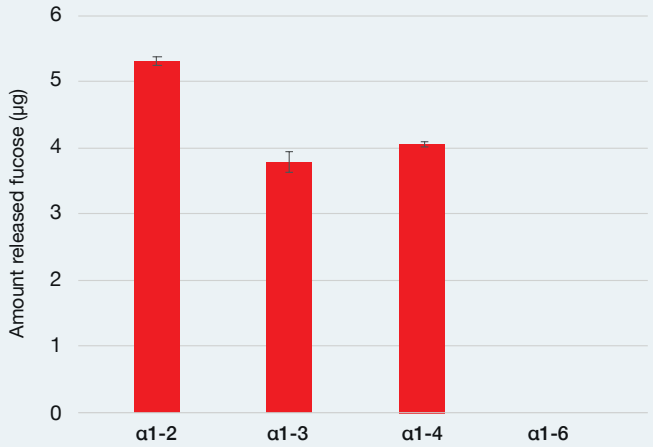
Immobilized FucosEXO™
Immobilized enzyme for defucosylation of glycoproteins in spin columns

FucosEXO™

PRODUCT	DESCRIPTION	ID
FucosEXO, 2000 units	Defucosylates 2 mg glycoprotein	G1-FM1-020

Substrate Specificity of FucosEXO™

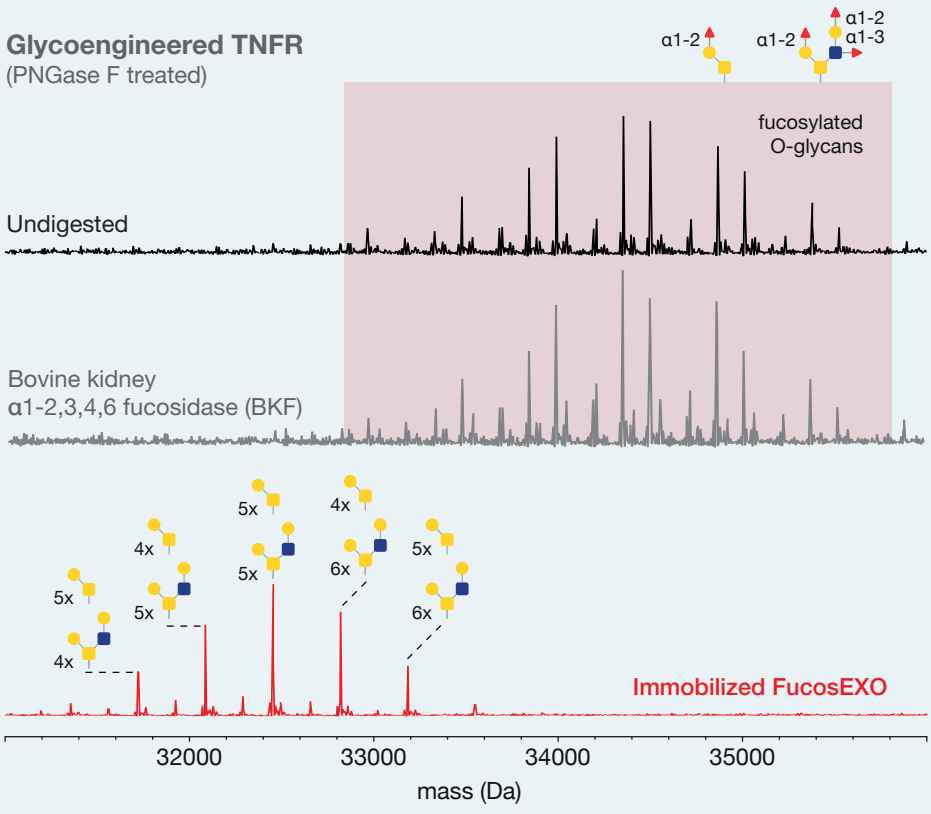
Fucose residues are attached to both N- and O-glycans with different linkages. α1-2, α1-3, and α1-4-linked fucose most commonly occurs in O-glycans and as antenna fucosylation of N-glycans, whereas the core α1-6-linked fucose is found as a modification of the N-glycan core. A panel of oligosaccharide substrates representing different linkages was here used to measure the release of fucose after FucosEXO incubation. FucosEXO was found to efficiently release α1-2, α1-3, and α1-4-linked fucose, whereas no activity was observed on α1-6-linked fucose residues.



FucosEXO substrate specificity. The substrate specificity of FucosEXO was analyzed on equal molar amounts of synthetic oligosaccharides; α1-2 (2'-fucosyllactose), α1-3 (3-fucosyllactose), α1-4 (Lewis a) and α1-6 (α1-6 fucosylated chitobiose). Substrates were incubated with FucosEXO for 30 min at 37°C and the amount of released fucose was measured spectrophotometrically using an L-Fucose Assay Kit (Megazyme).

Defucosylation of Native Glycoproteins using Immobilized FucosEXO™

Fucosylation of O-glycans is involved in the synthesis of functionally important glycan epitopes, including blood group antigens and the Lewis structures. The analysis of glycoproteins modified with such complex glycans can be challenging and requires specific and efficient enzymatic tools. Here, a glycoengineered TNFR protein carrying up to 11 O-glycans decorated with α1-2 and α1-3-linked fucose was treated with Immobilized FucosEXO, and the activity was compared to a commercially available α-fucosidase. Within 1 hour, a complete removal of fucose was achieved using FucosEXO, while treatment with the α-fucosidase only had a minor impact on the fucoses.



