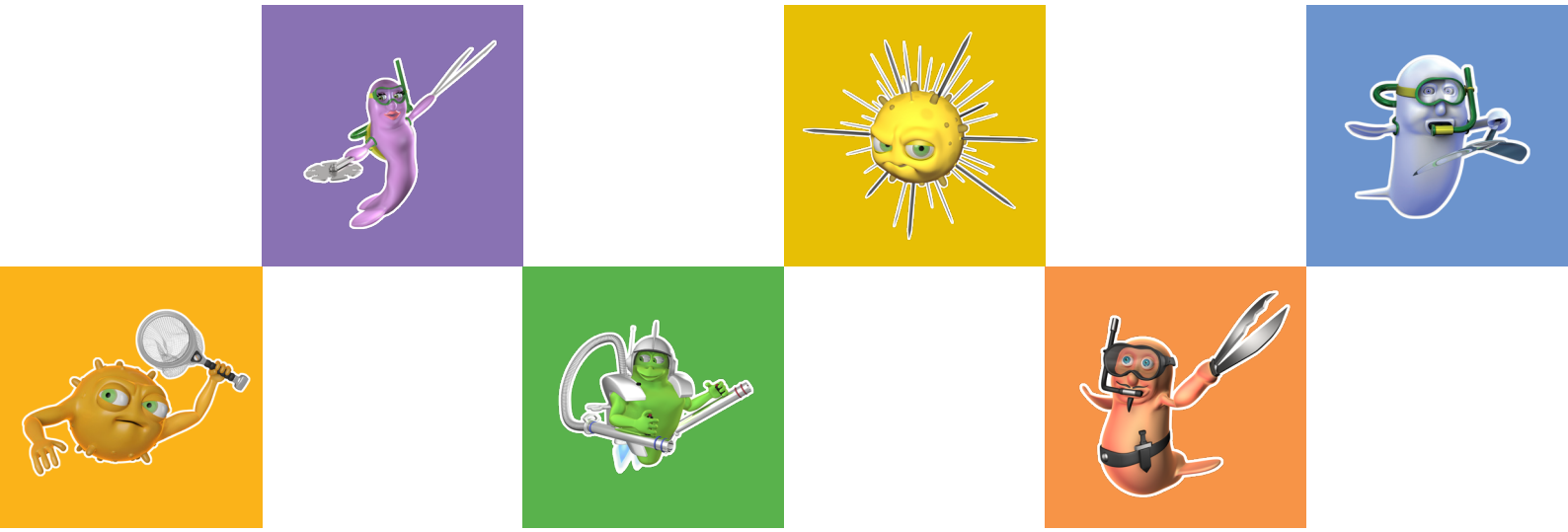
SmartEnzymes™

GENOVIS

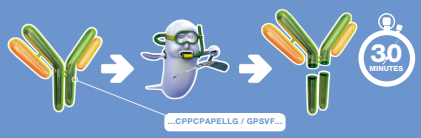


Nature offers a vast source of enzymes, perfected through evolution to perform defined reactions. At Genovis, we believe that enzymes with unique properties can be used as biological tools to support the research and development of complex biopharmaceuticals to help bring safe and effective medicines to patients in need. Our task is to identify new enzymes and give them names. We call them SmartEnzymes.

SmartEnzymes™

GENOVIS

FabRICATOR®



- Specific - one precise digestion site below the hinge of IgG
- F(ab')2 and Fc/2 fragments in 30 min
- Needs no reducing agents or co-factors
- Available in an HPLC column format for fast on-colum digestion

FabALACTICA®

- Specific - one digestion site above the hinge of human IgG1
- Generates intact Fab and Fc fragments
- Overnight digestion reaction
- Needs no reducing agents or co-factors

GingisREX®

- Arginine-specific protease
- Digests proteins and peptides C-terminally of arginine residues
- 60 min reaction
- Active in 6 M urea and 0.1% SDS

FragIT™

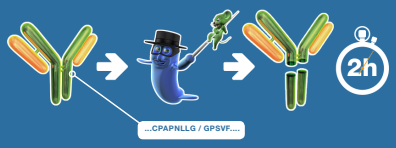
- Immobilized FabRICATOR enzyme on agarose beads
- Generates F(ab')2 and Fc/2 from IgG
- Convenient spin column format
- No enzyme in the final preparation
- Available as FragIT kit for purification of F(ab')2 and Fc/2 fragments

Immobilized FabALACTICA®

- Immobilized FabALACTICA enzyme on agarose beads
- Generates intact Fab and Fc fragments
- Convenient spin column format
- No enzyme in the final preparation
- Available as FabALACTICA Fab kit for purification of Fab and Fc fragments

CONJUGATION

FabRICATOR® Z



- Specific - one precise digestion site below the hinge of mouse IgG2a and IgG3
- Generates F(ab')2 and Fc/2 fragments
- 2 h reaction
- Needs no reducing agents or co-factors

GingisKHAN®

- One digestion site above the hinge of human IgG1
- Generates intact Fab and Fc fragments
- 60 min reaction
- Requires mild reducing agents (included)

GlyCLICK®

Alexa Fluor® 488 · Alexa Fluor® 555 · Alexa Fluor® 647

DFO · Biotin · Azide Activation

- Site-specific conjugation of IgG
- Active on human IgG1-4 and several other species
- The antibody retains antigen binding
- Quantitative labeling of 2 labels per antibody

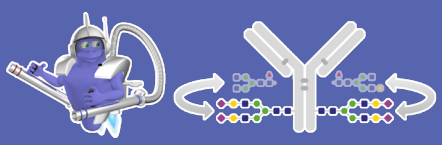
FragIT™ Z

- Immobilized FabRICATOR Z enzyme on agarose beads
- Generates F(ab')2 and Fc/2 from mouse IgG2a and IgG3
- Convenient spin column format
- No enzyme in the final preparation
- Available as FragIT Z kit for purification of F(ab')2 and Fc/2 fragments

FabULOUS™

- Digests IgG in the hinge region of several species and subclasses
- Generates Fab and Fc fragments
- 60 min reaction
- Requires reducing conditions

TransGLYCIT™



- Transglycosylation of IgG in three hours
- Generation of defined G2S2 glycoforms
- Active on human IgG1, 2 and 4
- Optional defucolsyation with FucosEXO 16

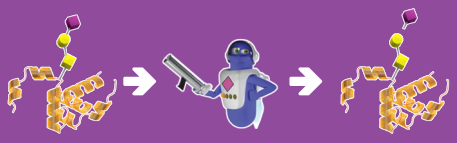
GlycINATOR®

- Hydrolyzes the N-glycan structure of the IgG Fc domain
- 30 min reaction
- Requires native IgG fold
- Hydrolyzes all Fc glycoforms of IgG

OpeRATOR®

- Digests mucin-type O-glycosylated proteins N-terminally of the O-glycosylation site
- 2 h to overnight (16-18 h) reaction
- Maps O-glycosylation site occupancy

SialEXO®



- SialEXO hydrolyzes all sialic acids and SialEXO 23 specifically hydrolyzes α2-3-linked sialic acids
- Available immobilized on agarose beads
- Active on both N- and O-linked glycans
- 1 - 2 h reaction
- Active on native glycoproteins

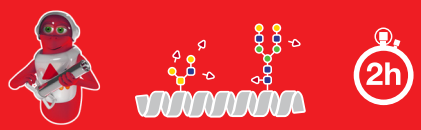
Immobilized GlycINATOR®

- Immobilized GlycINATOR enzyme on agarose beads
- Hydrolyzes all Fc glycoforms of IgG
- Convenient spin column format
- No enzyme in the final preparation

GlycOCATCH®

- Enriches mucin-type O-glycosylated proteins and peptides
- 30 min - 2 h binding
- Requires desialylation
- Applications in glycomics

FucosEXO™



- Hydrolyzes α1-2,3,4 linked Fucose on native glycoproteins
- α1-2,3,4 linked Fucose
- 1-2 h incubation
- No co-factors required

IgGZERO®

- Hydrolyzes the N-glycan structure of the IgG Fc domain
- 30 min reaction
- Requires native IgG fold
- Limited activity on high-mannose and hybrid-type Fc glycans

OglyZOR®

- O-glycosidase acting on O-glycans
- Hydrolyzes core 1 and to some extent core 3 type O-glycans on native glycoproteins
- 2-4 h reaction
- Requires pre-removal of sialic acids

GalactEXO™

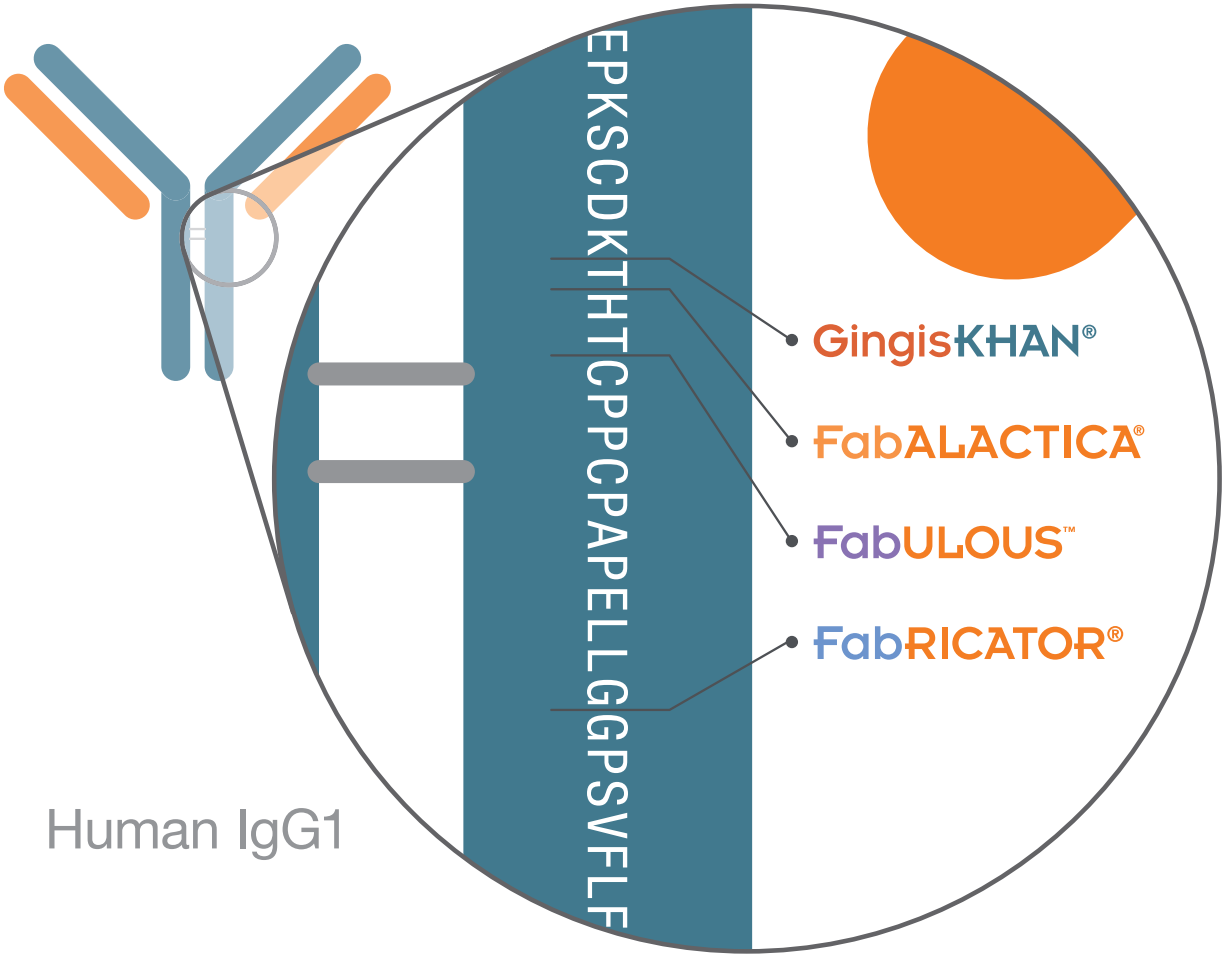
- Hydrolyzes galactcose residues on N- and O-glycosylated proteins
- Efficient removal of β1-3,4 linked galactose
- Active on both N- and O-linked glycans
- 2 h reaction
- For native glycoproteins and free glycans

deGlycIT™

- Immobilized IgGZERO enzyme on agarose beads
- Hydrolyzes IgG Fc N-glycans
- Convenient spin column format
- No enzyme in the final preparation

GalNAcEXO™

- Exoglycosidase for native O-glycoproteins
- Hydrolyzes α-linked GalNAcs
- 4h reaction
- Activity without need for any co-factors


EPKSCDKTHTCPPCPAPELLGGPSVFLF
GingisKHAN®
FabALACTICA®
FabULOUS™
FabRICATOR®
Human IgG1

Genovis IgG Proteases

Genovis provides unique enzymes and technologies used in characterization and conjugation of biopharmaceuticals such as monoclonal antibodies (mAbs), Fc-fusion proteins, biosimilars and antibody-drug conjugates (ADCs). The rise of monoclonal antibodies and other biomolecules into biotherapeutics has increased the analytical challenges significantly. To ensure safe and potent drugs, many different quality attributes of the large heterogeneous molecules need to be characterized. For this reason, the analysis of antibody subunits such as Fab, F(ab')2 and Fc/2 using liquid chromatography and high resolution mass spectrometry (LC-MS) has emerged as a new platform method for characterization. Traditional techniques are often time consuming and may induce artefacts in the sample, whereas the middle-level approach is faster and generates data that is easier to interpret.

The IgG proteases from Genovis are a group of poteolytic enzymes that digest antibodies from several species and subclasses into subunits. The enzymes FabRICATOR® (IdeS) and FabALACTICA® (IgdE) are specific proteases, digesting IgG at a single site below or above the hinge, respectively. Other proteases for digestion of IgG include FabRICATOR®Z (IdeZ), FabULOUS™ (SpeB) and GingisKHAN® (Kgp). An overview of the digestion sites of the enzymes in human IgG1 is presented in the figure on page 6, and a comparison of the IgG proteases is given in the table below.

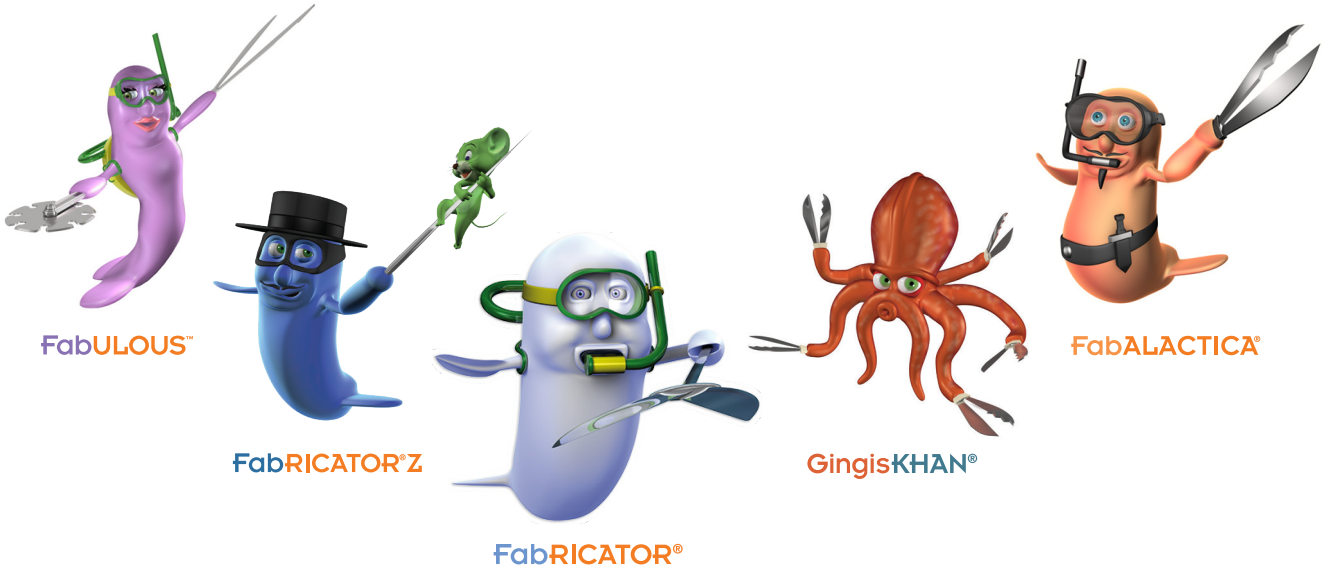
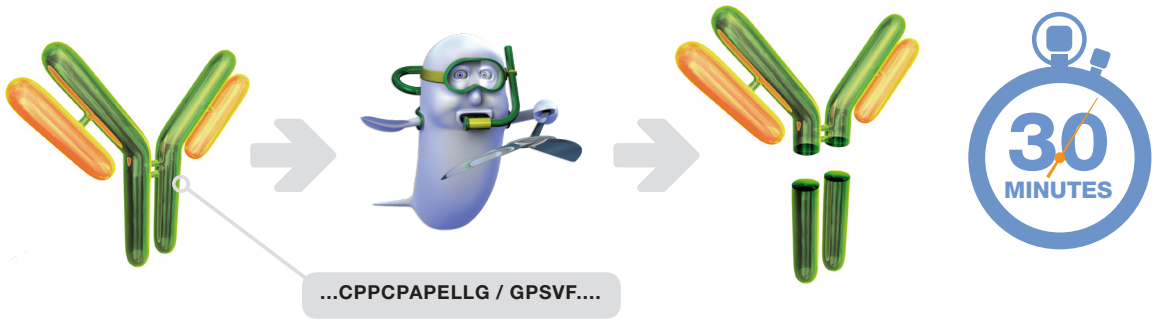


Table 1. Comparison of the Genovis IgG proteases.

Enzyme	FabRICATOR®	FabALACTICA®	GingisKHAN®	FabULOUS™	FabRICATOR®Z
IgG species and subclasses	Human IgG1-4, mouse IgG2a and IgG3, some classes of rat, monkey, rabbit and sheep	Human IgG1	Human IgG1	Human IgG, mouse, rat, goat, sheep and rabbit	Human IgG1-4, Mouse IgG2a and IgG3, some classes of monkey, rabbit and sheep
Digestion site (human IgG1)	LLG / GPS	DKT / HTC	CDK / THT	THT / CPP	LLG / GPS
Above / below hinge (human IgG1)	Below	Above	Above	Above	Below
Reaction requirements	Physiological buffers	Physiological buffers	2 mM cysteine	Reducing conditions	Physiological buffers
Reaction time	30 min	O/N	1 h	1 h	2 h
pH	5.5 - 8	6 - 8	8	6.5 - 8	5.5 - 8

FabRICATOR®



FabRICATOR® (IdeS) is a unique cysteine protease that digests IgG at a specific site below the hinge, enabling antibody subunit analysis.

FabRICATOR is an IgG-specific cysteine protease that digests antibodies at a single amino acid site below the hinge region, generating a homogenous pool of F(ab')2 and Fc/2 fragments within 30 minutes. Neutral pH and no requirements for co-factors make the enzyme easy to use and enable platform analytical workflows based on FabRICATOR without the need for optimization. FabRICATOR 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. A selection of publications using FabRICATOR is available on p. 50.

- Human IgG1-4, Fc-fusion proteins, ADCs, mouse IgG2a and IgG3*, IgG of some classes from monkey, rat, rabbit and sheep
- 30 min reaction
- No need for reducing agents or co-factors
- CPAPELLG / GPSVF (below the hinge)

Antibody Subunit Workflow

The FabRICATOR sample preparation of antibodies is a common workflow for subunit LC-MS analysis (*Fig. 1*). IgG is digested using FabRICATOR at 37°C for 30 minutes to generate F(ab')2 and Fc/2 fragments followed by reduction and denaturation. The generated ~25 kDa subunits allow increased mass resolution using LC-MS instrumentation and enable fast and accurate analysis of IgG glycans and other quality attributes such as oxidation, deamidation and pyroglutamination.

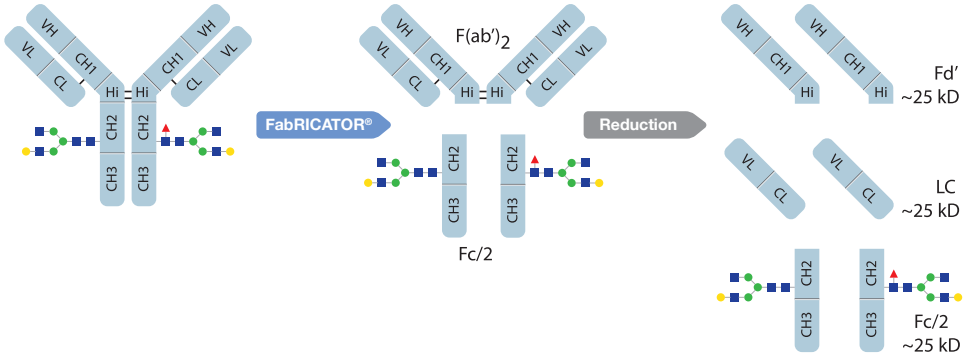
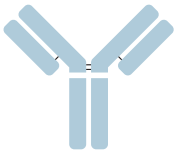
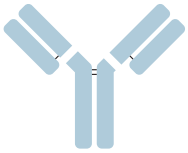
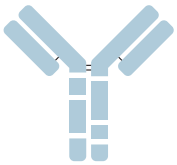


Figure 1. FabRICATOR digestion of IgG results in F(ab')2 and Fc/2 fragments that can be further reduced to antibody subunits.

* FabRICATOR has limited activity on mouse IgG2a and IgG3. For digestion of these antibodies, FabRICATOR® Z is recommended.