Defucosylation using Immobilized FucosEXO. Glycoengineered TNFR with core 1 and core 2 O-glycans decorated with both α1-2 and α1-3 fucose was incubated with bovine kidney fucosidase (1 h, 37°C) or Immobilized FucosEXO (1 h, RT). The processed protein was analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1 × 50 mm).

Immobilized FucosEXO™

PRODUCT	DESCRIPTION	ID
Immobilized FucosEXO, Microspin 5 × 0.5 mg	Defucosylates 5 × 0.5 mg glycoprotein	G1-FM6-025
Immobilized FucosEXO, Microspin 10 × 0.5 mg	Defucosylates 10 × 0.5 mg glycoprotein	G1-FM6-050

GalactEXO™

Hydrolysis of β1-3,4-linked Galactose

GalactEXO is a β-galactosidase mix that hydrolyses β1-3,4-linked terminal galactose in N- and O-glycosylated proteins. The enzyme is applicable for trimming of galactose on released glycan structures for exoglycosidase arrays or for the generation of antibodies with homogenous G0/G0F glycan profiles.

- Galactose on N- and O-glycosylated proteins
- Hydrolyzes β1-3 and β1-4-linked galactose
- 2 h reaction
- No co-factors needed

Degalactosylation Workflow

GalactEXO is a mix of β-galactosidases that facilitates efficient hydrolysis of β1-3,4-linked galactose on glycoproteins. The GalactEXO enzyme acts on both N- and O-glycosylated structures for complete hydrolysis of terminal galactose within two hours. The enzyme can be used for intact glycoproteins as well as on released glycans for applications in glycan sequencing or trimming of antibody Fc glycans.

Product Formats

GalactEXO™
Lyophilized enzyme for hydrolysis of β1-3,4-linked galactose

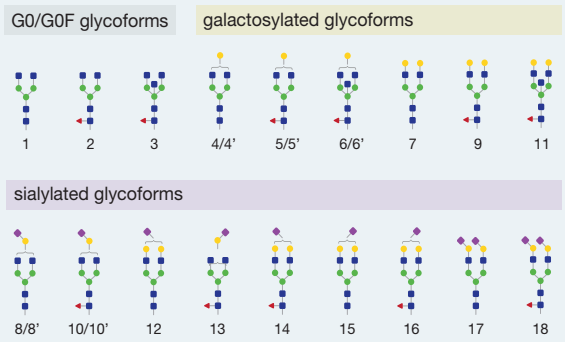
Immobilized GalactEXO™
Immobilized enzyme for hydrolysis of β1-3,4-linked galactose in spin columns

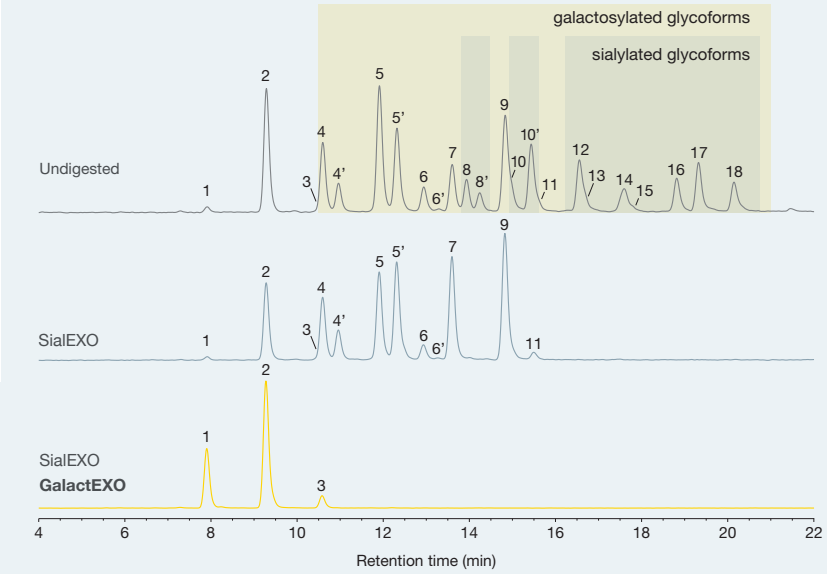
GalactEXO™

PRODUCT	DESCRIPTION	ID
GalactEXO, 2000 units	Degalactosylates 2 mg glycoprotein	G1-GM1-020

Released Glycan Analysis with GalactEXO™

Released glycan analysis using exoglycosidases requires specific and complete hydrolysis to minimize errors in data interpretation. A labeled N-glycan library was incubated with the SialEXO sialidase and GalactEXO for 1 h and analyzed with UHPLC. The HILIC-FLD separations show the complete removal of sialylated and galactosylated structures, allowing the remaining peaks to be easily identified as G0, G0F, or G0F with bisecting GlcNAc.

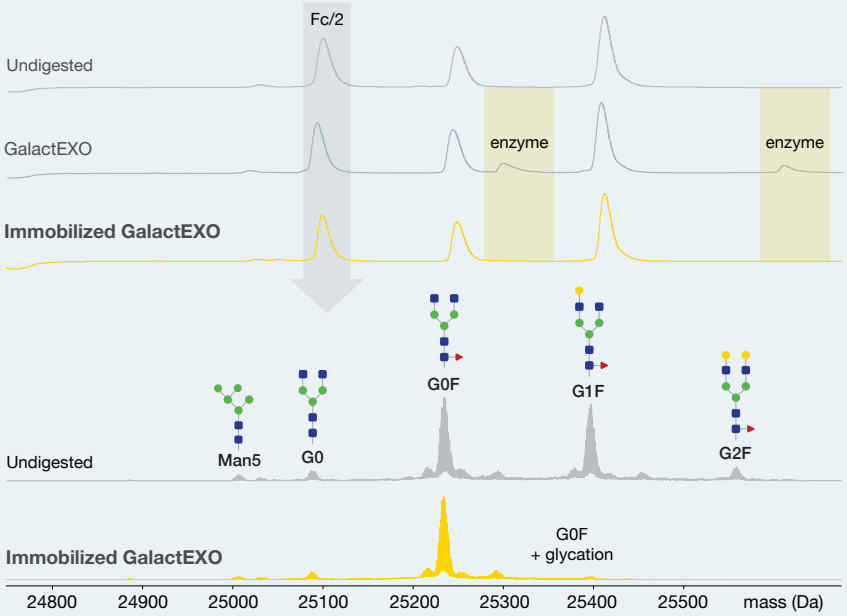




Released glycan analysis. HILIC-FLD UHPLC chromatograms of a 2-AB labeled glycan library analyzed undigested (top), after treatment with SialEXO (middle) or SialEXO and GalactEXO (bottom). Analyzed on Thermo Scientific Vanquish Duo UHPLC system equipped with Thermo Scientific Accurcore 150 Amide HILIC column (2.1 × 150 mm).

G0 Antibodies with Immobilized GalactEXO™

The presence of galactose impacts antibody effector functions, and to study this mode of action, a clean removal of galactose residues may be necessary. The β1-4 galactosidase activity of Immobilized GalactEXO was demonstrated on trastuzumab with a 30 min incubation prior to subunit digestion using FabRICATOR and analysis by LC-MS. The UV chromatograms clearly show the absence of the GalactEXO enzymes (yellow shading) in the Immobilized GalactEXO sample. The shift to G0F glycoforms can be seen in the mass spectra.



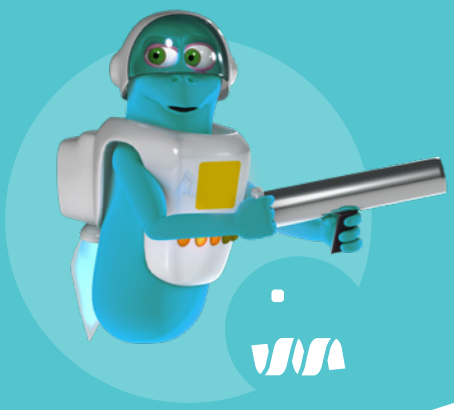
Degalactosylation of trastuzumab. Deconvoluted mass spectra of the Fc/2 fragment of trastuzumab treated with immobilized GalactEXO (bottom panel). The mAb was digested with FabRICATOR, separated by RP-HPLC (Waters™ BioResolve™ RP mAb (2.1 × 50 mm) and analyzed by ESI-Q-TOF MS (Bruker Impact II).

Immobilized GalactEXO™

PRODUCT	DESCRIPTION	ID
Immobilized GalactEXO, Microspin 5 × 0.5 mg	Degalactosylates 5 × 0.5 mg glycoprotein	G1-GM6-025
Immobilized GalactEXO, Microspin 10 × 0.5 mg	Degalactosylates 10 × 0.5 mg glycoprotein	G1-GM6-050

GalNAcEXO™

Hydrolysis of α-linked GalNAcs



The GalNAcEXO enzyme is an α-N-Acetylgalactosaminidase for efficient hydrolysis of terminal GalNAc residues on glycoproteins. GalNAcs linked to serine or threonine, referred to as Tn antigen, are quickly and efficiently hydrolyzed by GalNAcEXO, and the enzyme also displays activity on α1-3-linked terminal GalNAcs. The enzyme is a valuable tool that reduces sample heterogeneity for the analysis of complex O-glycoproteins that carry α-linked GalNAc residues as immature truncated core 1.

- α-linked GalNAc residues on O-glycoproteins
- Tn antigen and α1-3-linked terminal GalNAcs
- 4 h reaction
- No need for co-factors

Removal of GalNAc Residues

GalNAcEXO hydrolyzes GalNAcs on glycoproteins under native conditions and is active in pH ranging from 6.0 to 7.6. No co-factors or special buffers are required. Depending on the nature of the substrate, the GalNAcEXO reaction on glycoproteins is complete in two hours while more complex samples may require overnight incubation.

Product Formats

GalNAcEXO™
Lyophilized enzyme for hydrolysis of GalNAc residues

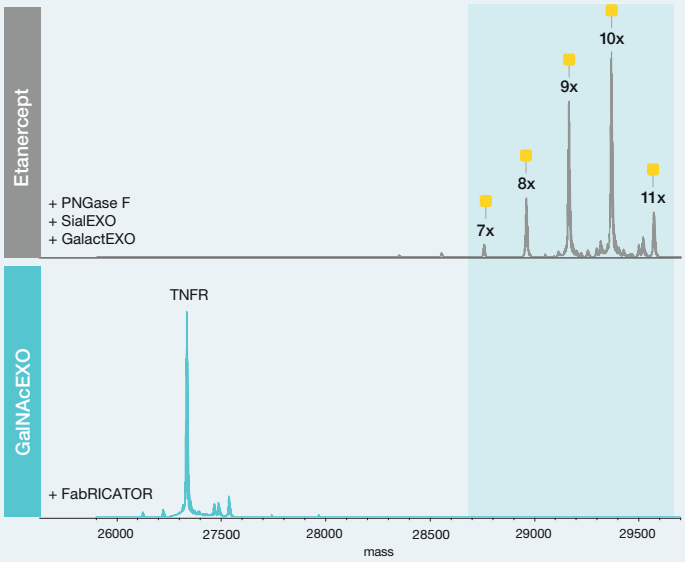
Immobilized GalNAcEXO™
Immobilized enzyme for hydrolysis of GalNAc residues in spin columns