Comparison to Other Common Enzymes

Enzyme	FabRICATOR	Papain/Ficin	Pepsin
Digestion site			
Specificity	IgG specific/ One digestion site	Unspecific/ Several digestion sites	Unspecific/ Several digestion sites
Selectivity	IgG (only known substrate)	Several proteins	Several proteins
Reaction conditions	No optimization	Requires optimization	Requires optimization
Reaction time	30 min	1-24 h	1-24 h

High Resolution LC-MS for Amino Acid Sequence Verification

Mass spectrometers with high resolving power allow for amino acid verification of mAbs.
FabRICATOR generates the precise antibody subunit fragments Fc/2, LC and Fd that can be mono-isotopically resolved and analyzed using LC-MS (*Fig. 2*).

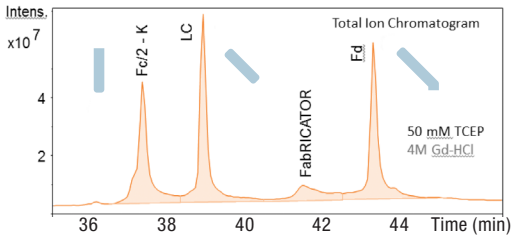


Figure 2. LC-MS on adalimumab

Determining the Degree of Oxidation

Oxidation of antibodies is a key quality attribute that may affect therapeutic antibody functionality.
FabRICATOR is a convenient tool to determine the degree of oxidation at the subunit level (*Fig. 3*). The shift in retention time in the chromatogram shows that the amount of oxidized antibody increases as the oxidation time is prolonged.

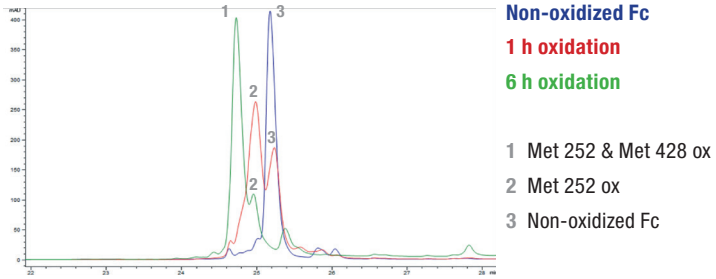


Figure 3. Reversed-phase chromatogram of bevacizumab exposed to oxidizing reaction conditions (0, 1 or 6 hours) before FabRICATOR digestion.

Glycan Profiling of Cetuximab

By analyzing antibody subunits generated by FabRICATOR, the mass resolution is significantly increased. This allows for fast and accurate glycan profiling of antibodies using LC-MS at the subunit level. Ayoub and colleagues (Ayoub, 2013, p. 42) used the subunit workflow to determine the glycan profile of the Fab and Fc domains of cetuximab (*Fig. 4*).

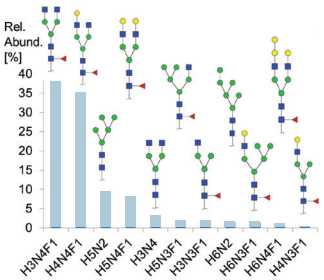


Figure 4. Relative abundance of glycans on the Fc domain of cetuximab. 11 glycans were quantified and no additional digestion or labeling were needed for similarity assessment.

FabRICATOR®

Lyophilized FabRICATOR for rapid antibody subunit generation is available in different sizes. FabRICATOR LE is a low endotoxin preparation and is suitable for cell/tissue-based assays, and the plates 8x100 and 96x100 units allow for a rapid antibody subunit generation in a high-throughput format.

Product ID	Description	Digestion	EUR	USD
A0-FR1-020	FabRICATOR, 2000 units	2 mg IgG	470	660
A0-FR1-050	FabRICATOR, 5000 units	5 mg IgG	920	940
A0-FR1-250	FabRICATOR, 5 x 5000 units	25 mg IgG	3,395	3,740
A0-FR1-096	FabRICATOR, 96x100 units	96 x 100 µg IgG	1,735	2,260
A0-FR1-008	FabRICATOR, 8x100 units	8 x 100 µg IgG	320	410
A0-FR8-020	FabRICATOR LE (low endotoxin), 2000 units	2 mg IgG	530	710
A0-FR8-050	FabRICATOR LE (low endotoxin), 5000 units	5 mg IgG	1,010	1,070

Validation Kit

Three different batches of lyophilized FabRICATOR are included in the FabRICATOR Validation kit for validation of FabRICATOR-based analytical methods.

Product ID	Description	Digestion	EUR	USD
A0-FR4-060	FabRICATOR, 3 x 2000 units	3 x 2 mg IgG	1,420	1,970

Anti-FabRICATOR®

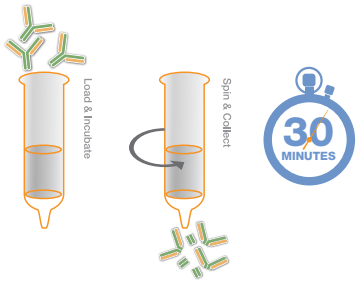
Anti-FabRICATOR is a goat polyclonal antibody that is used for detection of the FabRICATOR enzyme with western blot or ELISA.

Product ID	Description	Concentration	EUR	USD
A3-AF1-010	Anti-FabRICATOR, Protein G Purified, 0.1 ml	4 mg/ml	280	395
A3-AF2-010	Anti-FabRICATOR, Affinity Purified, 0.1 ml	1 mg/ml	630	750
A3-AF3-005	Anti-FabRICATOR, Affinity Purified Biotin Conjugated, 0.1 ml	0.5 mg/ml	750	895

FragIT™

FragIT™ (immobilized FabRICATOR®) digests IgG from several species and subclasses and generates F(ab')2 and Fc/2 fragments.

The FabRICATOR enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for digestion of 0.5 mg up to 100 mg of antibody or Fc-fusion protein. FragIT generates antibody subunits with no enzyme in the final preparation.

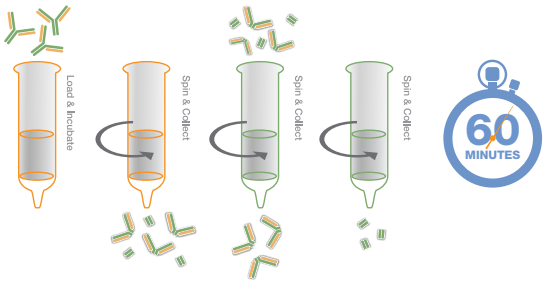


Product ID	Description	Digestion	EUR	USD
A0-FR6-010	FragIT Microspin	2 x 0.5 mg IgG	350	490
A0-FR6-025	FragIT Microspin	5 x 0.5 mg IgG	800	1,110
A0-FR6-050	FragIT Microspin	10 x 0.5 mg IgG	1,330	1,850
A0-FR6-100	FragIT Midispin	1-10 mg IgG	1,070	1,490
A0-FR6-1000	FragIT Maxispin	10-100 mg IgG	3,200	4,470

FragIT™ kit

FragIT™ kit easily generates and purifies F(ab')2 and Fc/2 fragments from IgG.

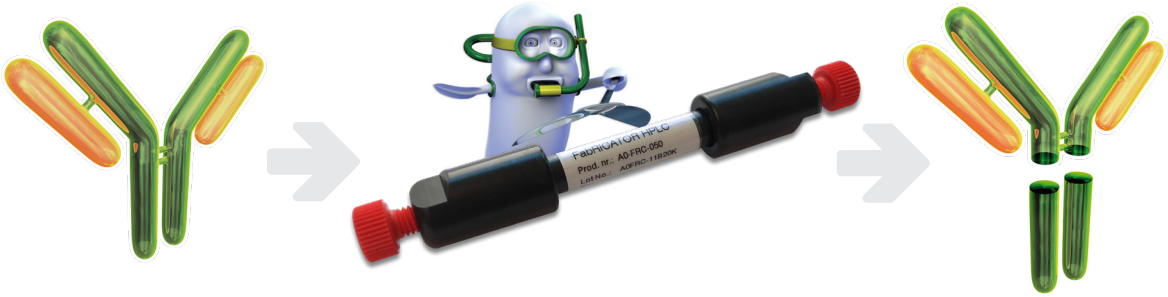
FragIT kit consists of spin columns of FragIT for antibody digestion and spin columns of CaptureSelect™* Fc resin for affinity binding of the Fc fragments. After digestion, the Fc fragments are captured in the affinity spin column and pure F(ab')2 fragments are obtained in the flowthrough.



Product ID	Description	Digestion	EUR	USD
A2-FR2-005	FragIT kit, Microspin	0.5 mg IgG	400	550
A2-FR2-025	FragIT kit, Microspin	5 x 0.5 mg IgG	1,070	1,490
A2-FR2-050	FragIT kit, Microspin	10 x 0.5 mg IgG	2,040	2,495
A2-FR2-100	FragIT kit, Midispin	10 mg IgG	1,340	1,860
A2-FR2-1000	FragIT kit, Maxispin	100 mg IgG	4,010	5,610

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

FabRICATOR®-HPLC



FabRICATOR-HPLC offers on-column digestion of monoclonal antibodies for rapid subunit generation in automated middle-level workflows.

FabRICATOR-HPLC contains the FabRICATOR (IdeS) enzyme immobilized on an HPLC compatible resin, generating F(ab')2 and Fc fragments without risk of over-digestion. In-house testing (*Fig. 2* and *3*) highlights the stability and reproducibility with the column delivering consistent digestion for 14 days at 37°C. More than 450 injections of mAb were made during testing and no carry-over was observed.

FabRICATOR-HPLC can be used in a standard LC-MS setup for routine analysis (*Fig. 1*). But more advanced configurations are possible, for example with 2D-LC. 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.

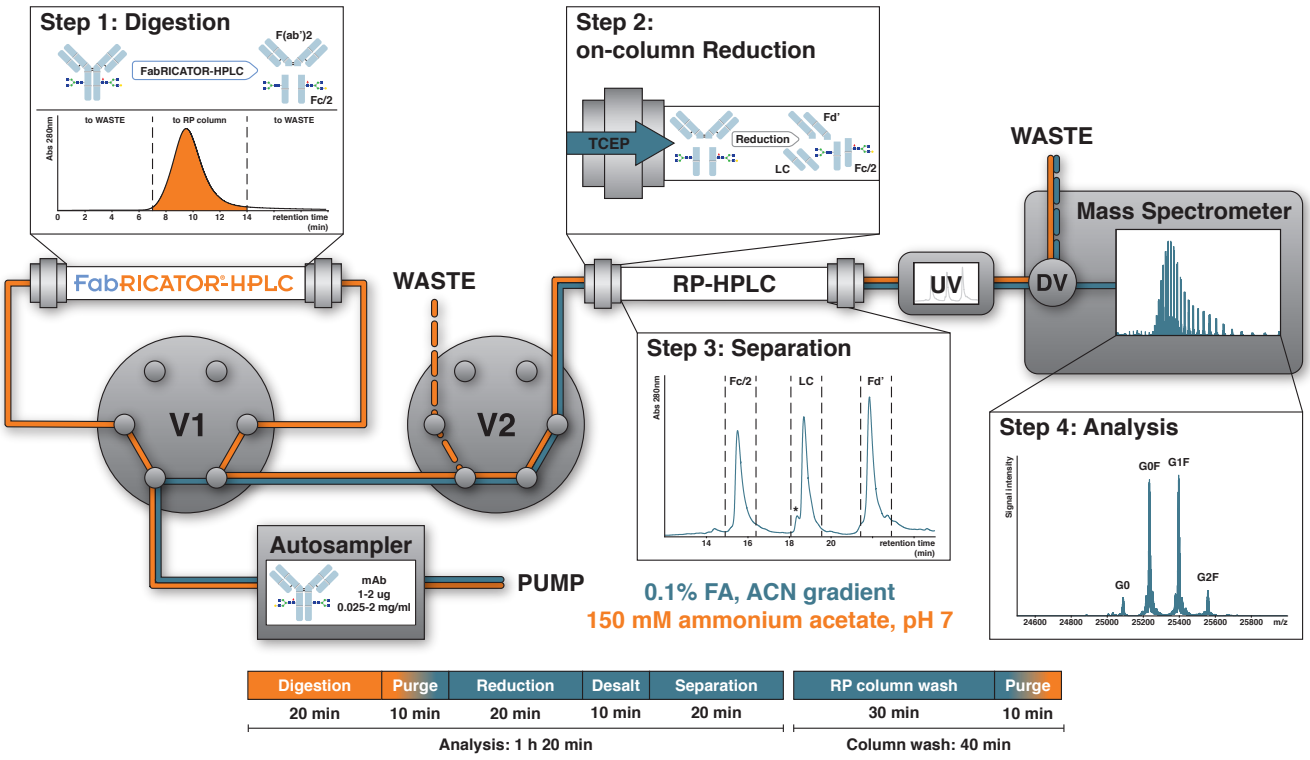


Figure 1. Potential set-up for an automated middle-level workflow using FabRICATOR-HPLC. FabRICATOR digests IgGs to F(ab')2 and Fc, which is well suited for high resolution MS analysis.

Robust On-column Performance

Digestion efficiency is maintained by FabRICATOR-HPLC during continuous operation at 37°C for up to 14 days. In our tests, 2 µg trastuzumab samples were injected every 4h under native conditions in 150 mM ammonium acetate, pH 7 at a flow rate of 25 µl/min. The resulting antibody fragments were reduced on-column and analyzed using the automated subunit analysis workflow described in *Fig. 1* (*Fig. 2a*). More than 95% of the antibody was digested during the entire 14-day period. (*Fig. 2b*).

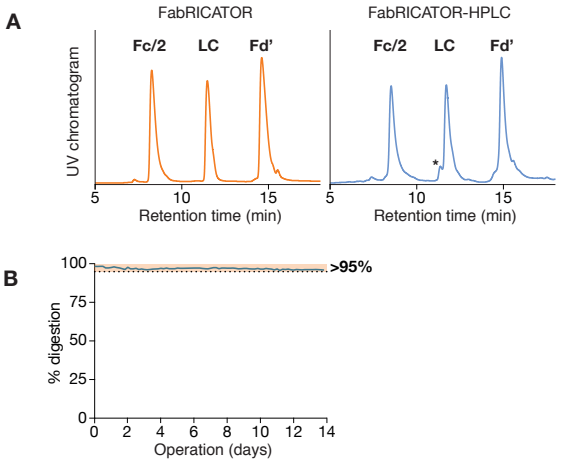


Figure 2. a) Comparison between digestion of trastuzumab using standard in-solution FabRICATOR protocol (left) and digestion using the automated FabRICATOR-HPLC workflow (right). The asterisk marks LC fragments that are not completely reduced with one intramolecular disulfide bridge intact. **b)** Quantification of digestion performance over a period of 14 days.

Reproducible Glycan Analysis

The performance and operational stability of the FabRICATOR-HPLC column are demonstrated here using analysis of trastuzumab Fc glycosylation as an example. Automated middle-level analysis using FabRICATOR-HPLC yielded mass spectra virtually indistinguishable from the one obtained from a standard in-solution FabRICATOR digestion workflow *(Fig. 3a)*. The resulting Fc glycosylation profiles were stable and reproducible during 14 days of continuous operation with standard deviations of less than 0.5% for all glycoforms (*Fig. 3b*).

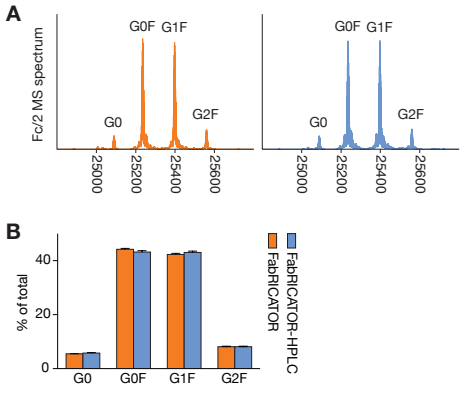


Figure 3. a) Deconvoluted mass spectra of the trastuzumab Fc/2 fragment from in-solution FabRICATOR digestion (orange), or FabRICATOR-HPLC (blue). **b)** Glycosylation profiles of trastuzumab generated by in-solution FabRICATOR digestion (orange, n=10) or FabRICATOR-HPLC (blue, n=28, 2 samples per day).

Product Overview

Column hardware: PEEK/biocompatible
Column dimensions: 2.1 mmD x 50 mmL
Support resin: POROS® (see Legal and Disclaimers, p. 39)
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

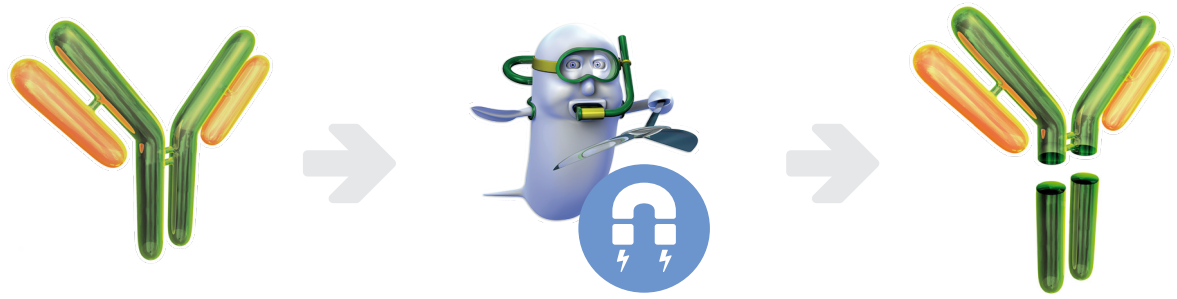
*Depending on specific application

FabRICATOR®-HPLC

FabRICATOR-HPLC contains the FabRICATOR enzyme immobilized in an HPLC column for fast on-column digestion of monoclonal antibodies.

Product ID	Description	EUR	USD
A0-FRC-050	FabRICATOR-HPLC	1,750	1,980

FabRICATOR® MagIC



FabRICATOR® MagIC enables parallel subunit generation of antibodies for automated middle-level workflows

FabRICATOR MagIC contains FabRICATOR (IdeS) enzyme immobilized on magnetic agarose beads for digestion of antibodies into of F(ab')2 and Fc/2 subunits in 20 min. The magnetic format enables parallel preparation of samples for the analysis of multiple antibodies in middle-level workflows with minimal hands-on time. FabRICATOR MagIC can be used in manual or automated setups for rutine analysis of IgG-based biopharmaceuticals (Fig. 1).

- Human IgG1-4, IgG from monkey, rabbit, sheep and rat IgG2b
- CPAPELLG / GPSVF (below the hinge)
- Over 95% digestion within 20 minutes
- No need for reducing agents or co-factors

Key Characteristics

- Antibody subunit generation in 20 min
- Parallel processing of up to 96 samples
- Optimized for automated workflows
- Digest and reduce in a one-pot reaction
- Only requires a single pipetting step

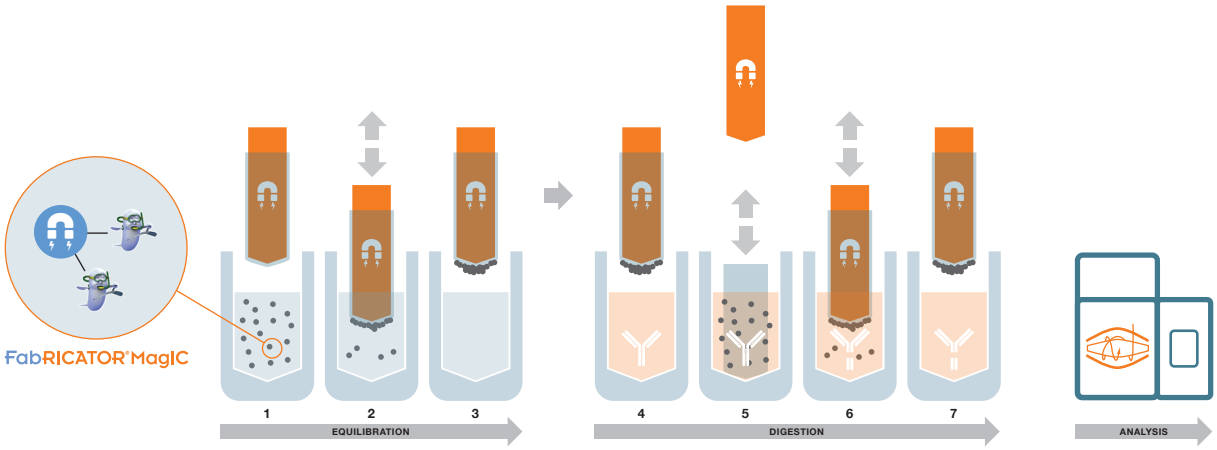


Figure 1. FabRICATOR MagIC workflow on Thermo Scientific KingFisher™ Purification System. IgG, FabRICATOR MagIC and PBS are dispensed into a 96-deepwell plate according to instructions for the KingFisher workflow. FabRICATOR MagIC beads are transferred (1) to wells containing PBS for equilibration (2). The beads are collected (3), added to the antibody samples (4) and incubated with mixing for 10-20 min at 37°C (5). The beads are then collected (6) and transferred to waste wells 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/2 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 of different anitbodies using middle-level workflows resulting in a better resolution compared to intact level approaches. Reverse phase LC-MS analysis of the resulting subunits or fragments from an antibody processed with FabRICATOR MagIC demonstrates the complete digestion and subsequent reduction of the generated subunits (Fig. 2).

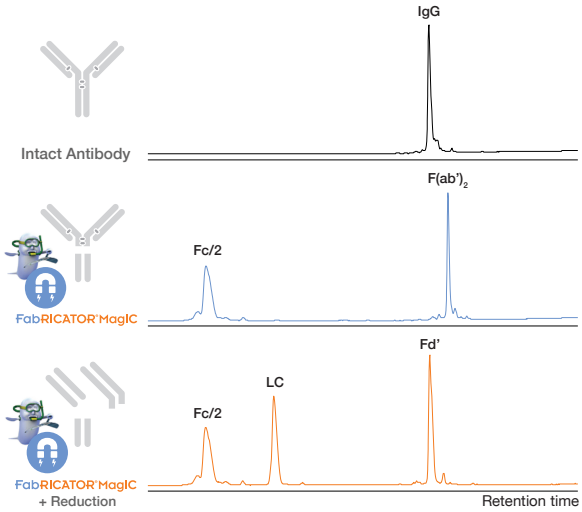


Figure 2. Analysis of a monoclonal antibody by RPLC-MS at the intact level (top), after FabRICATOR MagIC digestion (middle) or after treatment with FabRICATOR MagIC and 5 mM TCEP (bottom).