GalNAcEXO™

PRODUCT	DESCRIPTION	ID
GalNAcEXO, 2000 units	Hydrolyzes GalNAcs from 2 mg glycoprotein	G1-NA1-020

GalNAcEXO™ Performance on Complex Substrates

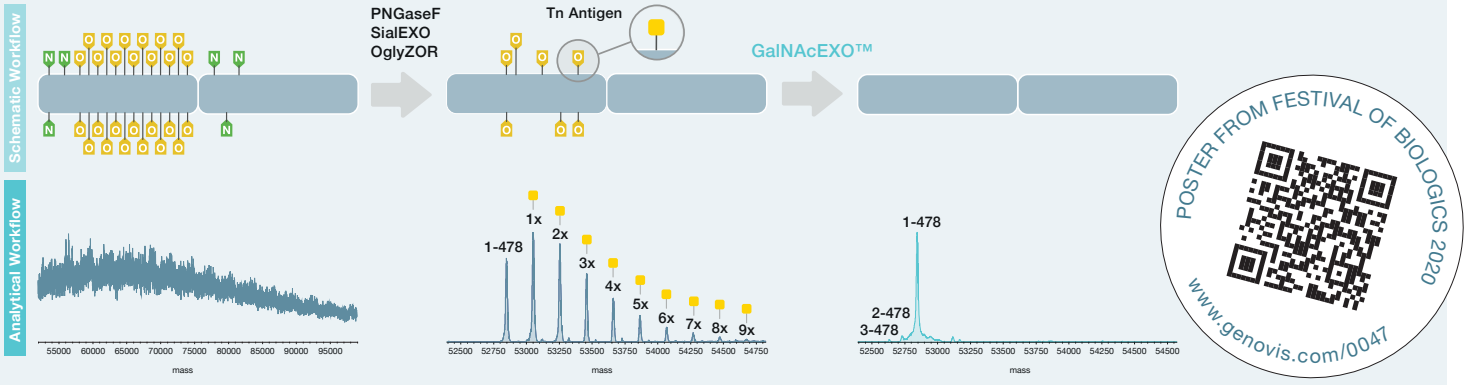
Etanercept is a complex biopharmaceutical carrying 13 O-glycan sites, of which an average of 9 to 10 are occupied. After N-glycan removal by PNGase F and trimming of O-glycans with SialEXO and GalactEXO, GalNAc-related peaks were observed in the mass spectra (top). After incubation with the GalNAcEXO enzyme, over 95% of the remaining α-GalNAcs were removed (bottom).



GalNAcEXO activity on etanercept with truncated O-glycans. Deconvoluted mass spectra of partially deglycosylated etanercept (top) and after treatment with GalNAcEXO (O/N at 37°C, bottom). Samples were FabRICATOR-digested, separated by RP-HPLC (Waters™ BioResolve™ RP mAb, 2.7 µm, 2.1 × 100 mm) and analyzed by ESI-Q-TOF MS (Bruker Impact II).

Deglycosylation of the C1 Inhibitor using GalNAcEXO™

During the production of O-glycosylated biopharmaceuticals, Tn antigens may appear as a result of incomplete processing of the core GalNAc residues. This appears as a repeating mass shift with a 203 Da HexNAc unit difference. To confirm the presence of Tn antigens on the heavily (26 sites) O-glycosylated C1 inhibitor, the following workflow was applied: first, the N-glycans were removed using PNGase F and core 1 O-glycans were removed using OglyZOR and SialEXO. A pattern of repeating peaks was observed in the mass spectra. The remaining GalNAc residues were completely removed using GalNAcEXO, leaving one single peak. This workflow allowed for quantification of the number of core GalNAcs and reduced the remaining heterogeneity, facilitating the confirmation of the intact mass of the protein as well as revealing truncated variants.



Total deglycosylation of the recombinant C1 inhibitor. The C1 inhibitor was analyzed by LC (Waters™ ACQUITY UPLC Protein BEH C4 column, 1.7 µm, 2.1 × 100 mm) and ESI-Q-TOF mass spectrometry (Bruker Impact II). In its intact form (left), the protein is highly complex, and deconvolution of the mass spectra only yielded noise. After removal of N- and core 1 O-glycans, the Tn antigens remained (middle) and could efficiently be removed by incubation with GalNAcEXO (O/N at 37°C, right).

Immobilized GalNAcEXO™

PRODUCT	DESCRIPTION	ID
Immobilized GalNAcEXO, Microspin 5 × 0.5 mg	Hydrolyzes GalNAcs from 5 × 0.5 mg glycoprotein	G1-NA6-025
Immobilized GalNAcEXO, Microspin 10 × 0.5 mg	Hydrolyzes GalNAcs from 10 × 0.5 mg glycoprotein	G1-NA6-050

OmniGLYZOR™

Hydrolysis of N- and Mucin-type O-glycans

OmniGLYZOR contains a mixture of immobilized enzymes for the removal of N- and simple mucin-type O-glycans on antibodies, fusion proteins and other glycosylated proteins. Removal of glycans is widely used to reduce heterogeneity to facilitate analysis of the protein by for example mass spectrometry. Deglycosylation can also be used to study the functional role of the glycans.

- N- and simple mucin-type O-glycans on glycoproteins
- Hydrolysis of N- and O-glycans from glycoproteins
- 1-4 hour reaction
- *Rapi*Gest™* and PNGase F included

Deglycosylation Workflow

OmniGLYZOR contains enzymes required for the removal of N-glycans and the most commonly occurring mucin-type O-glycans, namely mono- and disialyl core 1 and Tn antigen (α-GalNAc). PNGase F, O-glycosidase, sialidase and α-GalNAcase activities are included in OmniGLYZOR.

The glycoprotein sample is incubated with the OmniGLYZOR resin in a microspin column for 1-4 h under native reaction conditions. The deglycosylated glycoprotein is then easily collected by a centrifugation step. Some N-glycosylation sites are poorly or not accessible to PNGase F unless the substrate protein is denatured. Remaining N-glycans can therefore be removed by an additional deglycosylation step using lyophilized PNGase F under denaturing conditions. Lyohilized PNGase F and *Rapi*Gest SF is included in the OmniGLYZOR kit.

Product Formats

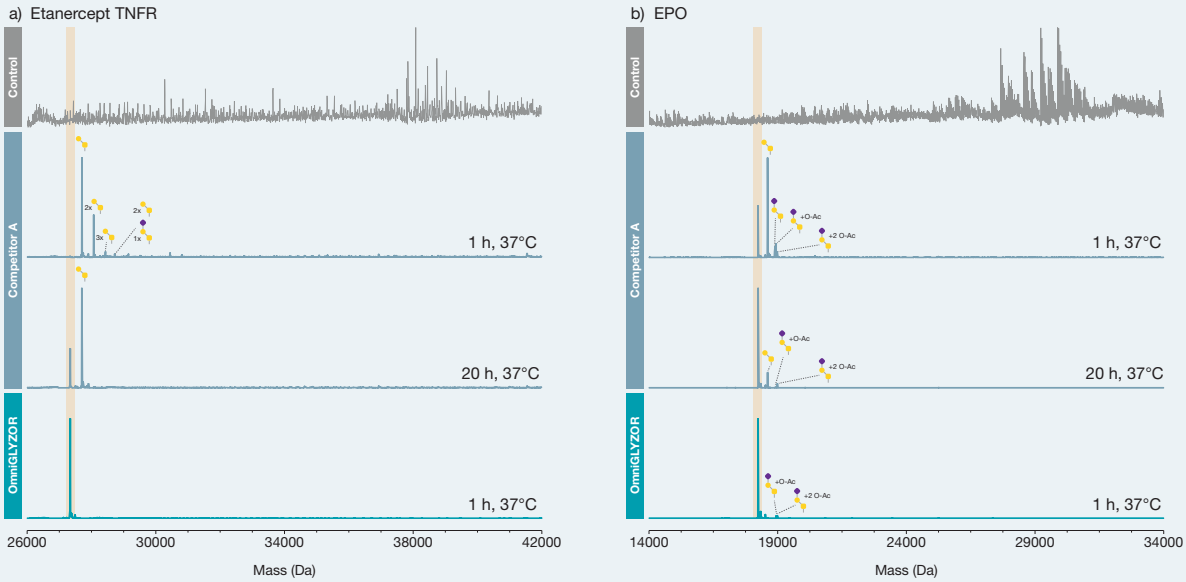
OmniGLYZOR™
A mix of immobilized enzymes in spin columns for deglycosylation of glycoproteins carrying N- and simple O-glycans

OmniGLYZOR™

PRODUCT	DESCRIPTION	ID
OmniGLYZOR, Microspin 5 x 50-100 µg	Deglycosylates 5 x 50-100 µg glycoprotein	G3-OM6-005
OmniGLYZOR, Microspin 10 x 50-100 µg	Deglycosylates 10 x 50-100 µg glycoprotein	G3-OM6-010

Efficient Removal of N- and Mucin-type O-glycans

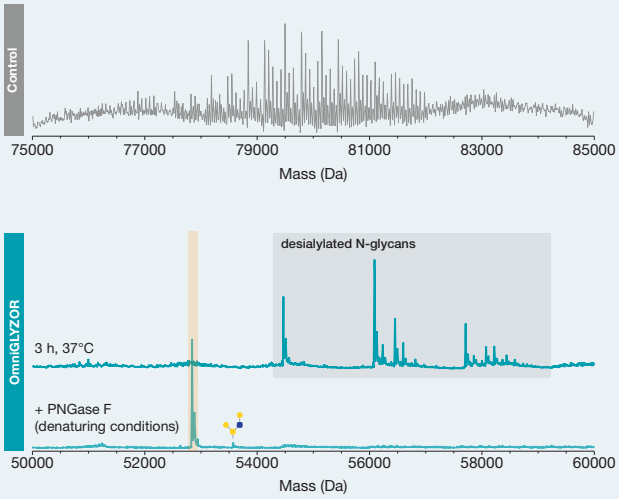
We demonstrate the performance of OmniGLYZOR using two therapeutic proteins as substrates, the heavily glycosylated Fc-fusion protein etanercept and erythropoietin (EPO). Due to the glycan heterogeneity, intact analyses resulted in complex mass spectra. By incubating the samples with OmniGLYZOR for 1 hour at 37°C, the N-and O-glycans were efficiently removed as indicated by single peaks corresponding to the unmodified protein. For EPO, a minor amount of O-glycans modified with acetylated sialic acids were left on the protein since those structures were inefficiently hydrolyzed by OmniGLYZOR. Both substrate proteins were also treated with another commercially available deglycosylation product according to the manufacturer's recommendation (O/N incubation at 37°C), and the data are shown for comparison.