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 degradtion was analyzed by LC-MS at the intact or the subunit level using FabRICATOR MagIC. The results show the ability to quickly detect and monitor such modifications in a complex mixture of mAbs using FabRICATOR MagIC (Fig. 3).

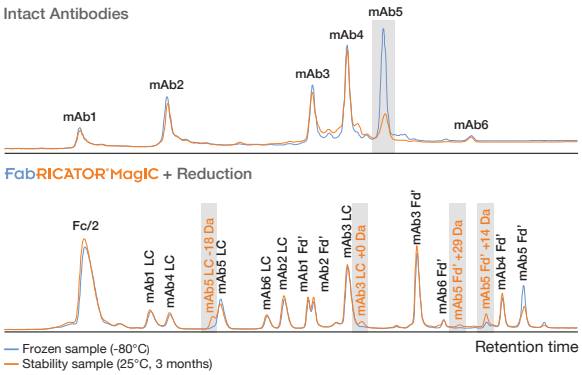


Figure 3. Analysis of tryptophan oxidation during forced degradation studies. Six mAbs were analyzed by RPLC-MS at the intact level (top panel) and after FabRICATOR MagIC with 5 mM TCEP (bottom panel). Highlighted differences between control (blue) and sample (orange).

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 (Fig. 4).

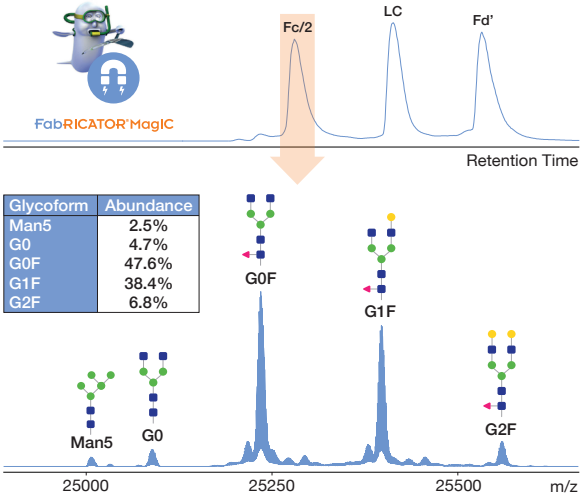
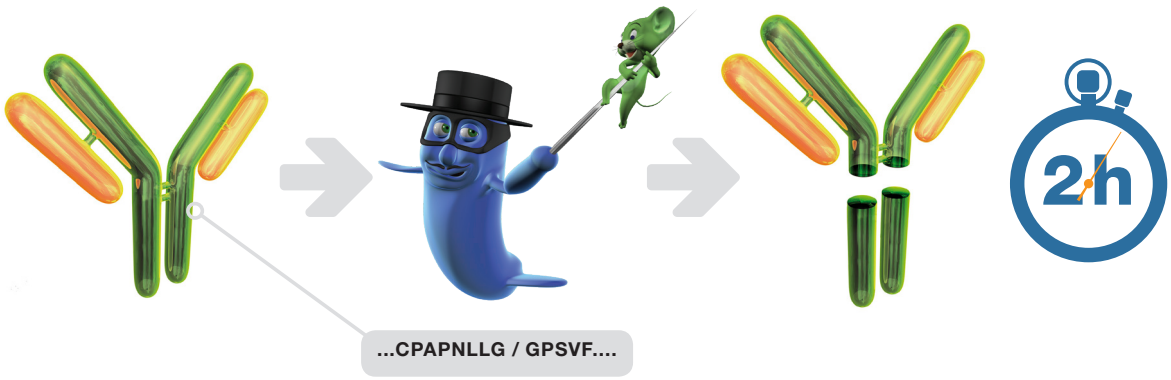


Figure 4. Analysis of Fc glycosylation by middle-level LC-MS using FabRICATOR MagIC (top) and quantification of glycoforms from a deconvoluted mass spectrum of the Fc/2 fragment (bottom).

FabRICATOR® MagIC

	Product ID	Description	EUR	USD
	A0-FRM-024	FabRICATOR MagIC 2 mL 24 samples	580	620
	A0-FRM-096	FabRICATOR MagIC 4 x 2 mL 96 samples	1,970	2,090

FabRICATOR®Z



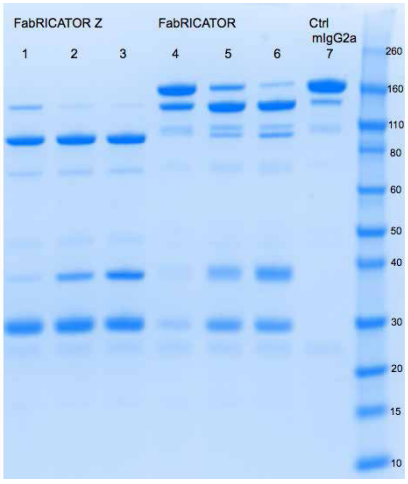
FabRICATOR® Z (IdeZ) is a cysteine protease that digests mouse IgG2a and IgG3 at a specific site below the hinge.

FabRICATOR Z digests mouse IgG2a and IgG3 and generates a homogenous pool of F(ab')2 and Fc/2 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 overdigestion because of the high specificity of the enzyme.

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

Digestion of Mouse IgG2a using FabRICATOR Z

Three different concentrations of FabRICATOR Z (IdeZ) and FabRICATOR (IdeS) were used to digest mouse IgG2a (*Fig. 1*). After 2 hours of incubation, FabRICATOR Z (IdeZ) readily digests mouse IgG2a, whereas FabRICATOR only digests a small amount of the antibody.



Lane 1-3: Mouse IgG2a digested with three different concentrations of FabRICATOR Z (IdeZ)

Lane 4-6: Mouse IgG2a digested with three different concentrations of FabRICATOR (IdeS)

Lane 7: Non-digested mouse IgG2a

Figure 1. Digestion of mouse IgG2a using FabRICATOR Z and FabRICATOR. F(ab')2 is detected at approximately 110 kDa and Fc fragments at approximately 30 kDa. The enzymes are detected at approximately 37 kDa.

FabRICATOR®Z

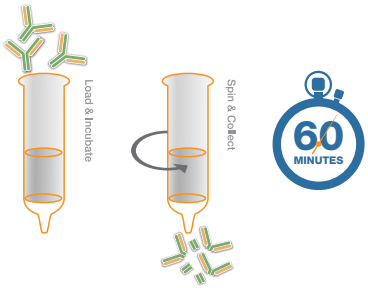
The FabRICATOR Z enzyme consists of 2000 units for digestion of 2 mg mouse IgG2a and IgG3. The enzyme is provided as a lyophilized powder.

Product ID	Description	Digestion	EUR	USD
A0-FRZ-020	FabRICATOR Z, 2000 units	2 mg IgG	470	660

FragIT™ Z

FragIT™ Z (immobilized FabRICATOR® Z) digests mouse IgG2a and IgG3 and generates F(ab')2 and Fc/2 fragments.

The FabRICATOR Z enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for digestion of 0.5 mg mouse IgG2a or IgG3. FragIT Z generates F(ab')2 and Fc/2 fragments with no enzyme in the final preparation.

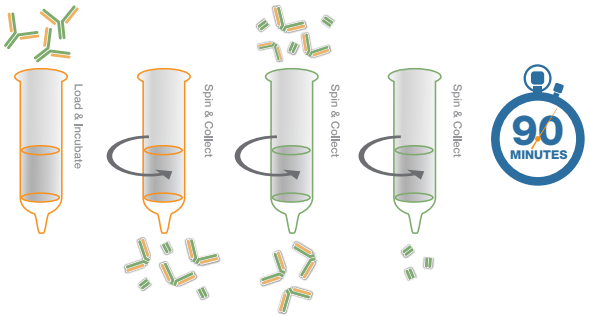


Product ID	Description	Digestion	EUR	USD
A0-FZ6-010	FragIT Z Microspin	2 x 0.5 mg IgG	350	490
A0-FZ6-025	FragIT Z Microspin	5 x 0.5 mg IgG	800	1,110
A0-FZ6-050	FragIT Z Microspin	10 x 0.5 mg IgG	1,330	1,850

FragIT™ Z kit

FragIT™ Z kit easily generates and purifies F(ab')2 and Fc/2 fragments from mouse IgG2a and IgG3.

FragIT Z kit consists of spin columns of FragIT Z for antibody digestion and spin columns of CaptureSelect™* Fc resin for affinity binding of the Fc fragments. After digestion, the Fc fragments are captured in the affinity spin column and pure F(ab')2 fragments are obtained in the flowthrough.



Product ID	Description	Digestion	EUR	USD
A2-FZ2-005	FragIT Z kit	0.5 mg IgG	400	550
A2-FZ2-025	FragIT Z kit	5 x 0.5 mg IgG	1,070	1,490

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

FabALACTICA®

FabALACTICA® (IgdE) digests human IgG1 at a specific site above the hinge without the need for reducing conditions.

FabALACTICA is a cysteine protease that specifically digests human IgG1 above the hinge without the need for reducing conditions or co-factors. FabALACTICA is used to generate intact and homogenous Fab and Fc fragments from human IgG1. 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 can be used to characterize intact and paired Fc glycosylation, bi- or multispecific antibodies, monovalent binding, higher order structures, disulphide scrambling (Faid, 2017, p. 50), and for subunit workflows on antibodies with mutated hinge regions.

- Human IgG1
- Overnight (O/N) reaction
- No need for reducing agents or co-factors
- KSCDKT / HTCPPCP (above the hinge)

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, 2mM cysteine	Yes	No
Reaction time	O/N (16-18 h)	1 h	1-24 h	1-24 h

Intact Fab and Fc Fragments from Therapeutic mAbs using FabALACTICA

FabALACTICA was used to digest the three therapeutic mAbs cetuximab, trastuzumab and adalimumab. *Fig. 1* shows that FabALACTICA generates intact Fab and Fc fragments from all three antibodies.

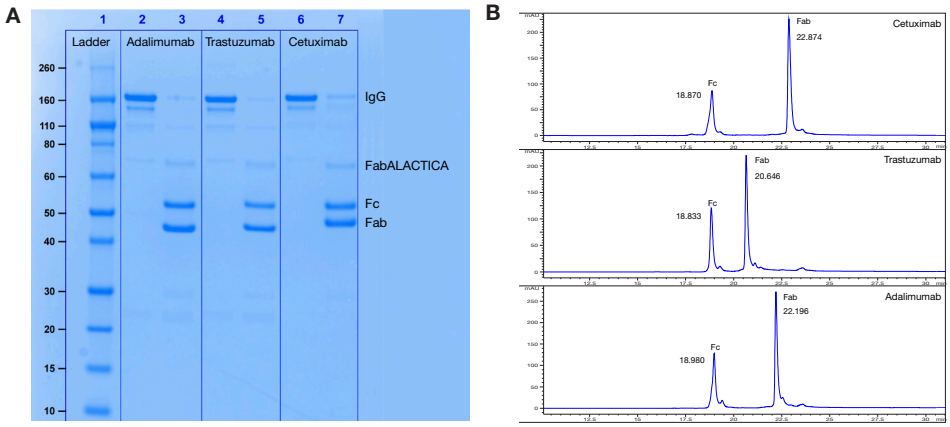


Figure 1. Digestion of cetuximab, trastuzumab, and adalimumab using FabALACTICA O/N at 37°C. **a)** Non-reduced SDS-PAGE. **b)** Separation of intact Fc and Fab fragments on RP-HPLC.

Paired Glycan and Intact Fab Analysis using FabALACTICA and LC-MS

The intact Fc fragment of ~53 kDa enables characterization of the two conserved Fc-glycosylation sites simultaneously with high mass accuracy (*Fig. 2a*). After FabALACTICA digestion, the mass of the intact Fab fragment can be analyzed to study modifications and light and heavy chain pairing for bispecific antibodies, or used for comparability assessment (*Fig. 2b*).

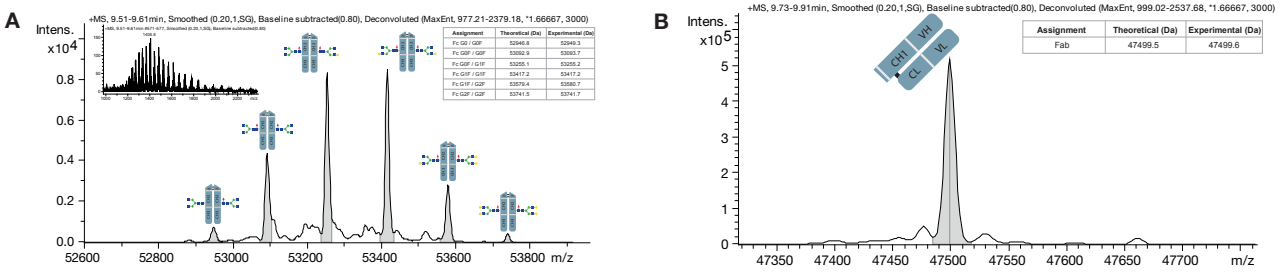


Figure 2. Trastuzumab was digested using FabALACTICA O/N at 37°C and intact Fc and Fab fragments were studied using LC-MS. **a)** Paired glycan analysis of trastuzumab Fc fragments. **b)** LC-MS of the intact Fab fragment of trastuzumab.

FabALACTICA®

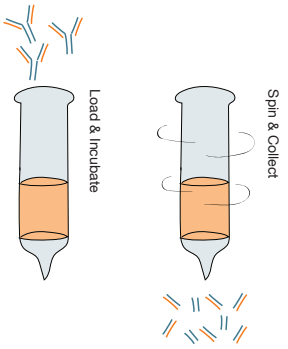
The FabALACTICA enzyme consists of 2000 units for digestion of 2 mg human IgG1. The enzyme is provided as a lyophilized powder.

Product ID	Description	Digestion	EUR	USD
A0-AG1-020	FabALACTICA, 2000 units	2 mg hIgG1	560	690

Immobilized FabALACTICA®

Immobilized FabALACTICA® digests human IgG1 and generates intact Fab and Fc fragments.

The FabALACTICA enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for digestion of 0.5 mg up to 100 mg of antibody.
Immobilized FabALACTICA generates antibody subunits with no enzyme in the final preparation.



Sample Preparation Workflow

The FabALACTICA enzyme can be used to generate subunits of human IgG1. The antibody is digested using the Immobilized FabALACTICA enzyme at room temperature overnight to generate intact Fab and Fc fragments.

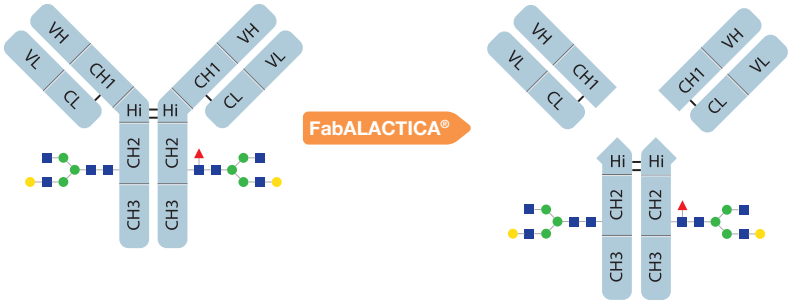


Figure 1. The FabALACTICA sample preparation workflow.

Immobilized FabALACTICA®

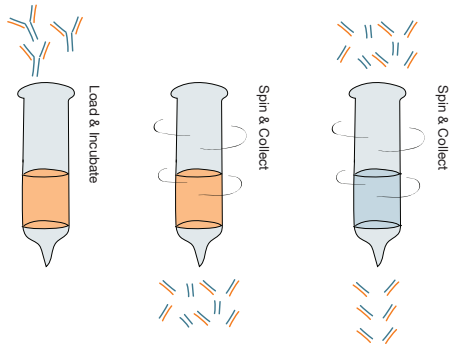
Immobilized FabALACTICA spin columns are provided for digestion of 0.5 mg up to 100 mg of human IgG1 antibody.

Product ID	Description	Digestion	EUR	USD
A0-AG6-010	Immobilized FabALACTICA Microspin	2 x 0.5 mg hIgG1	380	540
A0-AG6-050	Immobilized FabALACTICA Microspin	10 x 0.5 mg hIgG1	1,380	1,930
A0-AG6-100	Immobilized FabALACTICA Midispin	5-10 mg hIgG1	1,160	1,595
A0-AG6-1000	Immobilized FabALACTICA Maxispin	10-100 mg hIgG1	3,470	4,830

FabALACTICA® Fab kit

FabALACTICA® Fab kit easily generates and purifies intact Fab fragments from human IgG1.

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. After digestion, the Fc fragments are captured in the affinity spin column and intact, pure Fab fragments are obtained in the flowthrough.



Preparation of Pure Fabs

Three therapeutic mAbs were incubated with Immobilized FabALACTICA in spin columns. The Fc fragments were captured in the CaptureSelect™* Fc spin columns and the Fabs could easily be eluted by centrifugation (*Fig. 2*). The resulting Fab preparation is homogenous and pure.

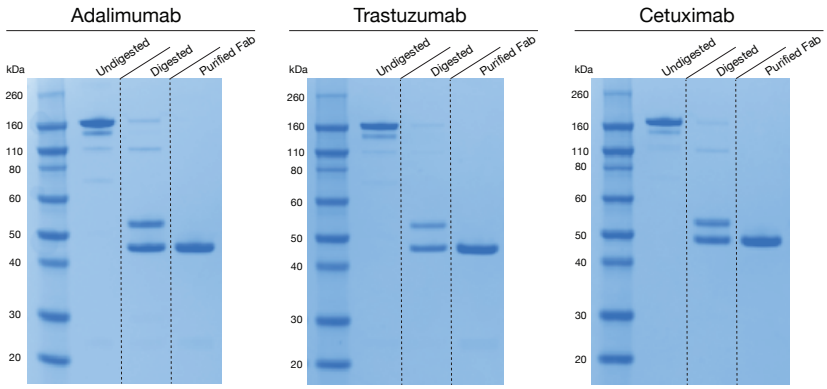


Figure 2. Adalimumab, trastuzumab and cetuximab digested by Immobilized FabALACTICA. Pure Fab fragments were obtained in a high yield from all three mAbs using the FabALACTICA Fab kit.

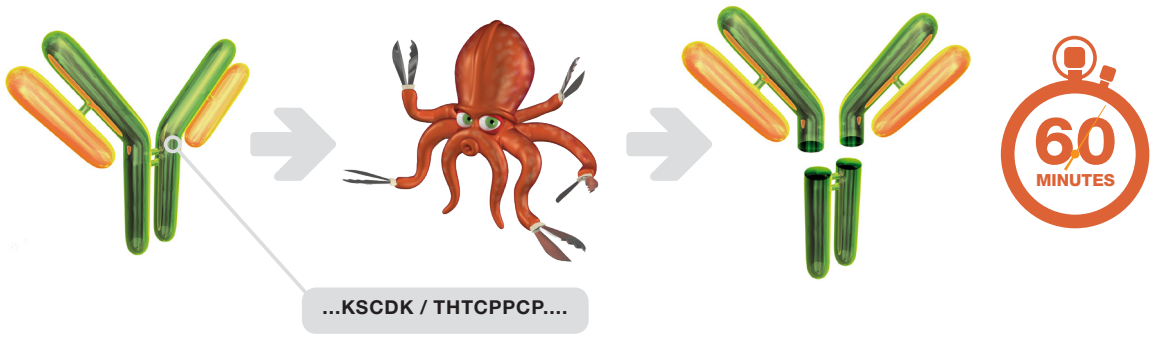
FabALACTICA® Fab kit

The FabALACTICA Fab kit consists of Immobilized FabALACTICA spin columns and CaptureSelect™* Fc affinity spin columns for easy generation and purification of Fab fragments from human IgG1 antibodies.

Product ID	Description	Digestion & Purification	EUR	USD
A2-AFK-005	FabALACTICA Fab kit	0.5 mg hIgG1	440	600
A2-AFK-025	FabALACTICA Fab kit	5 x 0.5 mg hIgG1	1,160	1,595
A2-AFK-100	FabALACTICA Fab kit	10 mg hIgG1	1,430	1,980
A2-AFK-1000	FabALACTICA Fab kit	100 mg hIgG1	4,350	6,120

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

GingisKHAN®



GingisKHAN® (Kgp) is a cysteine protease that digests human IgG1 at a specific site above the hinge.

Digestion of human IgG1 using GingisKHAN generates a homogenous pool of intact Fab and Fc fragments. Mild reducing reaction conditions, 2 mM cysteine, are required and ready-to-use reducing agent is provided together with the enzyme. GingisKHAN is used to characterize antibody-based biotherapeutics using LC-MS, and to study Fc glycan analysis, bispecific antibodies, affinity and avidity effects and general PTM identification. The activity on IgG1 hinge regions allows digestion of both monoclonal antibodies (trastuzumab and adalimumab) as well as Fc-fusion proteins (etanercept) carrying an IgG1 hinge region (*Fig. 1*). On other antibody subclasses, additional digestions at exposed lysines may occur. Publications using GingisKHAN to study bispecific antibodies are listed on p. 50.

- Human IgG1
- 60 min reaction
- 2 mM cysteine (included)
- KSCDK / THTCPPCP (above the hinge)

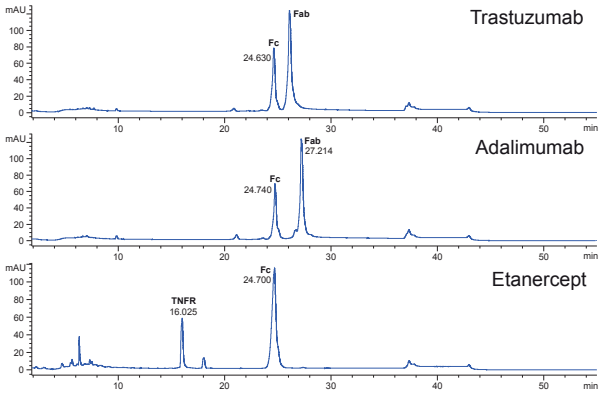


Figure 1. GingisKHAN digestion of trastuzumab, adalimumab and etanercept.