Complete hydrolysis of N- and O-glycans by OmniGLYZOR. Deglycosylation of a) etanercept and b) EPO. The substrate proteins were incubated on OmniGLYZOR Microspin columns for 1 h at 37°C, or with another commercially available deglycosylation product for 1 h and 20 h at 37°C. To simplify the analysis of etanercept, the deglycosylated protein was digested with FabRICATOR to separate the O-glycosylated TNFR domain from the Fc fragment. The resulting subunits were analyzed by reversed-phase LC-MS using a Waters™ BioAccord™ LC-MS system. EPO was analyzed in the same way in its intact state. The peaks corresponding to the fully deglycosylated substrate proteins are shaded in orange.

Deglycosylation under Denaturing Reaction Conditions

The C1-inhibitor is a human plasma-derived biotherapeutic that is modified with 6 N-glycans and up to 28 O-glycans consisting of mostly sialyl core1 structures. Without pretreatment of this highly heterogeneous protein, reversed-phase LC-MS analysis yielded a complex mass spectrum impossible to interpret in detail. Using OmniGLYZOR Microspin columns under native conditions, all O-glycans were efficiently removed within 3 hours. However, between 1 and 3 N-glycans remained on the protein. These inaccessible N-glycans were removed by an additional deglycosylation step under denaturing conditions using the lyophilized PNGase F and MS-compatible *Rapi*Gest™ SF surfactant included in the OmniGLYZOR kit. A complete removal of all glycans was observed, with the exception of the minor amount of core 2 O-glycans present on the molecule.

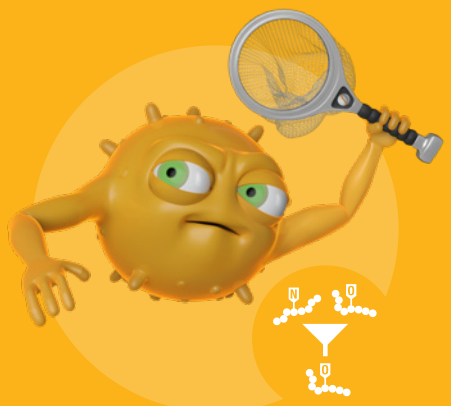


Complete deglycosylation of plasma-derived human C1 inhibitor. The protein was analyzed by reversed-phase LC-MS using a Waters™ BioAccord™ LC-MS system in its untreated state (top), after deglycosylation on an OmniGLYZOR Microspin column under native conditions (middle) and after additional deglycosylation using PNGase F under denaturing conditions (bottom). The peak corresponding to the deglycosylated substrate protein is shaded in orange.

* *Rapi*Gest™ SF Surfactant from Waters Corporation is included in OmniGLYZOR™. *Rapi*Gest™ is a trademark of Waters Corporation.

GlycOCATCH®

Enrichment of O-glycoproteins



GlycOCATCH is an affinity resin for purification of mucin-type O-glycosylated proteins and peptides. The affinity resin is based on inactive OpeRATOR enzyme that has been engineered to bind O-glycosylated proteins and peptides with high affinity. The applications of GlycOCATCH include specific enrichment or removal of O-glycoproteins and peptides, glycomics, studies of complex samples and characterization of biopharmaceuticals.

- Mucin-type O-glycosylated proteins and peptides
- Binds to glycoproteins and peptides carrying mucin-type O-glycans.
- 2 h workflow
- Desialylation using SialEXO (included) increases the binding

Affinity Purification Workflow

GlycOCATCH is provided in a spin column format to enable easy-to-use enrichment of mucin-type O-glycoproteins. The GlycOCATCH protein binds to O-glycoproteins with high affinity, and due to the strong interaction, elution is performed using 8 M urea. Alternatively, the elution can be performed with the included OpeRATOR enzyme, that digests the bound O-glycoproteins N-terminally of the O-glycan sites. In this case, peptides are recovered under native conditions. Since non-sialylated O-glycans are bound much more efficiently to the resin, the sialidase mix SialEXO is included.

Product Formats

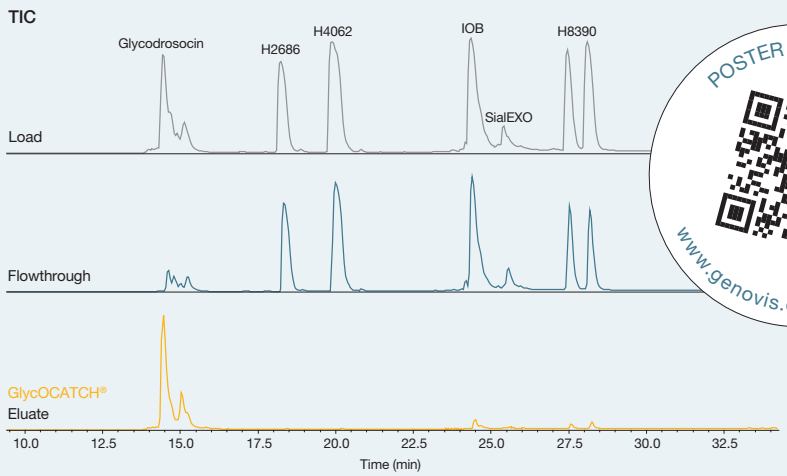
GlycOCATCH®
Enrichment resin for affinity purification of mucin-type O-glycoproteins