GingisKHAN®

2000 units of GingisKHAN is provided as a lyophilized powder together with 5 vials of lyophilized GingisKHAN reducing agent for digestion of 2 mg human IgG1.

Product ID	Description	Digestion	EUR	USD
B0-GKH-020	GingisKHAN, 2000 units	2 mg hIgG1	470	660

GingisKHAN® Fab kit

GingisKHAN® Fab kit generates and purifies Fab fragments from human IgG1.

The GingisKHAN Fab kit consists of lyophilized GingisKHAN enzyme for antibody digestion, and spin columns of CaptureSelect™* CH1 resin for affinity binding of the Fab CH1 domains. After digestion, the Fab fragments are captured in the affinity spin column and can easily be eluted.

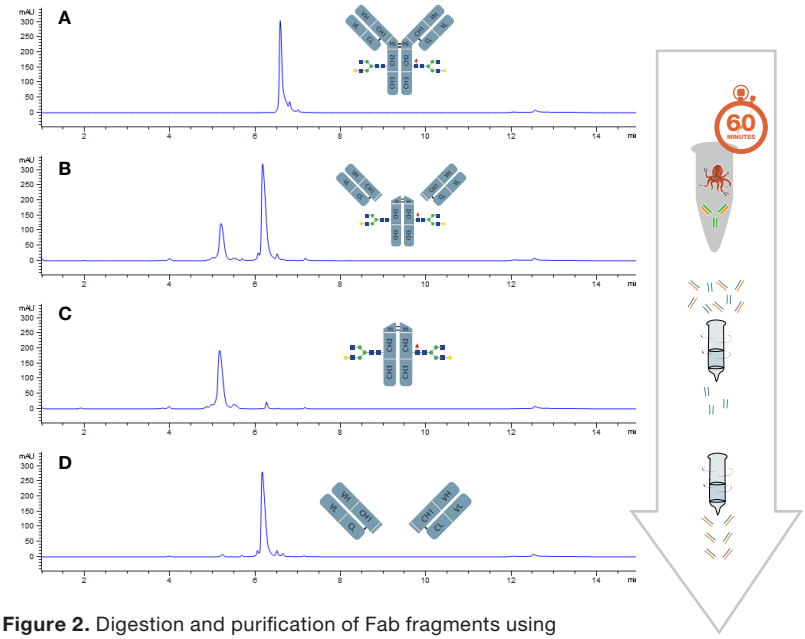


Figure 2. Digestion and purification of Fab fragments using GingisKHAN Fab kit. **a)** Intact antibody (human IgG1). **b)** Analysis of antibody fragments after GingisKHAN digestion. **c)** The flowthrough Fc. **d)** Elution of the purified Fab fragments.

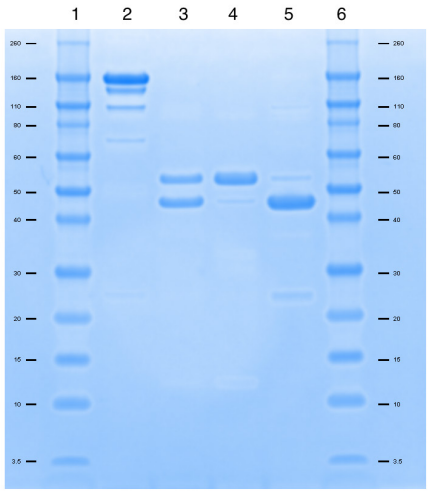


Figure 3. SDS-PAGE analysis of purified Fab fragments from trastuzumab using GingisKHAN Fab kit.
Lane 1 and 6: MW marker
Lane 2: Intact human IgG1
Lane 3: Fab and Fc fragments after GingisKHAN digestion
Lane 4: Flowthrough Fc fragments
Lane 5: Eluted Fab fragments

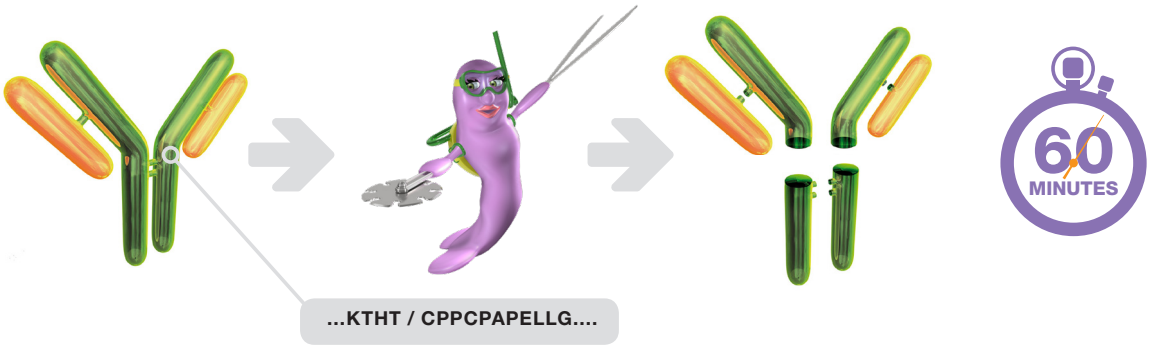
GingisKHAN® Fab kit

GingisKHAN Fab kit consists of 2000 units of the GingisKHAN enzyme, 5 x lyophilized reducing agent and 4 x CaptureSelect™* CH1 affinity spin columns for generation and purification of Fab fragments from human IgG1 antibodies.

Product ID	Description	Digestion	EUR	USD
B0-GFK-020	GingisKHAN Fab kit, 2000 units	2 mg hIgG1	1,070	1,150

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

FabULOUS™

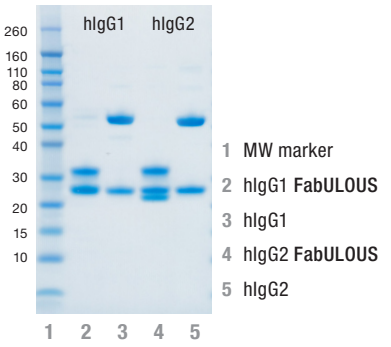


FabULOUS™ (SpeB) is a cysteine protease that digests in the hinge region of IgG from several different species and subclasses.

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 will also digest IgG from mouse, rat, goat, sheep and rabbit and can be used to generate intact Fab fragments from mouse IgG1, for instance. The enzyme requires reducing conditions for optimal activity, and if stronger reducing conditions are used, it is likely that interchain thiols will be reduced. Publications using FabULOUS are listed on p. 50.

- Human IgG and IgG from mouse, rat, goat, sheep and rabbit.
- 60 min reaction
- Requires reducing conditions (not included)
- Human IgG1: KTHT / CPPCPAP (above the hinge)

Human IgG



Mouse IgG

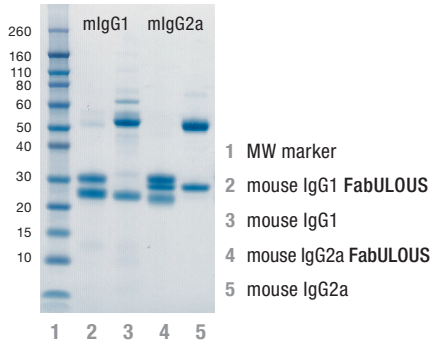


Figure 1. FabULOUS digestion of human (IgG1 and IgG2) and mouse (IgG1 and IgG2a) antibodies.

FabULOUS™

The FabULOUS enzyme consists of 2000 units for digestion of 2 mg IgG. The enzyme is provided as a lyophilized powder.

Product ID	Description	Digestion	EUR	USD
A0-PU1-020	FabULOUS, 2000 units	2 mg IgG	470	660

FabULOUS™ Fab kit

FabULOUS™ Fab kit generates and purifies Fab fragments from mouse IgG.

The FabULOUS Fab kit is designed to generate and purify Fab fragments from mouse IgG. The FabULOUS Fab kit consists of 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. CaptureSelect™* LC-Lambda (mouse) affinity columns are available upon request.
The antibody is digested within 60 minutes using the lyophilized FabULOUS enzyme, and the prepared Fab fragments bind to the CaptureSelect™* affinity spin columns and are easily eluted (*Fig. 3*). The prepared Fab fragments from mouse IgG1 are demonstrated in *Fig. 2* and *4* and can be used in affinity studies, studies of Fab glycosylation and structural studies.

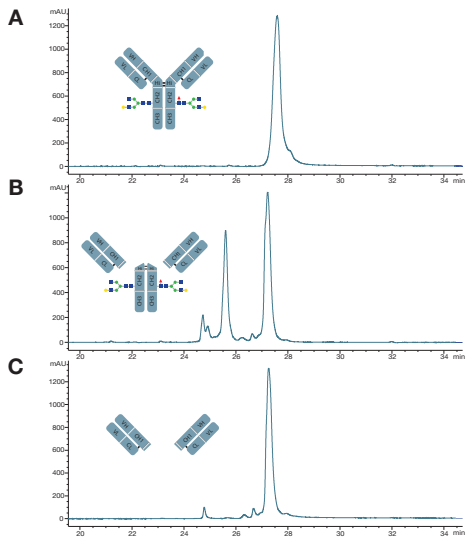


Figure 2. a) Intact monoclonal mouse IgG1 antibody. **b)** Analysis of the fragments after FabULOUS Fab kit digestion. **c)** Eluted Fab fragments from the CaptureSelect™* LC-Kappa (mur) column.

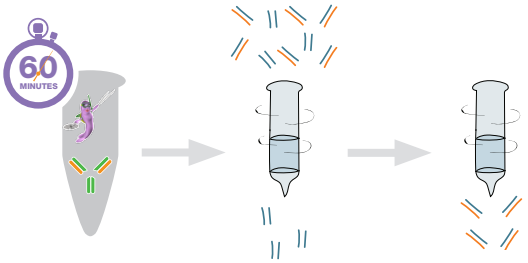


Figure 3. Schematic overview of the generation and purification of Fab fragments from mouse IgG using FabULOUS Fab kit.

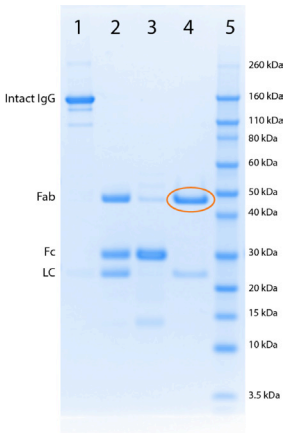


Figure 4. Non-reducing SDS-PAGE analysis of purified Fab fragments from monoclonal mIgG1 using FabULOUS Fab kit.
Lane 1: Intact mIgG1
Lane 2: Digested mIgG1
Lane 3: Flowthrough from CaptureSelect™* column
Lane 4: Eluted Fab fragments
Lane 5: MW marker

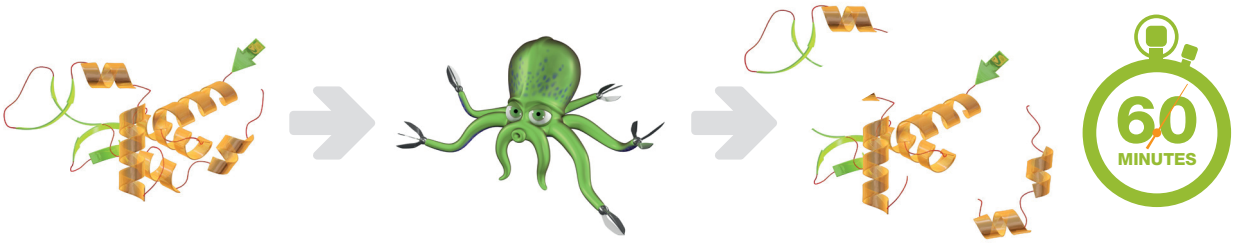
FabULOUS™ Fab kit

FabULOUS Fab kit consists of 2000 units of the FabULOUS enzyme and 4 x CaptureSelect™* LC-kappa (mur) affinity spin columns for generation and purification of Fab fragments from mouse IgG.

Product ID	Description	Digestion	EUR	USD
A1-PFK-020	FabULOUS Fab kit mouse, 2000 units	2 mg mIgG	795	870

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

GingisREX®



GingisREX® (RgpB) is a protease that digests proteins C-terminally of arginine residues.

GingisREX specifically digests peptides and proteins C-terminally of arginine residues. The protease does not have activity at lysines, as commonly observed using Arg-C (*Fig. 1* and Table 1). The enzymatic activity of GingisREX includes digestion of Arg-Pro linkages that are difficult to digest with other enzymes. The enzyme is active at a broad pH range of 5.5-9.0, but is inhibited by guanidine hydrochloride.

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

Unique Specificity for Arginine Residues

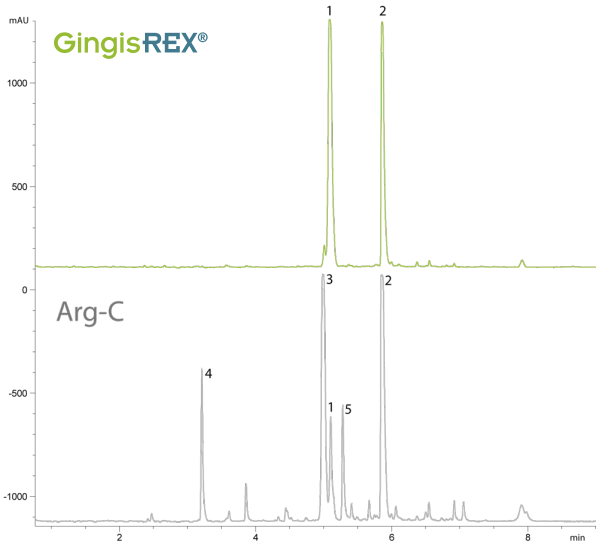


Figure 1. Oxidized insulin β-chain digested O/N at 37°C with GingisREX or Arg-C. The resulting sequences are presented in Table 1.

Table 1. Sequences of oxidized insulin β-chain digested by GingisREX or Arg-C, as indicated in *Fig. 1*. Green color indicates arginine residues and red color indicates lysine residues.

Peptide No.	Amino Acid Sequence
Intact protein	FVNQHLCGSHLVEALYLVCGERGFFYTPKA
1	GFFYTPKA
2	FVNQHLCGSHLVEALYLVCGER
3	GFFYTPK
4	FVNQHLCGSH
5	LVEALYLVCGER + Na

Applications of GingisREX

The GingisREX enzyme can be used to analyze proteins by mass spectrometry for the use in peptide mapping, protein fingerprinting and sequence analysis. It generates larger peptides with more charge per peptide, which is beneficial for mass spectrometric analyses. Using this workflow, the mass-to-charge of longer peptides can be resolved, resulting in increased sequence coverage and identification of particular post-translational modifications.

Peptide Mapping of Trastuzumab

On a large and complex sample, such as a therapeutic antibody, the digestion at arginine residues gives larger peptides and results in fewer peaks and a less complicated peptide map. This is beneficial for e.g. data interpretation in mass fingerprint analyses. As an example, the GingisREX and Arg-C digestion profiles of trastuzumab are presented in *Fig. 2*.

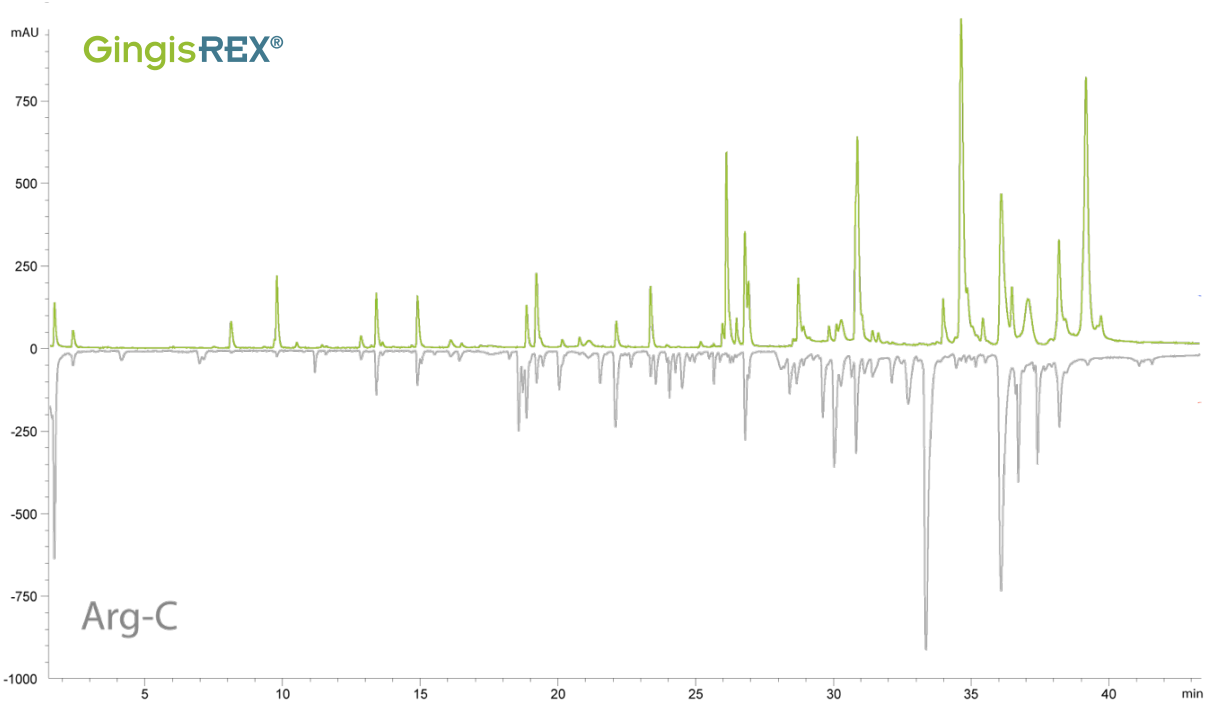


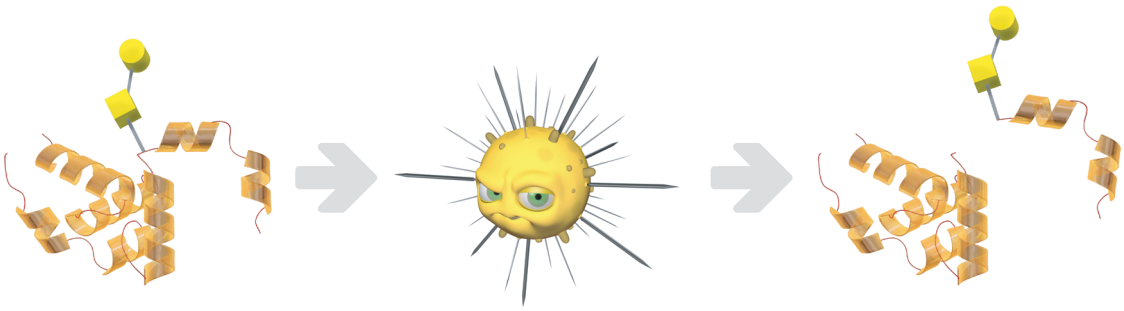
Figure 2. Peptide maps of trastuzumab after digestion using GingisREX or Arg-C.

GingisREX®

GingisREX is an arginine-specific protease that digests proteins C-terminally of arginine residues. The enzyme is provided as a lyophilized powder in vials of 5 µg enzyme.

Product ID	Description	Enzyme:Protein ratio	EUR	USD
B0-GRX-005	GingisREX, 5 µg enzyme	1:20 - 1:200	475	530

OpeRATOR®



OpeRATOR® is an O-glycan-specific protease that digests proteins carrying mucin-type O-glycans N-terminally of Ser and Thr glycosylation sites.

The OpeRATOR enzyme is a novel tool for analysis of mucin-type O-glycans on glycoproteins and glycopeptides. The enzyme binds to O-glycans (core 1) and digests the amino acid backbone N-terminally of the serine and threonine (S and T) glycosylation sites (*Fig. 2*). This generates glycopeptides carrying O-glycans and enables O-glycan profiling, O-glycopeptide mapping and site occupancy determination, as well as middle-level approaches using mass spectrometry. The OpeRATOR enzyme is most active towards sites with asialylated core 1 glycans. Sialylated sites and sites with core 3 O-glycans are digested with significantly reduced activity. SialEXO® (p. 30), a sialidase mix for efficient and complete removal of sialic acids, is provided with OpeRATOR.

- Digests native proteins with mucin-type O-glycosylation
- 2 h to overnight (16-18 h) reaction
- Desialylation using SialEXO® (included) increases performance
- N-terminally of O-glycosylated serine and threonine residues

Effect of SialEXO Pretreatment on OpeRATOR Enzymatic Activity

OpeRATOR displays some activity on sialylated O-glycoproteins, but the activity is much higher when the sialic acids are removed using SialEXO (*Fig. 1*).

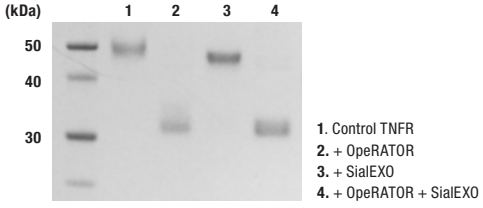


Figure 1. The native O-glycosylated TNF receptor (TNFR) was incubated with OpeRATOR with or without the addition of SialEXO, and the reactions were analyzed by SDS-PAGE.

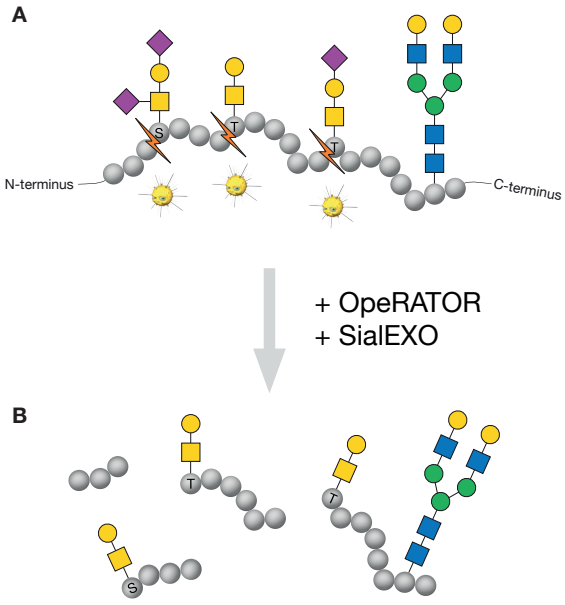


Figure 2. a) Schematic overview of OpeRATOR activity. O-glycans are required for OpeRATOR activity and the enzyme is not active at N-glycans. **b)** With OpeRATOR and SialEXO treatment, the O-glycosylated protein is digested into peptides carrying O-glycans. The digestion occurs N-terminally of the O-glycosylation sites.

O-glycan Site-specific Digestion of EPO using OpeRATOR

Erythropoietin (EPO) is a ~30 kDa glycoprotein with a single O-glycosylation site. The protein was used as a substrate to demonstrate the specific activity of OpeRATOR (*Fig. 3* and *4*). The O-glycosidase OglyZOR® (p. 29) was used to demonstrate the O-glycan-dependent activity of OpeRATOR. As can be seen in Lane 3 in *Fig. 4*, there is no OpeRATOR digestion of EPO when the O-glycan has been removed.

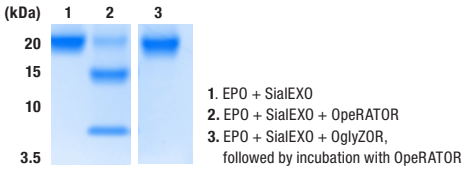


Figure 4. EPO was incubated with SialEXO to remove sialic acids (Lane 1), and with OpeRATOR to digest the protein N-terminally of the O-glycans (Lane 2). EPO was incubated with OglyZOR® to remove the O-glycan before OpeRATOR incubation (Lane 3). No OpeRATOR activity was observed in the absence of O-glycans.

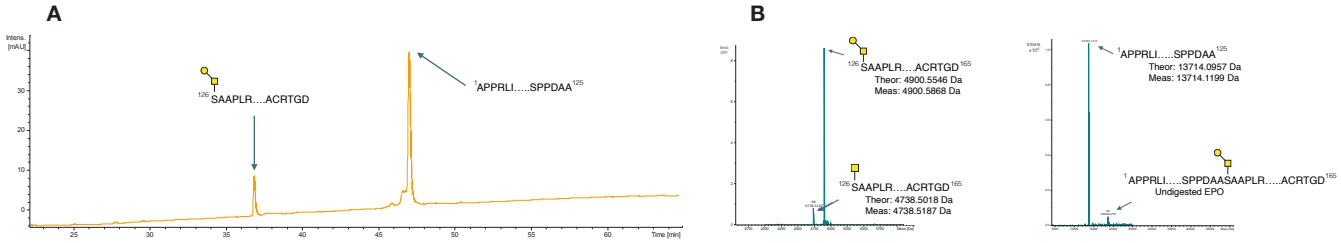


Figure 3. N-glycans were removed from EPO using PNGaseF and sialic acids were removed using SialEXO. In parallel, OpeRATOR hydrolyzed the protein N-terminally of the serine O-glycan (core 1) site. After reduction of disulfide bridges with DTT, the resulting two fragments were separated on a RP C4 column and intact mass was analyzed with a Bruker Impact II ESI QTOF MS. **a)** UV trace and **b)** QTOF MS.

LC-MS Analysis of Etanercept using OpeRATOR Maps O-glycan Sites

Etanercept, a highly O-glycosylated Fc fusion protein, was incubated with OpeRATOR and the resulting glycopeptides were analyzed using LC-MS/MS. Due to the heterogeneity in the O-glycan pattern of the protein and the OpeRATOR specificity for O-glycan structures, overlapping peptides were formed, making it possible to acquire a complete map of the O-glycan sites (*Fig. 5*).

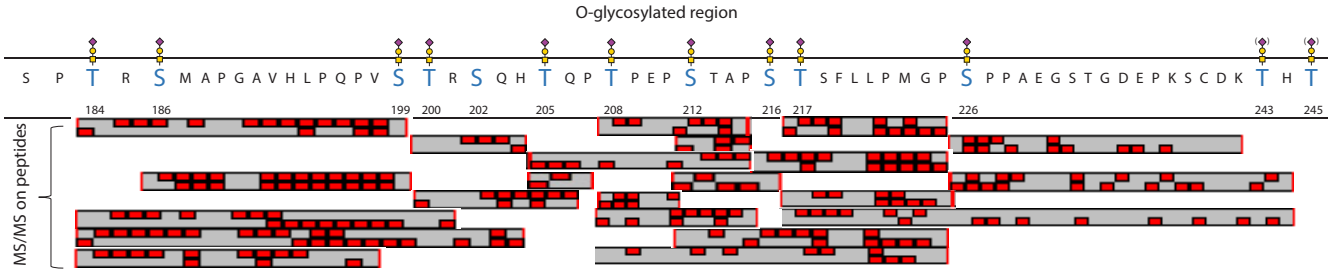


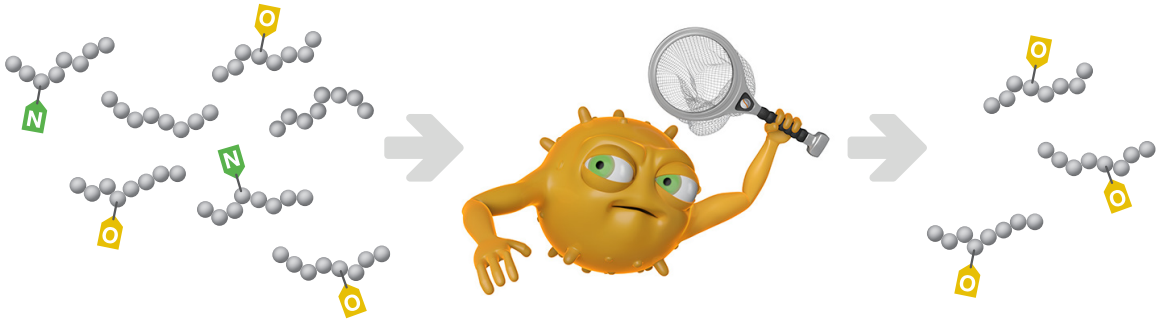
Figure 5. OpeRATOR digestion of the O-glycosylated hinge region of etanercept. OpeRATOR generates overlapping peptides, making it possible to map the O-glycan sites.

OpeRATOR®

The OpeRATOR enzyme consists of 2000 units for digestion of 2 mg O-glycoprotein. The enzyme is provided together with 2000 units of SialEXO for optional sialic acid removal. Both enzymes are provided as lyophilized powders.

Product ID	Description	Digestion	EUR	USD
G2-OP1-020	OpeRATOR, 2000 units	2 mg O-glycoprotein	920	1030

GlycOCATCH®



GlycOCATCH® is an enrichment resin for affinity purification of mucin-type O-glycosylated proteins and peptides.

GlycOCATCH is designed to specifically bind mucin-type O-glycosylated proteins and peptides. The affinity resin is based on inactive OpeRATOR® enzyme that has been engineered to bind core 1 O-glycosylated proteins and peptides with high affinity. GlycOCATCH is provided in a spin column format to allow easy-to-use enrichment of O-glycoproteins. Due to the strong interaction between the GlycOCATCH resin and O-glycoproteins/peptides, the elution is performed with 8 M urea. Alternatively, the elution can be performed with the included OpeRATOR enzyme. For optimal performance, the sialic acids of the glycoprotein need to be removed using the included SialEXO® sialidase mix (p.30). The applications of GlycOCATCH include specific enrichment or removal of O-glycoproteins and peptides, glycomics, studies of complex samples and characterization of biopharmaceuticals.
In *Fig. 1*, the O-glycan specificity of GlycOCATCH was studied on the peptide level. A selection of non-glycosylated and O-glycosylated (core 1) peptides was used. The peptides were 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 shows selective purification of the glycodrosocin peptide, carrying a core 1 O-glycan (*Fig. 1*).

- Binds proteins and peptides with mucin-type O-glycosylation
- 30 min - 2 h binding
- Desialylation using SialEXO® (included) increases binding
- Glycoproteins and peptides carrying mucin-type O-glycans

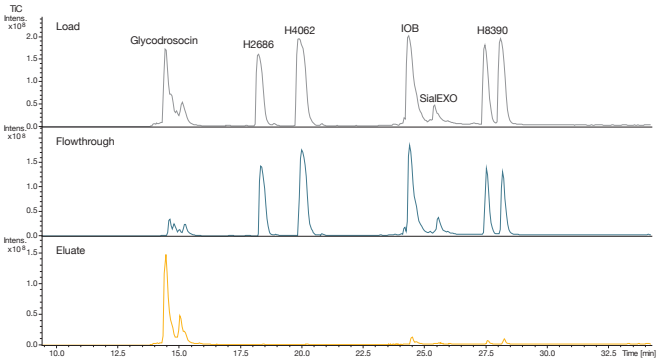


Figure 1. Selective purification of an O-glycosylated peptide (glycodrosocin) carrying a core 1 O-glycan using GlycOCATCH.

GlycOCATCH®

The GlycOCATCH box contains 4 microspin columns of GlycOCATCH affinity resin, 200 units of SialEXO and 200 units of OpeRATOR for enrichment of up to 200 µg O-glycoprotein.

Product ID	Description	Enrichment	EUR	USD
G3-OC6-002	GlycOCATCH	200 µg O-glycoprotein	625	740

OglyZOR®

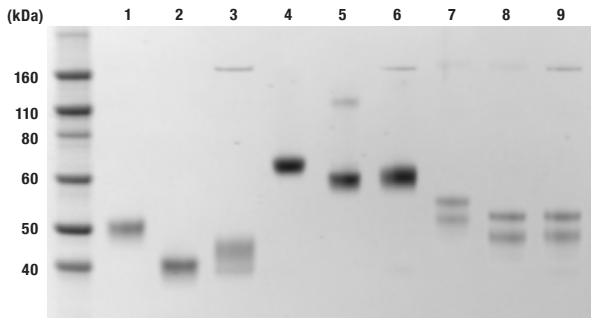
OglyZOR® is an O-glycosidase that specifically hydrolyzes core 1 type O-glycans on native glycoproteins.

OglyZOR is an O-glycosidase that catalyzes the removal of core 1 and to some extent core 3 type O-linked disaccharides from native glycoproteins. The enzyme does usually not require denaturation of the substrate. The sialic acids of the O-glycans need to be removed for OglyZOR activity. Therefore, the OglyZOR enzyme is provided together with the sialidase SialEXO® (p. 30). OglyZOR is used for removal of O-glycans for glycan analysis, confirmation of O-glycan presence and reduction of sample heterogeneity.

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

OglyZOR and SialEXO Compared to Other Enzymes

In *Fig. 1*, the enzymatic activities of OglyZOR and SialEXO are compared to other commercially available endoglycosidases and sialidases.



1. TNF receptor
2. + SialEXO and OglyZOR
3. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
4. Etanercept
5. + SialEXO and OglyZOR
6. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
7. Fetuin
8. + SialEXO and OglyZOR
9. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)

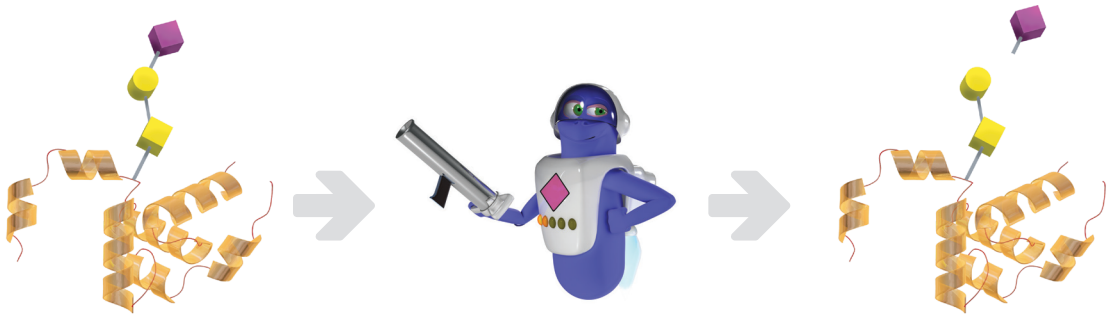
Figure 1. Comparison of the enzymatic activities of OglyZOR and SialEXO to commercially available endoglycosidases and sialidases. All incubations (4 h) were performed according to the manufacturers instructions, and the samples were all separated on SDS-PAGE.

OglyZOR®

The OglyZOR enzyme consists of 2000 units for hydrolysis of O-glycans on 2 mg glycoprotein. The enzyme is provided together with 2000 units of SialEXO for sialic acid removal. Both enzymes are provided as lyophilized powders.

Product ID	Description	Deglycosylation	EUR	USD
G2-OG1-020	OglyZOR, 2000 units	2 mg glycoprotein	795	920

SialEXO®



SialEXO® is a sialidase mix for complete removal of sialic acids from native glycoproteins.

SialEXO is used for removal of sialic acids on native glycoproteins, and it works on both O- and N-linked glycans. It is a combination of two sialidases acting on α2-3, α2-6 and α2-8 linkages (*Fig. 1*). SialEXO can be used to pretreat an O-glycosylated protein prior to digestion with OpeRATOR® (p. 26), or prior to deglycosylation with OglyZOR® (p.29). By using SialEXO in combination with the above-mentioned enzymes, the activities of the enzymes are enhanced. Applications of SialEXO include removal of all sialic acids and it can also be used in exoglycosidase arrays.

By quantifying liberated sialic acids after SialEXO treatment of the synthetic substrates 3'-sialyllactose (α2-3 bonds), 6'-sialyllactose (α2-6 bonds) and colominic acid (α2-8 bonds), the specificity of SialEXO was determined. As can be seen in *Fig. 1*, SialEXO is active on all sialic acid linkages.

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

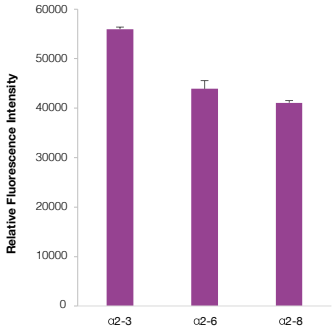


Figure 1. SialEXO activity on sialic acid linkages from the substrates 3'-sialyllactose (α2-3 bonds), 6'-sialyllactose (α2-6 bonds), and colominic acid (α2-8 bonds).

Table 1. Summary of key attributes for general α2-3, 6, 8 sialidases, including SialEXO.

	SialEXO®	Other Sialidase 1	Other Sialidase 2
Species	*Akkermansia muciniphila*	*Arthrobacter ureafaciens*	*Arthrobacter ureafaciens*
Reaction time	2 h	1 h or O/N	1 h or more if branched
pH range (optimal)	6.5 to 9.0 (6.8)	(6.0)	4.5 to 8.0 (6.0)
Molecular weight (kDa)	43 + 66	100	51 - 88

SialEXO®

The SialEXO mix consists of 2000 units for hydrolysis of sialic acids on 2 mg glycoprotein. The mix is provided as a lyophilized powder.

Product ID	Description	Desialylation	EUR	USD
G1-SM1-020	SialEXO, 2000 units	2 mg glycoprotein	570	690

SialEXO® 23

SialEXO®23 is an α2-3-specific sialidase, allowing targeted analysis of α2-3 linked sialic acids.

In contrast to SialEXO which is a sialidase mix, SialEXO 23 is an α2-3 specific sialidase, allowing targeted analysis of α2-3 linked sialic acids only. Efficient α2-3 desialylation of O- and N-glycosylated proteins can be achieved within one hour.
Hydrophilic interaction liquid chromatography (HILIC) was used to analyze two glycan libraries; one containing released glycans with α2-3-linked sialic acids and the other glycans modified with α2-6-linked sialic acids. Treatment with SialEXO or SialEXO 23 can be compared (*Fig. 2*). The shift in peaks clearly shows the release of sialic acids only linked by α2-3 with SialEXO 23 and of both α2-3 and α2-6 linked sialic acids by SialEXO.

- Hydrolyzes sialic acids on N- and O-linked glycans
- 60 min reaction
- Requires no co-factors
- α2-3-linked sialic acids

Table 2. Summary of key attributes for specific α2-3 sialidases, including SialEXO 23.

	SialEXO® 23	Other Sialidase 3	Other Sialidase 4
Species	*Akkermansia muciniphila*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
Reaction time	1 h	1 h	1 h
pH range (optimal)	7.0 to 9.0 (7.5)	(5.5)	(6.0)
Molecular weight (kDa)	66	74	75

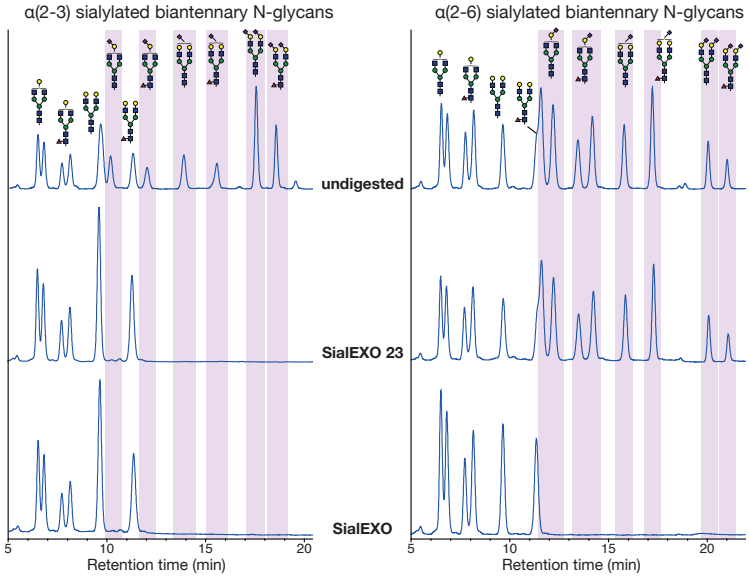


Figure 2. HILIC analysis of released glycans with α2-3- or α2-6-linked sialic acids after incubation with SialEXO 23 and SialEXO respectively. Sialylated glycan structures are shaded in purple.

SialEXO® 23

The SialEXO 23 enzyme consists of 500 units for hydrolysis of sialic acids on 0.5 mg glycoprotein. The enzyme is provided as a lyophilized powder.

Product ID	Description	Desialylation	EUR	USD
G1-SD2-005	SialEXO 23, 500 units	0.5 mg glycoprotein	470	540

Immobilized SialEXO®

Immobilized SialEXO® is a sialidase mix for complete removal of sialic acids from native glycoproteins within 30 minutes.

SialEXO hydrolyzes sialic acids on native glycoproteins, and is active on both O- and N-linked glycans. It is a combination of two sialidases acting on α2-3, α2-6 and α2-8 linkages. Immobilized SialEXO is a resin with a mixture of the two sialidases covalently coupled to agarose beads for complete removal of sialic acids with no enzyme in the final preparation. 0.5 mg glycoprotein is desialylated in 30 min at room temperature.

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

Efficient Desialylation of a Monoclonal Antibody

Antibodies with additional glycans in their Fab regions are often sialylated to a significant extent. Cetuximab carries a Fab glycan which is partially modified with N-Glycolylneuraminic acid (Neu5Gc). These sialic acids can be removed rapidly using Immobilized SialEXO (*Fig. 1*), demonstrating the utility of this enzyme for analysis of monoclonal antibodies and its activity towards non-human sialic acids.

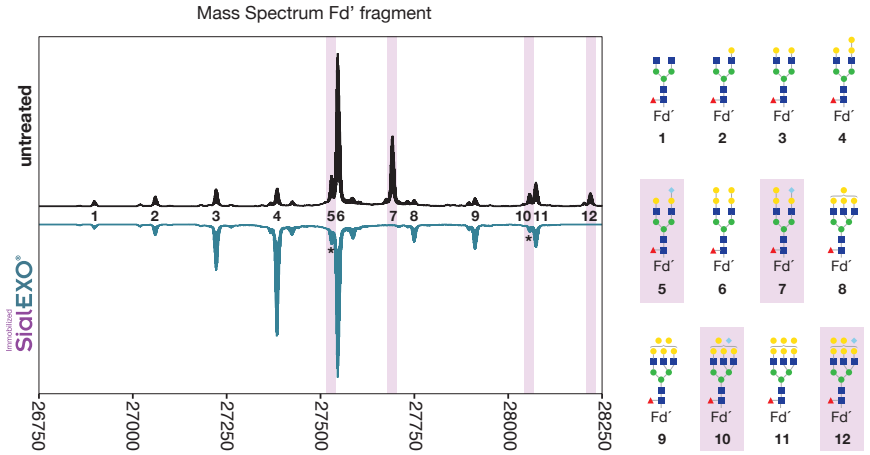


Figure 1. Deconvoluted mass spectra of the Fd' fragment of cetuximab, showing the Fab glycosylation profile. Sialylated structures (Neu5Gc, light blue diamond) are highlighted in purple and are absent in the lower spectrum. The asterisk marks peaks originating through neutral loss during ionization rather than remaining sialylated Fd' fragments.

Efficient Desialylation of a Complex Glycoprotein

We tested the performance of SialEXO and Immobilized SialEXO on the human C1 inhibitor. This glycoprotein is a challenging substrate with 6 N- and up to 26 O-glycans, modified with both α2,3 and α2,6-linked sialic acid. Analysis of released N-glycans demonstrates a complete desialylation by both SialEXO in solution and Immobilized SialEXO (*Fig. 2*) .