GlycOCATCH®

PRODUCT	DESCRIPTION	ID
GlycOCATCH	Enrichment of 0.2 mg O-glycoprotein	G3-OC6-002

Selective Purification of O-glycosylated Peptides using GlycOCATCH®

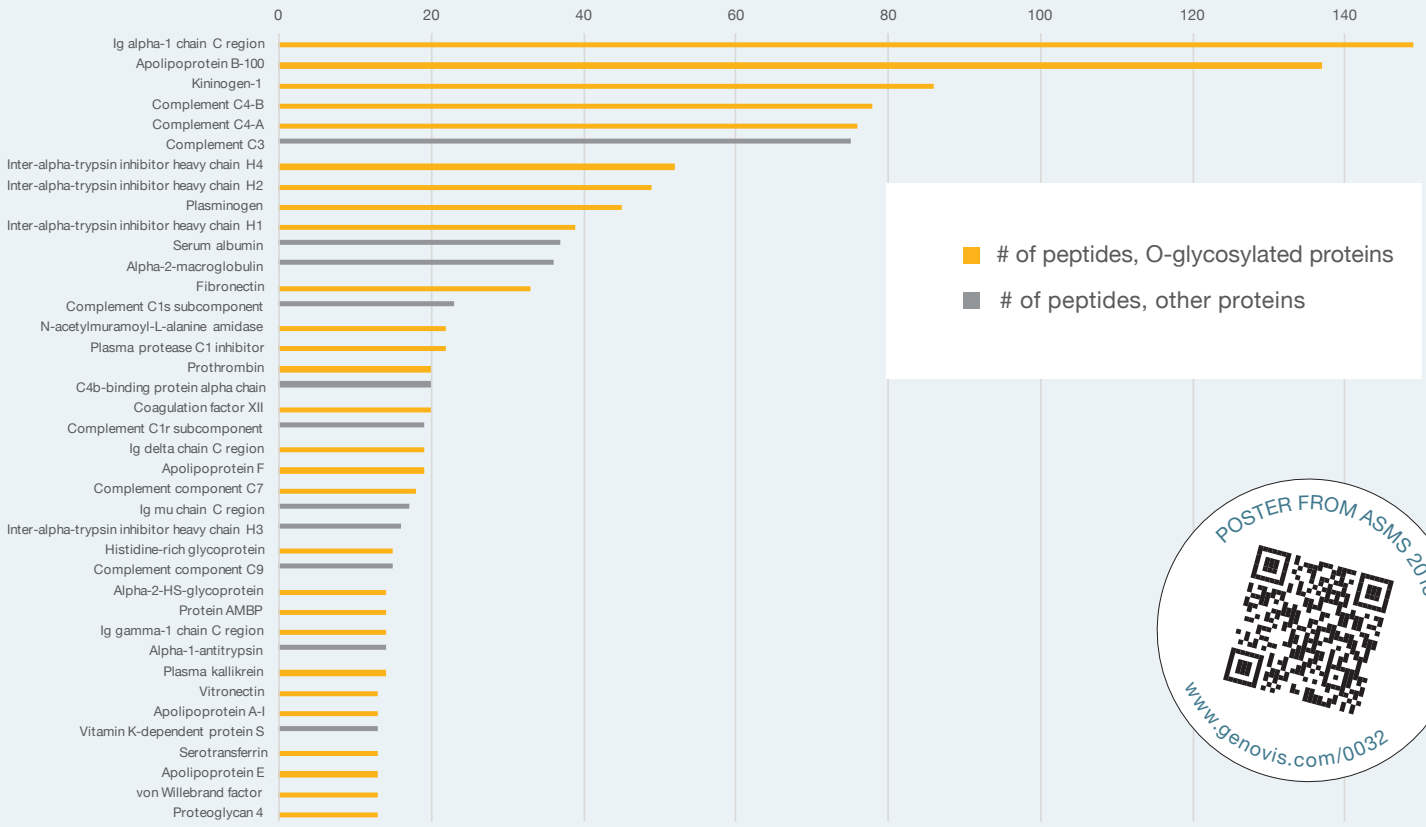
Here, the O-glycan specificity of GlycOCATCH was studied at the peptide level. An O-glycosylated peptide was spiked into a mixture of non-glycosylated peptides and loaded onto GlycOCATCH, the resin was washed, and the bound peptides were eluted with 8 M urea. After purification, the samples were separated and analyzed by RP-LC-MS. The data show selective purification of the glycodrosocin peptide, carrying a core 1 O-glycan.



Affinity purification using GlycOCATCH. Selective purification of an O-glycosylated peptide (glycodrosocin) carrying a core 1 O-glycan.

Affinity Purification of O-glycosylated proteins using GlycOCATCH®

GlycOCATCH can be used for specific enrichment of O-glycosylated peptides and proteins prior to MS analysis. The strong binding ability of GlycOCATCH was effectively used to enrich O-glycosylated proteins from a complex matrix such as human serum.