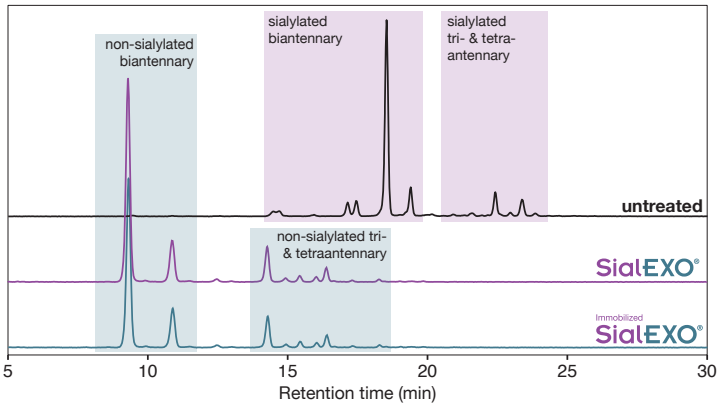


Figure 2. The C1 inhibitor was either treated with SialEXO in solution for 2 hours at 37°C, or with Immobilized SialEXO for 30 min at room temperature. N-glycans were released from the resulting desialylated protein using PNGase F and the resulting free glycans were labeled with 2-AB and analyzed by HILIC-FLD HPLC.

Simplified Charge Variant Analysis of Biologics

Capillary electrophoresis is commonly used to determine charge variants during characterization and quality control of biologics. The inherent charge of sialic acids might complicate charge variant profiles, masking other important modifications. The removal of sialic acids therefore facilitates the study of underlying charge variants in the protein. Cetuximab and etanercept were desialylated using Immobilized SialEXO and the separation was improved in imaged isoelectric focusing (icIEF; *Fig. 3*), using Maurice from ProteinSimple.

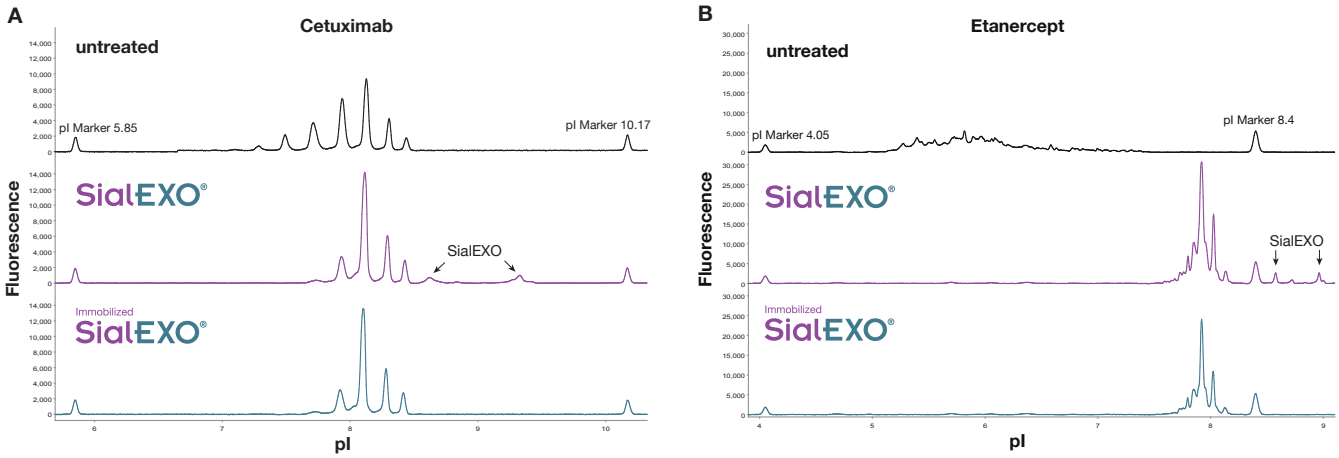


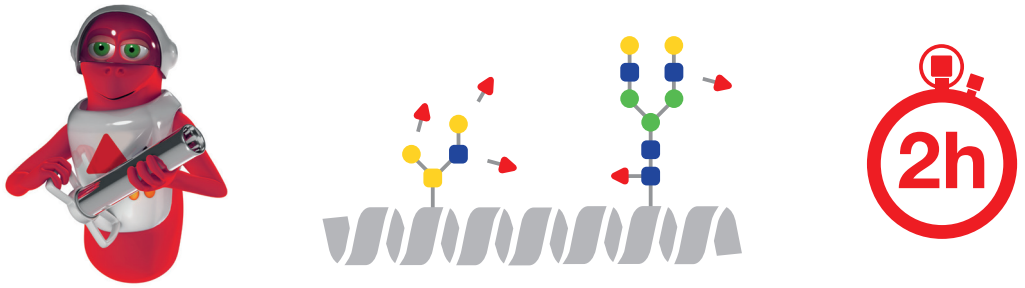
Figure 3. Desialylation of cetuximab **(a)** and etanercept **(b)** using Immobilized SialEXO followed by imaged isoelectric focusing. Data obtained in collaboration with ProteinSimple.

Immobilized
SialEXO®

The Immobilized SialEXO Microspin columns contain SialEXO covalently coupled to agarose beads, for desialylation of up to 0.5 mg native glycoproteins without enzyme in the final preparation.

Product ID	Description	Desialylation	EUR	USD
G1-SM6-010	Immobilized SialEXO Microspin	2 x 0.5 mg	380	470
G1-SM6-025	Immobilized SialEXO Microspin	5 x 0.5 mg	810	1,040
G1-SM6-050	Immobilized SialEXO Microspin	10 x 0.5 mg	1,350	1,830

FucosEXO™



Hydrolysis of α1-2,3,4 linked fucose on glycoproteins or oligosaccharides.

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. The two enzymes are modified with His-tags and expressed in E. *coli.* The molecular weight of the enzymes are 87 kDa and 64 kDa, respectively. FucosEXO is active in neutral pH and does not require any co-factors or special buffers. The exoglycosidase activity of FucosEXO allows for the complete hydrolysis of α1-2,3,4 linked fucose on up to 2 mg of glycoprotein. FucosEXO is a valuable tool for glycan structure analysis on *N*- and *O*-glycoproteins or in exoglycosidase arrays on oligosaccharides.

- Hydrolyzes α1-2,3,4 linked Fucose on native glycoproteins
- α1-2,3,4 linked Fucose
- 1-2 h incubation
- No co-factors required

Key Characteristics

- Efficient hydrolysis of α1-2,3,4 linked fucose
- For native glycoproteins or oligosaccharides
- Activity without the need for co-factors

Specific Hydrolysis of Fucose Linkages

Fucose can be attached to both *N*- and *O*-glycans. The α1-2, α1-3 and α1-4 linkages most commonly occur in *O*-glycans or as antenna fucosylation of *N*-glycans, whereas α1-6 linked fucose is found as a modification of the *N*-glycan core. We measured the release of fucose using FucosEXO from a panel of oligosaccharide substrates representing different linkages. FucosEXO efficiently releases α1-2, α1-3 and α1-4 linked fucose, without activity on α1-6 linked fucose residues (Fig. 1).

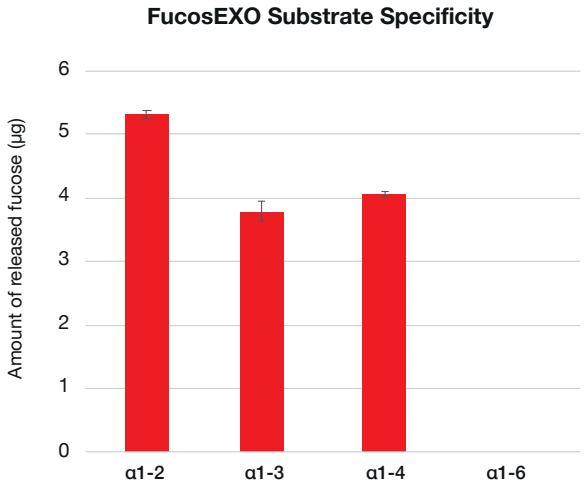


Figure 1. The substrate specificity of FucosEXO was analyzed on equal molar amounts of synthetic oligosaccharides; α1-2 (2'- fucosyllactose,) α1-3 (3-fucosyllactose), α1-4 (Lewis a) and α1-6 (α1-6 fucosylated chitobiose). Substrates were incubated with FucosEXO for 30 min at 37°C and the amount of released fucose measured spectrometrically using an L-Fucose Assay Kit (Megazymes).

Defucosylation of Native Glycoproteins

Fucosylation of *O*-glycans is involved in synthesis of functionally important glycan epitopes such as 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. We tested FucosEXO on a glycoengineered TNFR protein carrying up to 11 *O*-glycans decorated with α1-2 and α1-3 linked fucose and compared the activity to a commercially available α-fucosidase. Within 1 hour, complete removal of fucose on the TNFR protein was achieved with FucosEXO, while treatment with the other fucosidase only had a minor impact on fucoses (Fig. 2). Hydrolysis of all fucose residues from the TNFR protein was also achieved using Immobilized FucosEXO.

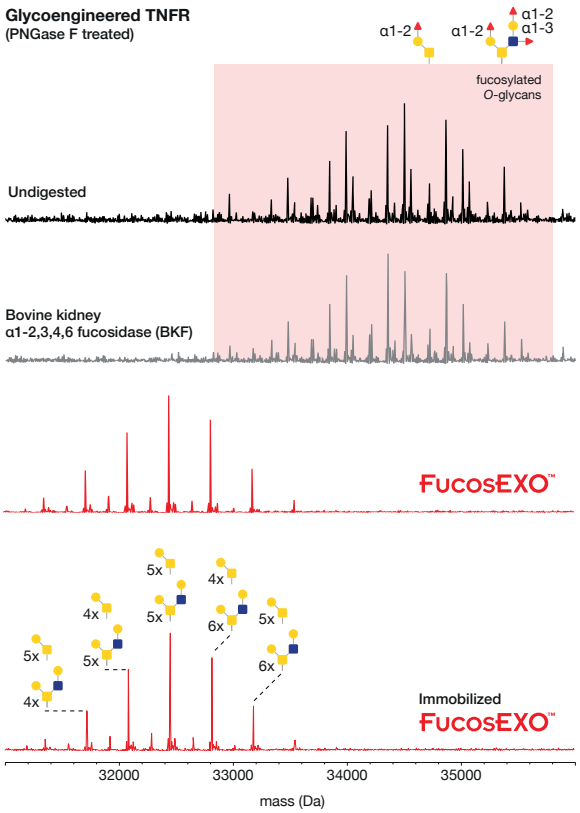


Figure 2. Glycoengineered TNFR with core 1 and core 2 *O*-glycans decorated with both 1-2 and 1-3 fucose was incubated with bovine kidney fucosidase (1h, 37°C), FucosEXO (1h, 37°C) or Immobilized FucoseEXO (1h, RT). The resulting protein was analyzed by reverse phase LC-MS on a Waters BioAccord system equipped with a Waters BioResolve RP mAb column (2.1 x 50 mm).

No Additives or Co-factors Required

FucosEXO is active in physiological buffers at a neutral pH and the activity is not dependent on any co-factors, making FucosEXO compatible with most samples without the risk of impairing LC-MS analysis (Table 1). Depending on the nature of the substrate, FucosEXO hydrolyzes terminal α1-2,3,4 linked fucose residues on glycoproteins within 1 to 2 hours, while longer incubation may be required for very complex samples. FucosEXO is also available immobilized on agarose beads in a spin column format for convenient processing without residual enzyme in the final preparation.

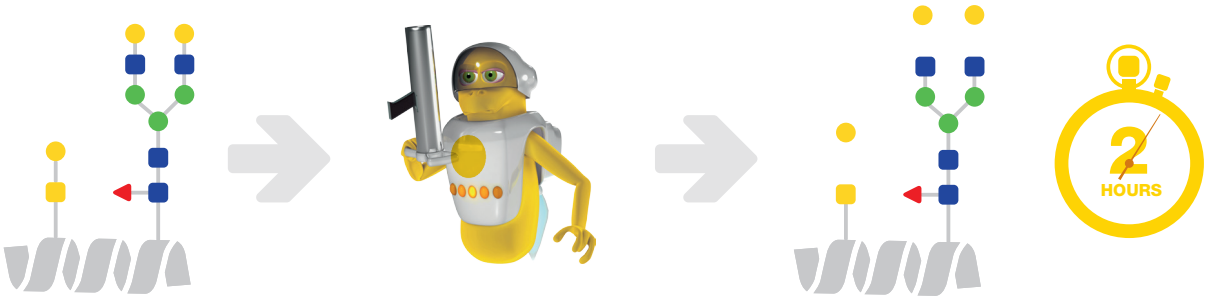
Table 1. Key characteristics of FucosEXO.

Enzyme Feature	FucosEXO
Incubation time	1-2 hours
pH range	6.0 - 8.0
MS compatibility	Yes
Special buffers	No
Co-factors	No
Additives or BSA	No

FucosEXO™

Product ID	Description	EUR	USD
G1-FM1-020	FucosEXO, 2000 units	750	845
G1-FM6-025	Immobilized FucosEXO, 5 x 0.5 mg	795	995
G1-FM6-050	Immobilized FucosEXO, 10 x 0.5 mg	1,295	1,745

GalactEXO™



GalactEXO™ is a β-galactosidase for efficient hydrolysis of galactose from glycoproteins and released glycans

GalactEXO is a β-galactosidase mix for complete hydrolysis of galactose residues on *N*- and *O*-linked glycans on native glycoproteins. The enzymes were discovered and characterized from *Akkermansia muciniphila* for efficient hydrolysis of β1-3 and β1-4 linked galactoses. The combined enzymatic activities will completely hydrolyze all galactoses on 2 mg of native glycoprotein. GalactEXO can be used for trimming of the released glycans in exo-glycosidase array sequencing experiments where β1-3 and β1-4 linked galactose are removed within 1 h of incubation. GalactEXO can be applied to obtain antibodies with homogenous G0 glyco-sylation profiles.

- Hydrolyzes galactcose residues on N- and O-glycosylated proteins
- β1-3 and β1-4 linked galactose
- 2 h incubation
- No co-factors required

Key Characteristics

- Efficient removal of β1-3,4 linked galactose
- Acitvity on both *N*- and *O*-glycan structures
- For native glycoproteins and free glycans

Hydrolysis of β1-4 Galactose on a mAb

The β-galactosidase activity of GalactEXO was demonstrated on trastuzumab carrying galactose residues in the Fc domain of the antibody. After a 2 h incubation with GalactEXO or with competing enzymes from other sources, the antibodies were digested into subunits using FabRICATOR® and analyzed using LC-MS (Fig. 1). The shift to G0F can be seen using GalactEXO whereas enzymes from other sources results in incomplete digestion.

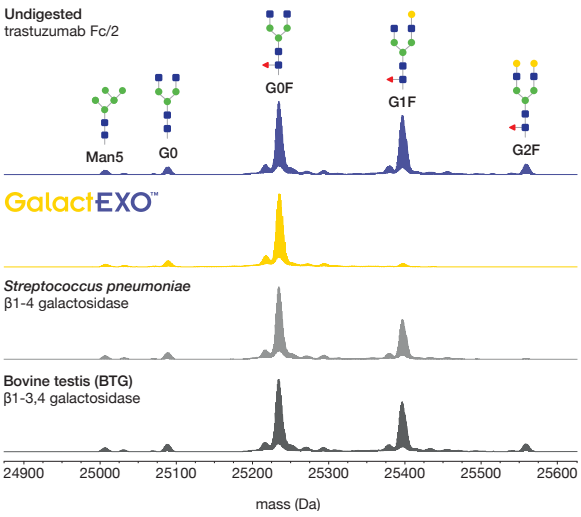


Figure 1. Deconvoluted mass spectra of the Fc/2 of trastuzumab treated with three different β-galactosidases. The antibody was digested with FabRICATOR, separated by reverse phase HPLC (Waters BioResolve RP, 2.1x100 mm) and analyzed by ESI-Q-TOF mass spectrometry (Bruker Impact II).

Activity on β1-3 Gal from Etanercept

GalactEXO also shows exogalactosidase activity for β1-3 galactose, present on *O*-glycosylated biopharmaceuticals. Etanercept was incubated with GalactEXO or galactosidases from other sources and the TNFαR fragment was analyzed using LC-MS after digestion with FabRICATOR to remove the Fc fragment (Fig. 2). The results show efficient galactosidase activity from GalactEXO compared to the enzymes from other sources.

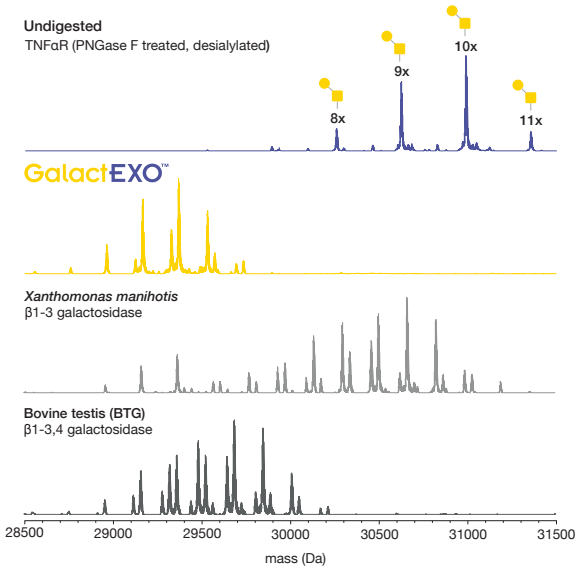
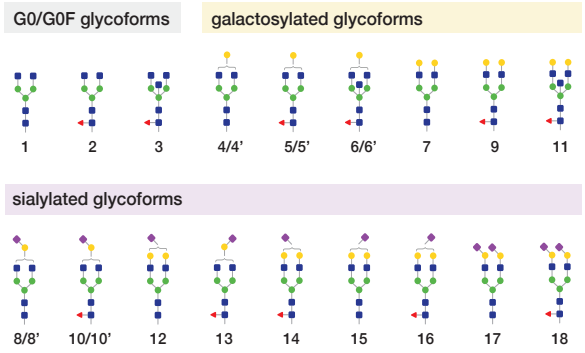


Figure 2. Deconvoluted mass spectra of the TNFαR fragment of etanercept treated with three different beta-galactosidases. The protein was digested with FabRICATOR, separated by reverse phase HPLC (Waters BioResolve RP, 2.1x100 mm) and analyzed by ESI-Q-TOF mass spectrometry (Bruker Impact II).

GalactEXO for Released Glycan Trimming

When analyzing released glycan structures using exoglycosidases it is crucial to obtain complete hydrolysis to minimize errors in data interpretation. A labeled *N*-glycan library was incubated with the SialEXO® sialidase and GalactEXO galactosidase for 1 h and analyzed for exoglycosidase acitvities (Fig. 3). The HILIC-FLD separations show complete hydrolysis of both the sialylated and galactosylated structures and the remaining peaks can easily be identified as G0, G0F or G0F with bisecting GlcNAc.



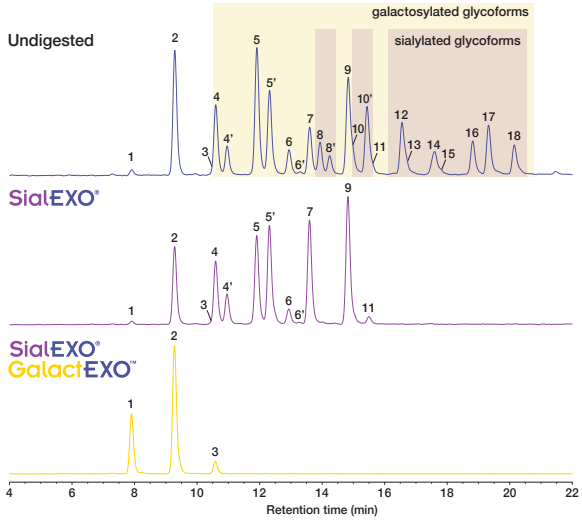
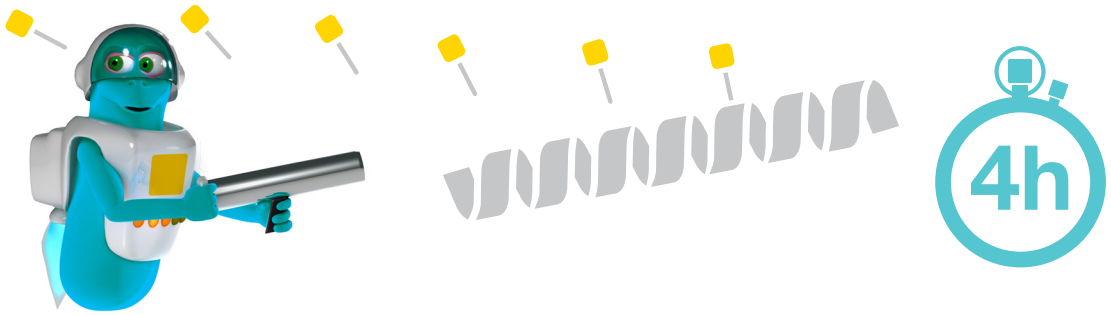


Figure 3. HILIC-FLD UHPLC chromatograms of a 2-AB labeled glycan library analyzed undigested (top), after treatment with SialEXO (middle) or both SialEXO and GalactEXO (bottom). Analysis was performed on a Thermo Scientific Vanquish Duo UHPLC system equipped with a Thermo Scientific Accurcore 150 Amide HILIC column (2.1 x 150 mm).

GalactEXO™

Product ID	Description	EUR	USD
G1-GM1-020	GalactEXO, 2000 units	750	850
G1-GM6-025	Immobilized GalactEXO Microspin, 5 x 0.5 mg	795	995
G1-GM6-050	Immobilized GalactEXO Microspin, 10 x 0.5 mg	1,295	1,750

GalNAcEXO™



GalNAcEXO™ is an efficient α-GalNAcase for hydrolysis of α-linked GalNAcs.

GalNAcEXO is an α-GalNAcase that hydrolyses the reducing *N*-acetylgalactosamines (GalNAcs), known as Tn antigens, on native *O*-glycoproteins. The enzyme has been derived from *Akkermansia muciniphila* and recombinantly expressed for the hydrolysis of α-GalNAcs on *O*-glycoproteins. The enzyme is active in neutral pH without the need for any co-factors. The exoglycosidase acitivty of GalNAcEXO allows for the removal of α-linked GalNAcs on up to 2 mg of native *O*-glycosylated protein. The GalNAcEXO enzyme is a valuable tool that reduces sample heterogeneity for the analysis of complex *O*-glycoproteins that carry α-linked GalNAc resudies as immature truncated *O*-glycoforms.

- Hydrolyzes α-linked GalNAc residues on native *O*-glycoproteins
- α-linked GalNAcs on Ser or Thr sites
- 4 h incubation
- No co-factors required

Key Characteristics

- Efficient hydrolysis of α-linked GalNAcs
- Exoglycosidase for native *O*-glycoproteins
- Activity without need for any co-factors

Performance on Complex Substrates

Etanercept is a model biopharmaceutical carrying 13 *O*-glycan sites of which 9 to 10 are occupied on average. After *N*-glycan removal by PNGaseF and truncation of *O*-glycans with SialEXO and GalactEXO, GalNAc related peaks were observed in the mass spectra (Fig. 1A). After incubation with the GalNAcEXO enzyme, over 95% of the remaining α-GalNAcs were removed (Fig. 1B).

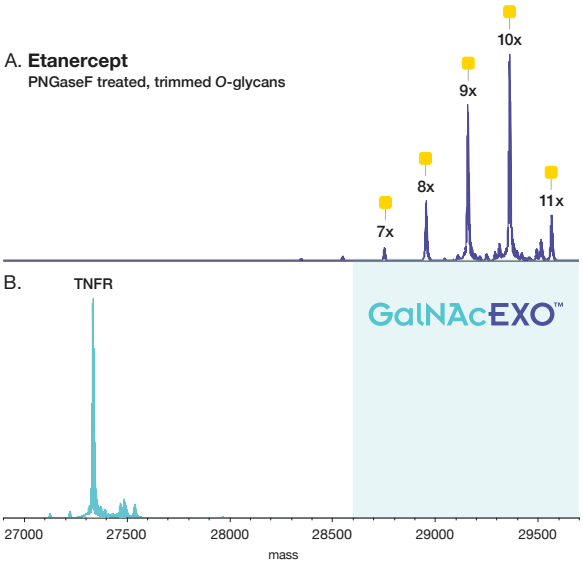


Figure 1. Deconvoluted mass spectra of partially deglycosylated etanercept (A), and after treatment with GalNAcEXO (B, O/N at 37 °C). Samples were FabRICATOR digested, separated by RP-HPLC (Waters BioResolve RP mAb, 2.7 µm, 2.1 x 100 mm) and analyzed by ESI-Q-TOF MS (Bruker Impact II).

Deglycosylation of the C1 Inhibitor

During production of *O*-glycosylated biopharmaceuticals, Tn antigens may appear as a result of incomplete processing of the core GalNAc. This appears as repeating mass shifts with a 203 Da HexNAc unit difference. To confirm the presence of Tn antigens, the following workflow can be applied, demonstrated on the heavily (26 sites) *O*-glycosylated C1 inhibitor (Fig 1A). At first, the glycoprotein was trimmed of the *N*-glycans by PNGaseF and core 1 *O*-glycans using OglyZOR and SialEXO. A pattern of repeating peaks was observed in the mass spectra (Fig. 2B). Secondly, the GalNAcEXO enzyme completely removed all remaining GalNAcs leaving a single peak (Fig. 2C). This workflow is further beneficial for analysis of PTMs not related to protein *O*-glycosylation.

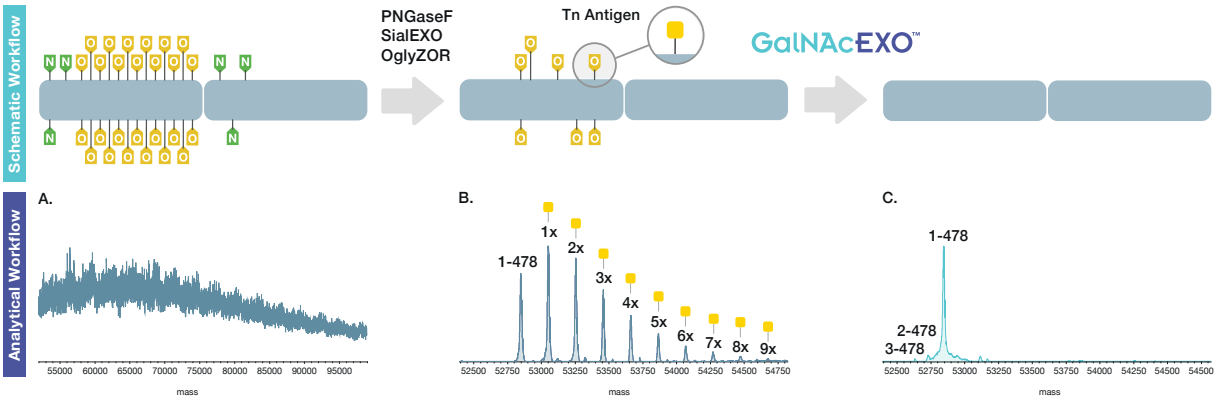


Figure 2. Total deglycosylation of recombinant C1 inhibitor. The C1 inhibitor was analyzed by LC (Waters ACQUITY UPLC Protein BEH C4 column, 1.7 µm, 2.1 x 100 mm) and ESI-Q-TOF mass spectrometry (Bruker Impact II). In its intact form (A) the protein is too complex and deconvolution of the mass spectra only yielded noise. After removal of *N*- and core 1 *O*-glycans the Tn antigens remain (B) and can efficiently be removed by incubation with GalNAcEXO (C, over night at 37 °C).

Efficient Hydrolysis without Co-factors

GalNAcEXO is active in physiological buffers in a neutral pH range, making it compatible with most samples. The activity is not dependent on co-factors, such as BSA, that can impair LC-MS analysis (Table 1). Depending on the substrate, the reaction for *O*-glycoproteins is complete in 4 hours while more complex samples may require incubation over night. GalNAcEXO is available immobilized in spin columns for easy processing without residual enzyme in the final sample.

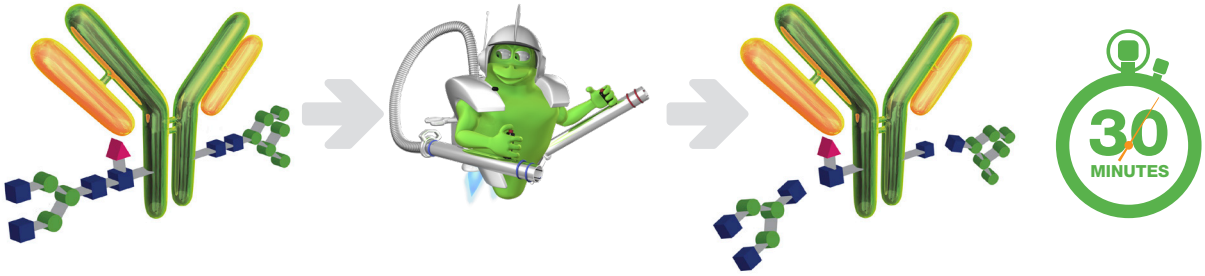
Table 1. Key characteristic features of the GalNAcEXO enzyme.

Enzyme Feature	GalNAcEXO
Incubation time	2 to 18 hours
pH range	6.0 - 7.6
MS compatibility	Yes
Special buffers	No
Co-factors	No
Additives or BSA	No

GalNAcEXO™

Product ID	Description	EUR	USD
G1-NA1-020	GalNAcEXO, 2000 units	750	850
G1-NA6-025	Immobilized GalNAcEXO, 5 x 0.5 mg	795	995
G1-NA6-050	Immobilized GalNAcEXO, 10 x 0.5 mg	1,295	1,750

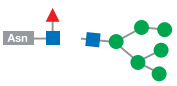
GlycINATOR®



GlycINATOR® (EndoS2) is an IgG-specific endoglycosidase that rapidly hydrolyzes all Fc glycans.

The GlycINATOR enzyme is an Fc-specific endoglycosidase that effectively hydrolyzes all IgG glycoforms, including complex, high-mannose, hybrid and bisected glycans. The enzyme acts on the chitobiose core and all Fc-glycans are digested after the inner GlcNAc. The reaction is fast and optimal at physiological conditions. The specificity for the Fc glycosylation site involves a protein-protein interaction, and for this reason, the native fold of the Fc is required. The Fc glycosylation site is conserved among many species and GlycINATOR deglycosylates antibodies from a range of species. The applications of GlycINATOR include rapid assessment of afucosylation levels by LC-MS (Liu, 2016, p. 42), reduction of sample complexity, inactivation of antibodies in immunoassays and site-specific conjugation using GlyCLICK®.

- Human IgG1-4, Fc-fusion proteins, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- 30 min reaction
- Requires native IgG fold
- All Fc glycoforms of IgG

Enzyme	GlycINATOR	IgGZERO	PNGase F	Endo H
Digestion site				
Works on native IgG	Yes	Yes	Yes/No	Yes/No
pH optimum	7.4	7.4	7.5	5-6
Reaction time	30 min	30 min	6-24 h	1-16 h
IgG specific	Yes*	Yes	No	No
Glycoform specificity	All Fc glycoforms	Complex Fc glyco-forms, limited activity on high-mannose and hybrid-type glycans	All glycoforms except core 1,3 alpha-fucose	High-mannose and some hybrid-type glycans

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

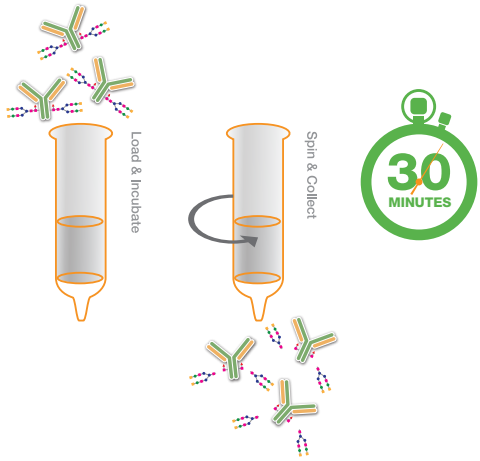
GlycINATOR®

The GlycINATOR enzyme consists of 2000 units for deglycosylation of 2 mg IgG. The enzyme is provided as a lyophilized powder.

Product ID	Description	Deglycosylation	EUR	USD
A0-GL1-020	GlycINATOR, 2000 units	2 mg IgG	480	660
A0-GL8-020	GlycINATOR LE (low endotoxin), 2000 units	2 mg IgG	520	690

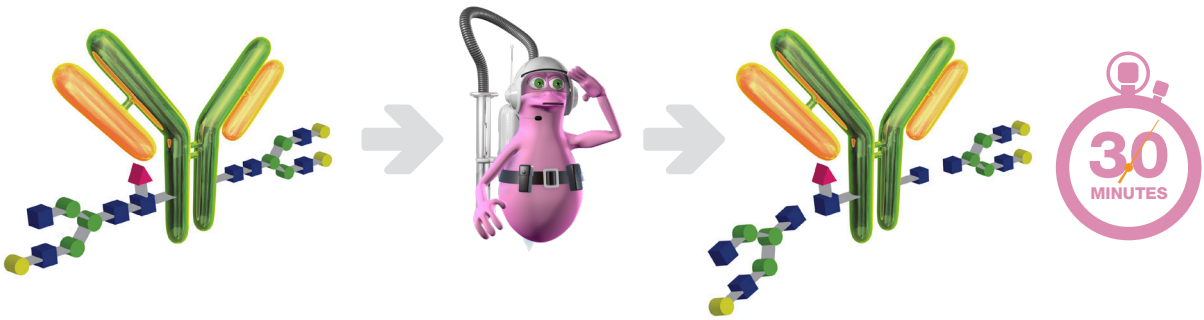
Immobilized GlycINATOR®

The GlycINATOR enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for deglycosylation of Fc glycans on 0.5 mg up to 100 mg of antibody or Fc-fusion protein.
Immobilized GlycINATOR deglycosylates the Fc domain with no enzyme in the final preparation.



Product ID	Description	Deglycosylation	EUR	USD
A0-GL6-010	Immobilized GlycINATOR Microspin	2 x 0.5 mg	350	490
A0-GL6-025	Immobilized GlycINATOR Microspin	5 x 0.5 mg	800	1,110
A0-GL6-050	Immobilized GlycINATOR Microspin	10 x 0.5 mg	1,330	1,850
A0-GL6-100	Immobilized GlycINATOR Midispin	1-10 mg	1,070	1,490
A0-GL6-1000	Immobilized GlycINATOR Maxispin	10-100 mg	3,200	4,470

IgGZERO®



IgGZERO® (EndoS) is an IgG-specific endoglycosidase that hydrolyzes Fc glycans.

The IgGZERO enzyme hydrolyzes N-glycans specifically at the Fc glycosylation site on IgG. In contrast to GlycINATOR, IgGZERO has limited activity on high-mannose and hybrid-type glycoforms. The hydrolysis of complex glycans is fast and carried out under native and physiological conditions, as the enzyme requires native IgG fold for activity. The IgGZERO enzyme is used to rapidly reduce sample complexity, in immunoassays to reduce antibody mediated effector functions, or as a tool to improve imaging by reducing Fc interactions (Gao, 2014, p. 50).

- Human IgG1-4, Fc-fusion proteins, IgG from mouse, rat, monkey, sheep, goat, cow and horse
- 30 min reaction
- Requires native IgG fold
- Complex Fc glycoforms, limited activity on high-mannose and hybrid-type glycans

IgGZERO and GlycINATOR Deglycosylation of Cetuximab Fc glycans

The glycoform specificity of IgGZERO and GlycINATOR was compared by monitoring the released glycans after incubation with cetuximab, a therapeutic antibody containing high-mannose glycans (Sjögren, 2015, p. 42). The data shows specific release of Fc-glycans, and GlycINATOR effectively hydrolyzed high-mannose glycans after a 30 min reaction time whereas IgGZERO did not (*Fig. 1*).

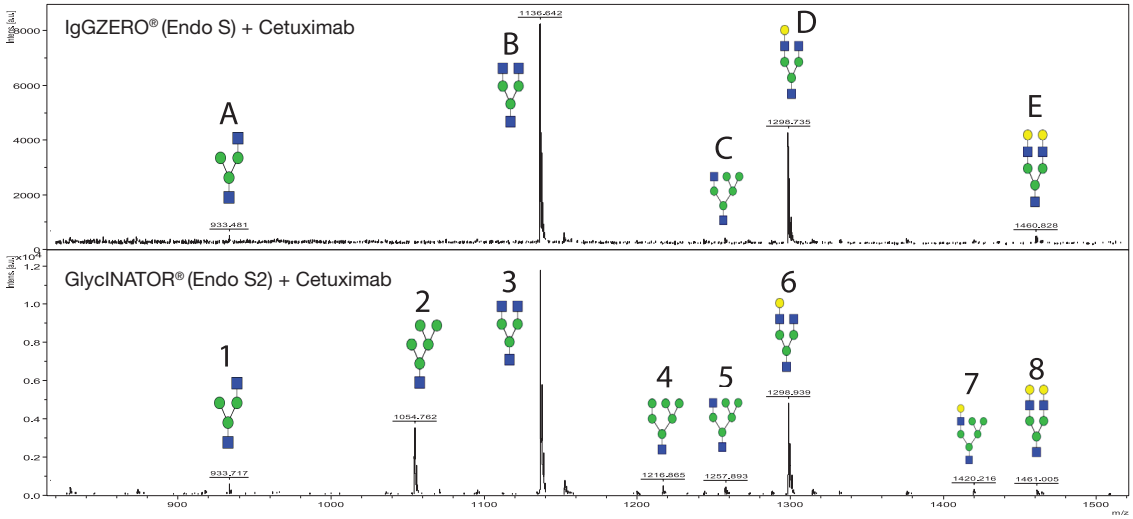


Figure 1. The N-glycans released by IgGZERO (A-E) and GlycINATOR (1-8) were analyzed by MALDI-TOF. High-mannose structures (2, 4 and 7) were readily hydrolyzed by GlycINATOR, but not by IgGZERO.

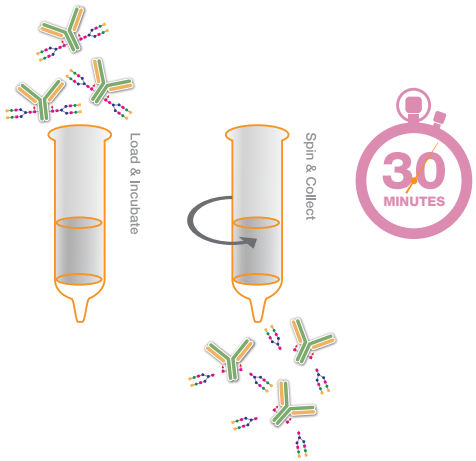
IgGZERO®

The IgGZERO enzyme consists of 2000 units for deglycosylation of 2 mg IgG. The enzyme is provided as a lyophilized powder.

Product ID	Description	Deglycosylation	EUR	USD
A0-IZ1-010	IgGZERO, 1000 units	1 mg IgG	250	340
A0-IZ1-050	IgGZERO, 5000 units	5 mg IgG	960	1,330
A0-IZ8-020	IgGZERO LE (low endotoxin), 2000 units	2 mg IgG	530	690

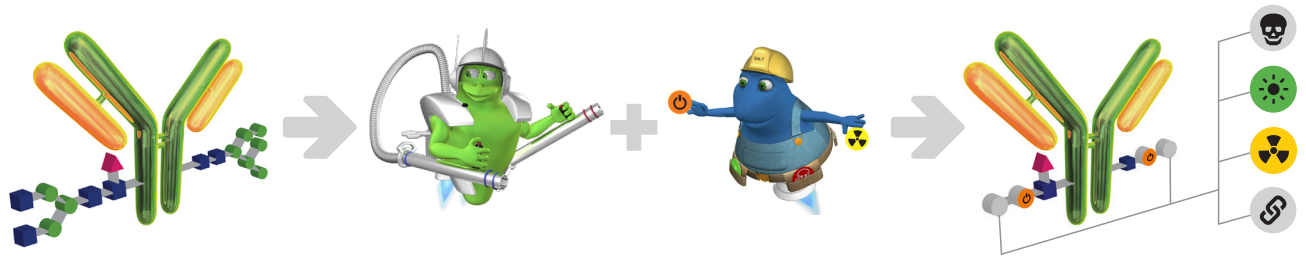
deGlycIT™

The IgGZERO enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for deglycosylation of Fc glycans on 0.5 mg up to 100 mg of antibody or Fc-fusion protein.
deGlycIT deglycosylates the Fc domain with no enzyme in the final preparation.



Product ID	Description	Deglycosylation	EUR	USD
A0-IZ6-010	deGlycIT Microspin	2 x 0.5 mg	350	490
A0-IZ6-025	deGlycIT Microspin	5 x 0.5 mg	800	1,110
A0-IZ6-050	deGlycIT Microspin	10 x 0.5 mg	1,330	1,850
A0-IZ6-100	deGlycIT Midispin	1-10 mg	1,070	1,490
A0-IZ6-1000	deGlycIT Maxispin	10-100 mg	3,200	4,470

GlyCLICK®



GlyCLICK® is a site-specific conjugation technology for IgG.

GlyCLICK is a three-step conjugation technology for site-specific and quantitative conjugation of IgG from several species and subclasses (*Fig. 1*). The technology utilizes Fc glycan remodeling by the GlycINATOR® endoglycosidase for complete deglycosylation of all Fc glycoforms followed by azide activation. Biocompatible click-chemistry (SPAAC) then enables complete and robust labeling of the antibody using a cyclooctyne-functionalized label of choice.
The unique combination of enzymes in GlyCLICK results in a degree of labeling (DOL) of 2 labels per IgG, generating stable and homogenous conjugates suitable for sensitive and versatile applications.
The GlyCLICK technology is available in various kit formats to facilitate tailored and site-specific labeling of antibodies.

- Human IgG1-4, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- Labels: Alexa Fluor®, biotin and DFO. Azide activation kits for custom conjugation
- GlycINATOR® hydrolyzes all Fc glycoforms

Available GlyCLICK Formats and sDIBO Labels

Kit format	Kit size	Name	Examples of Applications
Fluorophore	250 µg, 2 mg	AlexaFluor® 488	Immunofluorescence Microscopy, Flow cytometry
Fluorophore	250 µg, 2 mg	AlexaFluor® 555	Immunofluorescence Microscopy, Flow cytometry
Fluorophore	250 µg, 2 mg	AlexaFluor® 647	Immunofluorescence Microscopy, Flow cytometry
Affinity	250 µg, 2 mg	Biotin	Immuno assays, ELISA, WB
Chelator	250 µg, 2 mg	Deferoxamine	*In vivo* immuno imaging
Azide Activation	250 µg, 2 mg, 10 mg	No label	Custom conjugation

GlyCLICK Conjugation Overview

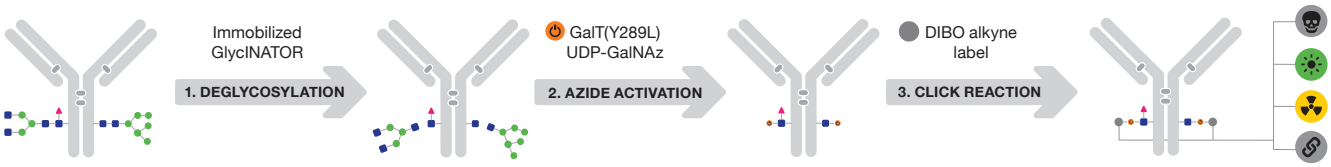


Figure 1. Schematic presentation of the GlyCLICK conjugation process.

1. **Deglycosylation**
   The IgG-specific endoglycosidase GlycINATOR (EndoS2) digests Fc glycans, exposing the core GlcNAc. The enzyme removes all glycoforms, including high-mannose, hybrid, complex, and bisecting type glycans.

2. **Azide activation**
   Azide-containing UPD-GalNAz is enzymatically attached to the exposed GlcNAc at the Fc glycan sites by the enzyme β-1,4-Galactosyltransferase, GalT(Y289L), making the antibody azide-activated for conjugation.