Enrichment of O-glycoproteins from human serum. Human serum was treated with SialEXO and incubated with the GlycOCATCH resin. The eluted proteins were denatured, reduced, alkylated and trypsin digested. Peptides were analyzed using RP-LC-MS/MS, and searched against the Swiss Prot database. Identified O-glycosylated proteins and other proteins with > 12 matching peptides and a MASCOT score > 200 are listed.

Anti-FabRICATOR®

Detection of the FabRICATOR® Enzyme

Anti-FabRICATOR (Anti-IdeS) is a goat polyclonal antibody detecting the FabRICATOR (IdeS) enzyme. It is available in three formats; affinity purified, biotin-conjugated affinity purified and purified on protein G. Anti-FabRICATOR can be used to detect the IdeS enzyme in Western blot and ELISA assays, as for example in preclinical gene therapy applications.

- Detects the FabRICATOR (IdeS) enzyme
- Source: goat
- Polyclonal
- Concentrations ranging from 0.5 mg/ml to 4 mg/ml

Product Formats

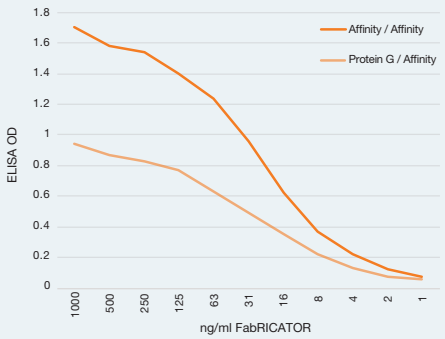
Anti-FabRICATOR®
Affinity Purified
Affinity purified goat polyclonal antibody

Anti-FabRICATOR®
Affinity Purified Biotin Conjugated
Affinity purified, biotin-conjugated goat polyclonal antibody

Anti-FabRICATOR®
Protein G Purified
Protein G-purified goat polyclonal antibody

Anti-FabRICATOR® Affinity Purified Performance

The Anti-FabRICATOR products are sensitive reagents for detecting the FabRICATOR (IdeS) enzyme. The Anti-FabRICATOR Affinity Purified displays a higher affinity to the FabRICATOR analyte as compared to the Anti-FabRICATOR Protein G Purified. This was demonstrated by a sandwich ELISA, where the plate was coated with Anti-FabRICATOR Protein G Purified or Anti-FabRICATOR Affinity Purified respectively, prior to the binding of the FabRICATOR enzyme. The biotinylated Anti-FabRICATOR Affinity Purified was used as the secondary antibody, and the readout was detected by HRP-labeled streptavidin.



Performance of Anti-FabRICATOR Affinity Purified. To display the difference in performance between the Anti-FabRICATOR Protein G Purified and the Anti-FabRICATOR Affinity Purified, wells were coated with either Anti-FabRICATOR Protein G Purified or Anti-FabRICATOR Affinity Purified at 2 µg/ml. The FabRICATOR enzyme was added in dilutions from 1 µg/ml to 1 ng/ml. Anti-FabRICATOR Affinity Purified Biotin Conjugated was then added at 1 µg/ml. HRP-Streptavidin was added in a 1:2000 dilution, followed by visualization using ABTS.

Anti-FabRICATOR®

PRODUCT	DESCRIPTION	ID
Anti-FabRICATOR Affinity Purified	Frozen solution of 0.1 ml, concentration 1 mg/ml	A3-AF2-010
Anti-FabRICATOR Affinity Purified, Biotin Conjugated	Frozen solution of 0.1 ml, concentration 0.5 mg/ml	A3-AF3-005
Anti-FabRICATOR Protein G Purified	Frozen solution of 0.1 ml, concentration 4 mg/ml	A3-AF1-010

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TransGLYCIT™
TransGLYCIT contains a technology licensed from GlycoT Therapeutics, LLC.

Immobilized PNGase F Denaturing
*Rapi*Gest™ SF Surfactant from Waters Corporation is included in Immobilized PNGase F Denaturing. *Rapi*Gest™ is a trademark of Waters Corporation.

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SmartEnzymes™

The SmartEnzymes portfolio consists of enzymes and technologies designed to improve the efficacy and throughput in analytical or preparative workflows for complex biopharmaceuticals such as antibodies, Fc-fusion proteins, complex glycoproteins and antibody conjugates.

FabRICATOR®
Below hinge digestion of IgG

FabALACTICA®
Above hinge digestion of human IgG1

FabDELLO™
Above hinge digestion of human IgG1

FabRICATOR® Z
Below hinge digestion of mouse IgG

FabULOUS™
Above hinge digestion of IgG

GingisKHAN®
Above hinge digestion of human IgG1

GlySERIAS™
Hydrolysis of Flexible Linkers

GlycINATOR®
Hydrolysis of IgG Fc glycans

IgGZERO®
Hydrolysis of complex Fc glycans

GlyCLICK®
Site-specific conjugation of native IgG

TransGLYCIT™
Transglycosylation of IgG

GingisREX®
Arginine-specific protein digestion

PNGase F
Hydrolysis of N-glycans

OpeRATOR®
O-glycan-specific protein digestion

OglyZOR®
Hydrolysis of core-1 O-glycans

SialEXO®
Hydrolysis of sialic acids

FucosEXO™
Hydrolysis of α1-2,3,4 fucose

GalactEXO™
Hydrolysis of β1-3,4-linked galactose

GalNAcEXO™
Hydrolysis of α-linked GalNAcs

OmniGLYZOR™
Hydrolysis of N- and Mucin-type O-glycans

GlycOCATCH®
Enrichment of O-glycoproteins

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