3. **CLICK reaction**
   Azide-activated antibodies are conjugated at the Fc glycan sites using a DBCO or sDIBO-label through Strain-Promoted Copper-free Click-Chemistry (SPAAC), resulting in a stable attachment of one label per Fc/2.

See Legal and Disclaimers, p. 43.

GlyCLICK Characteristics

Site-specific and Modular Technology

GlyCLICK enables quantitative and complete conjugation with a constant degree of labeling (DOL) of one label per Fc/2. The unique combination of enzymes allows Fc glycan-specific remodeling that restricts labeling to the Fc glycan sites without interference with the antigen binding domain of the antibody. GlyCLICK is a versatile and scalable tool for conjugation of several species and subclasses of IgG with a wide range of functionalized labels (*Fig. 2*). All conjugates will have the same reproducible mode of incorporation, independent on the choice of label or payload.

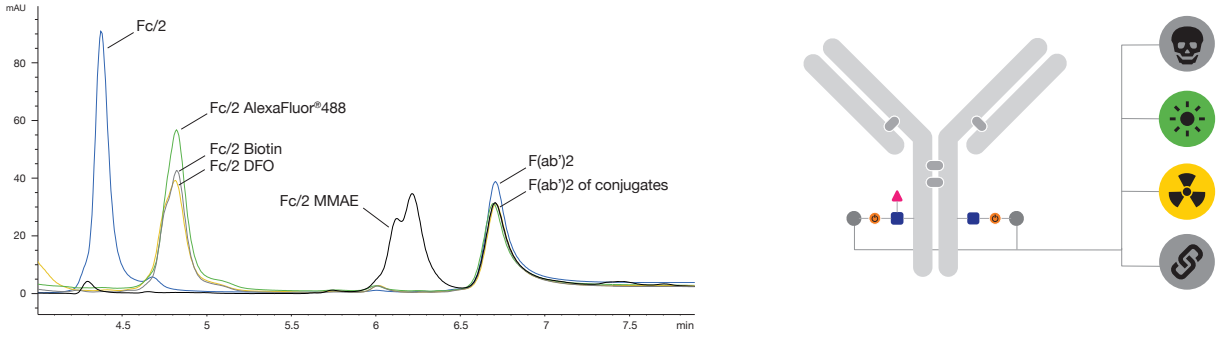


Figure 2. RP-HPLC analysis of panitumumab unmodified and conjugated with DFO, AlexaFluor488, Biotin or MMAE digested with FabRICATOR. RP-HPLC was performed on Agilent 1290 using Waters Acquity UPLC® BEH C4, 1.7 µm, 2.1 x 100 mm column.

Preserved Immunoreactivity

Site-specific conjugation at the Fc glycan sites ensures intact immunoreactivity by preserving the antigen-binding capability of the antibody. Surface Plasmon Resonance (SPR) was performed on trastuzumab with various GlyCLICK conjugates and compared to random conjugates. The results show overlapping curves between GlyCLICK-conjugates and native trastuzumab (*Fig. 3*). Although the affinity is unchanged for randomly conjugated material, the level of binding is severely impared.

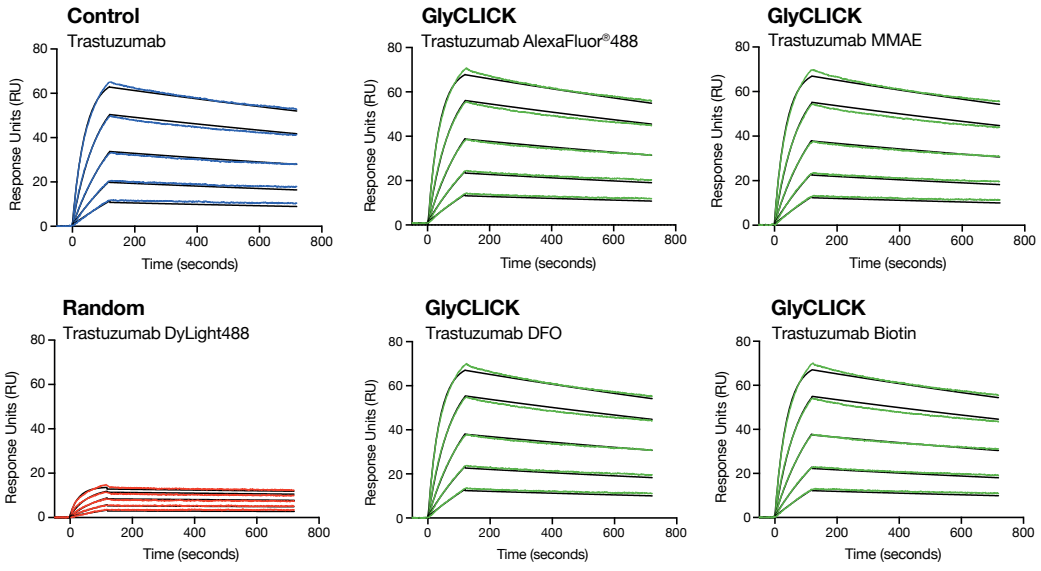


Figure 3. Affinity analysis of native and conjugated trasuzumab. Anti-human IgG (Fc) was used as the capturing molecule for trastuzumab, RandomDyLight™488 (DOL=10) conjugates and GlyCLICK conjugates: AlexaFluor488, Biotin, DFO and MMAE (DOL=2).HER2 was injected at a range ensuring sufficient curvature. All data was fitted against a 1:1 mathematical model.

GlyCLICK Applications

Immunofluorescent Microscopy

Site-spectific conjugation of primary antibodies using GlyCLICK ensures quantitative labeling for high-quality dynamic imaging of the antigen distribution in cells and tissue sections (*Fig. 4*). The constant DOL of 2 enables improved quantitation possibilities at optimal intensity levels for minimal saturation or loss of signal with less noise from background staining and cross-reactivity as compared to secondary detection. The modular GlyCLICK technology allows custom conjugation using a range of fluorescent labels for imaging applications.

"This technology benefits the fields of basic research and drug development and may form the basis for exciting diagnostic tools in clinical histopathology."

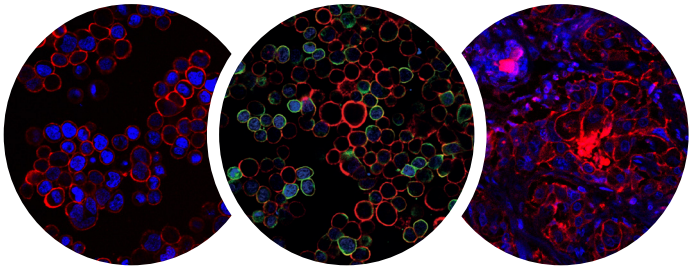


Figure 4. HER2(+) cells or tissue. **Left:** PFA-fixed cells incubated with DAPI (0.2 uM), GlyCLICK-AlexaFluor647 (1 ug/ml). **Middle:** PFA-fixed cells incubated with DAPI (0.2 uM), GlyCLICK-AlexaFluor647 (5 ug/ml), phallodinAF488. **Right:** paraffin embedded, BSA blocked tissue incubated with DAPI (0.2 uM), GlyCLICK-AlexaFluor647 (10 ug/ml).

Flow Cytometry

Directly labeled antibodies for primary detection offer advantages in flow cytometry applications but the right combination of antibody and label may not be available. The risk of unspecific binding and cross-reactivity using widely available secondary antibodies often require intricate experimental designs with cross-adsorbed material from various species. GlyCLICK enables robust and specific conjugation for increased flexibility in primary antibody selection for sensitive or multiplexed analyses. Primary detection was evaluated using GlyCLICK-conjugates and compared to an optimized secondary detection method using flow cytometry. The GlyCLICK-conjugates displayed higher separation index and reduced background compared to detection using secondary antibodies (*Fig. 5*).

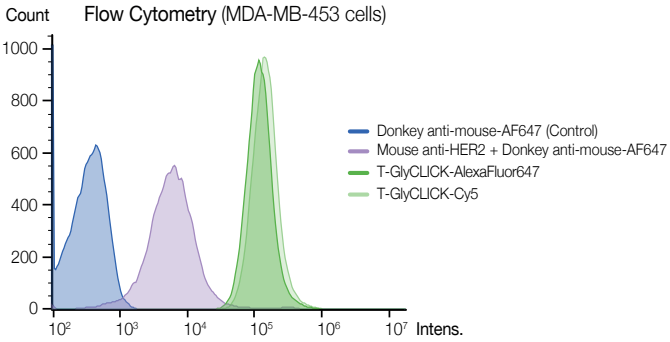


Figure 5. Flow cytometry of Fc-blocked HER2(+) cells incubated with trastuzumab-GlyCLICK-AlexaFluor647 or Cy5 (3.7 ug/ml) 30 min or mouse primary antibody (3.7 ug/ml) 30 min and secondary donkey anti-mouse (1:200) 30 min. Cells were resuspended (PBS 1%BSA) and analyzed with CytoFlex flow cytometer.

Immunoimaging *In Vivo*

Radioactively labeled antibodies are excellent tracers for immuno imaging *in vivo*. The *in vivo* effect of GlyCLICK conjugated trastuzumab-DFO labeled with ^{89}Zr was analyzed and compared to random labeling at lysines (DOL=0-6). Tumor-bearing mice injected with GlyCLICK-conjugates show a fivefold increase in tumor uptake as well as a significantly longer circulation time compared to randomly conjugated trastuzumab (*Fig. 6*).

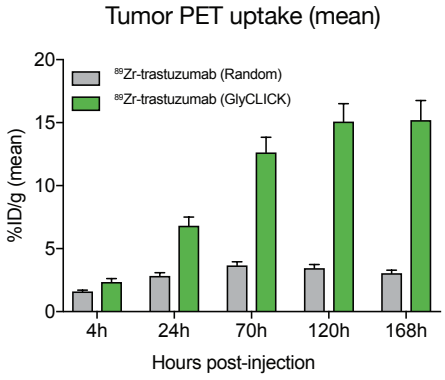


Figure 6. PET/CT imaging analysis of SK-OV-3 tumor-bearing mice showing mean tumor uptake over a time interval of 0-168 hours post-injection.

ADC Development

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 technology results in complete conjugation that incorporates two linker-payloads per antibody for a DAR of 2.0 (Fig. 7).

The GlyCLICK ADC kits generate homogenous ADCs with two-step cleavable linker-payloads for intracellular drug release. The GlyCLICK ADC kits produce conjugates that show cytotoxic effect in the pM range, indicating functional and highly potent ADCs (Fig 8).

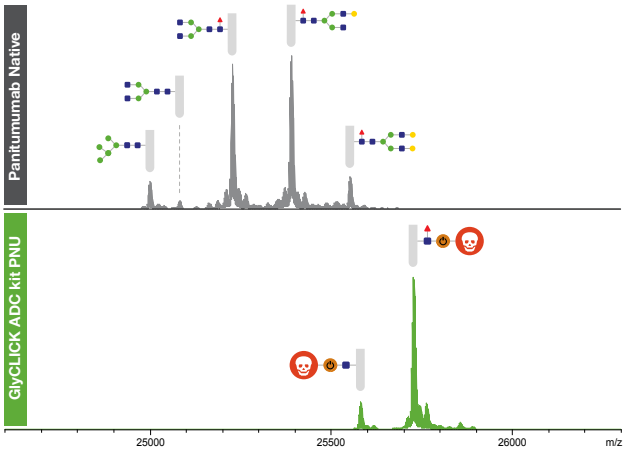


Figure 7. LC/MS analysis of panitumumab conjugated with the PNU linker-payload using GlyCLICK. The antibody was analyzed after FabRICATOR digestion determine the native Fc/2 glycan profile (top) and site-specific labeling after conjugation using GlyCLICK ADC kit PNU (bottom).

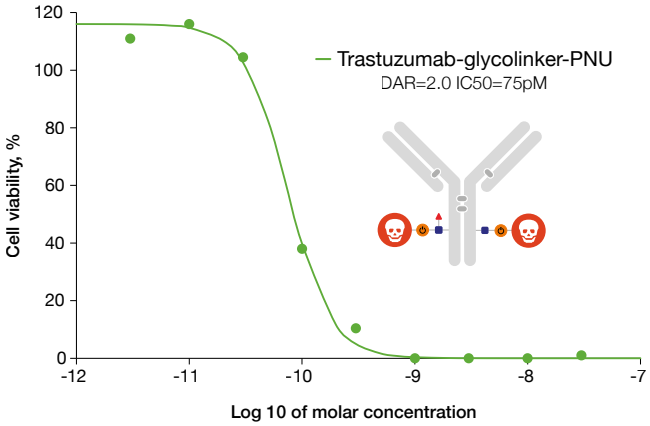


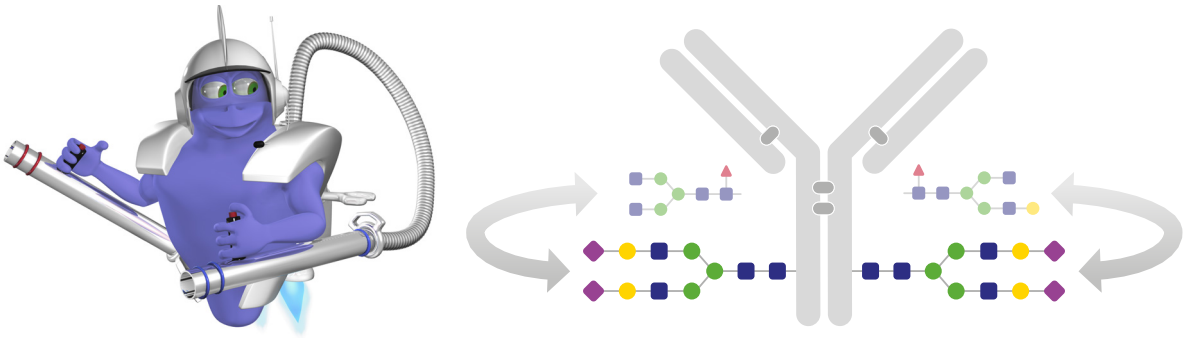
Figure 8. In vito cytotoxicity analysis of trastuzumab conjugated with the PNU linker-payload using GlyCLICK ADC kit PNU. Her2+ SK-BR-3 cells were incubated with the ADC and cell viability measured with PrestoBlue after 5-6 days. Curves represent the average result from three replicates.

GlyCLICK®

GlyCLICK contains all reagents and materials needed to azide activate or label the IgG.

Product ID	Description	Size	EUR	USD
L1-F01-025	GlyCLICK Alexa Fluor® 488	Conjugates 250 µg IgG	925	990
L1-F01-200	GlyCLICK Alexa Fluor® 488	Conjugates 1 x 2 mg IgG	1,570	1,620
L1-F02-025	GlyCLICK Alexa Fluor® 555	Conjugates 250 µg IgG	925	990
L1-F02-200	GlyCLICK Alexa Fluor® 555	Conjugates 1 x 2 mg IgG	1,710	1,780
L1-F03-025	GlyCLICK Alexa Fluor® 647	Conjugates 250 µg IgG	925	990
L1-F03-200	GlyCLICK Alexa Fluor® 647	Conjugates 1 x 2 mg IgG	1,710	1,780
L1-C01-025	GlyCLICK DFO	Conjugates 250 µg IgG	925	990
L1-C01-200	GlyCLICK DFO	Conjugates 1 x 2 mg IgG	1,570	1,620
L1-A01-025	GlyCLICK Biotin	Conjugates 250 µg IgG	925	990
L1-A01-200	GlyCLICK Biotin	Conjugates 1 x 2 mg IgG	1,570	1,620
L1-T02-200	GlyCLICK ADC kit MMAE	Conjugates 1 x 2 mg IgG	1,750	1,990
L1-T01-200	GlyCLICK ADC kit PNU	Conjugates 1 x 2 mg IgG	1,990	2,350
L1-AZ1-025	GlyCLICK Azide Activation	Activates 250 µg IgG	825	880
L1-AZ1-200	GlyCLICK Azide Activation	Activates 1 x 2 mg IgG	1,310	1,420
L1-AZ1-100	GlyCLICK Azide Activation	Activates 1 x 10 mg IgG	5,250	6,450

TransGLYCIT™

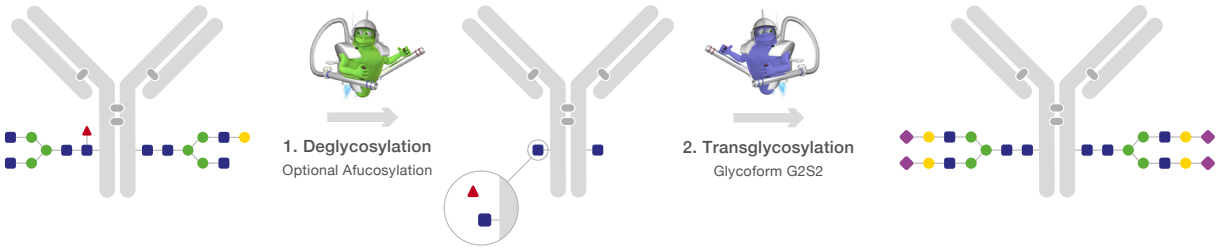


Efficient transglycosylation of native IgG using enzymatic Fc glycan remodeling

TransGLYCIT enables specific IgG glycan remodeling and prepares antibodies with defined and homogenous glycoforms. The technology platform is based on enzymatic transglycosylation using the GlycINATOR® (EndoS2) and TransINATOR™ (mutated EndoS2) enzymes for rapid and site-specific remodeling of IgG Fc-glycans. Using TransGLYCIT, IgG with defined glycoforms can be obtained within three hours and with the optional FucosEXO™ 16 enzyme, it is possible to generate afucosylated antibodies for direct comparison of antibodies with or without the core fucose. The enzymes in TransGLYCIT are expressed in E. coli with His-tags and the technology is provided in kits for processing of up to 1 mg of native IgG. TransGLYCIT is a valuable tool with applications in preparative and analytical workflows to study the impact of glycan profiles on structural and functional properties of therapeutic antibodies.

- Human IgG1, IgG2 and IgG4
- 3 hour workflow
- Glycoform G2S2 included
- Optional defucosylation with FucosEXO 16

The Transglycosylation Workflow



1. **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. With the option to include Immobilized FucosEXO 16, the α1-6 linked core fucose is also hydrolyzed to obtain afucosylated glycoforms.
2. **Transglycosylation:** The engineered glycosynthase TransINATOR catalyzes the transglycosylation reaction between the oxazoline reactive G2S2 glycoform and the exposed core GlcNAc.

TransGLYCIT™

Product ID	Description	EUR	USD
T1-S2F-010	TransGLYCIT G2S2 1 mg	995	1,195
T1-S2A-010	TransGLYCIT G2S2 Afucosylated 1 mg	1,350	1,650

Transglycosylation of Native IgG

Glycoengineering of the IgG glycan profile is important for the development of next-generation therapeutic antibodies with enhanced or silenced Fc effector functions. The generation of homogenous preparations is however challenging and often requires a complex bioprocess development or time-consuming cell-engineering strategies.

Using the *TransGLYCIT G2S2 kit*, the glycan profile of a therapeutic antibody was remodeled within three hours and analyzed at subunit level using LC-MS. The mass spectra show the heterogenous glycan profile of native trastuzumab (top) and the enzymatic glycan remodeling of the TransGLYCIT workflow. The mass shifts show complete deglycosylation to the core GlcNAc (middle) and transglycosylation of the antibody to generate homogenous G2S2 and G2S2F glycoforms using TransGLYCIT (bottom).

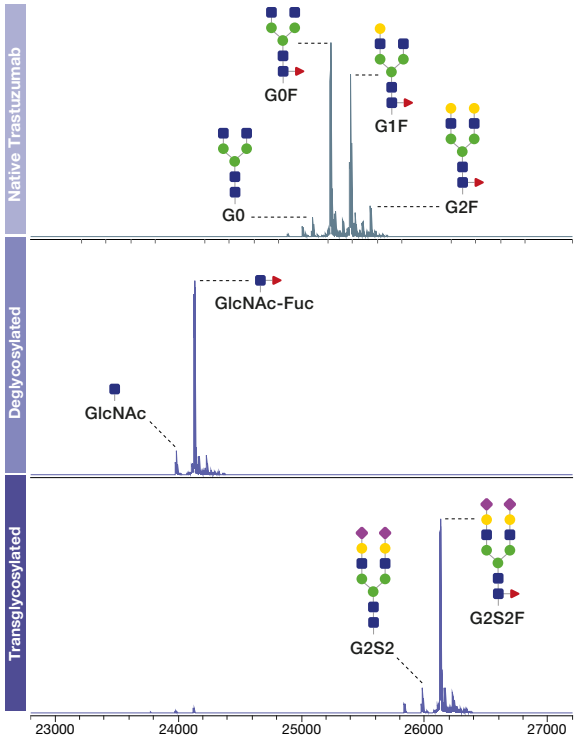


Fig. 1. Deconvoluted mass spectra of the Fc/2 fragment of native (top), deglycosylated (middle), and transglycosylated (bottom) trastuzumab display the N-glycan profile throughout the TransGLYCIT workflow. The mAb was digested with FabRICATOR and the subunits analysed with LC-MS using reversed phase chromatography (Agilent 1290; Waters BioResolve RP mAb 2.1 x 50 mm) and ESI-Q-TOF mass spectrometry (Bruker Impact II).

Afucosylated IgG Glycans using TransGLYCIT

Structural studies have shown that the antibody glycan profile impacts the binding to Fcγ receptors and immunological responses including antibody dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Afucosylated antibodies lacking the core fucose show increased binding to activating Fcγ IIIa receptors and thus an elevated ADCC response, providing a potential higher clinical efficacy for indications where this activity is required.

To generate an antibody with afucosylated glycans, the *TransGLYCIT G2S2 Afucosylated kit* was used to remodel the glycan profile of trastuzumab and analyzed by LC-MS at subunit level. The resulting mass spectra show the heterogenous glycan profile of native trastuzumab (top), initially carrying both afucosylated and fucosylated Fc glycans. The mass shifts indicate a complete deglycosylation and afucosylation to the core GlcNAc (middle). A homogenous and afucosylated G2S2 glycoform was generated using TransGLYCIT (bottom).

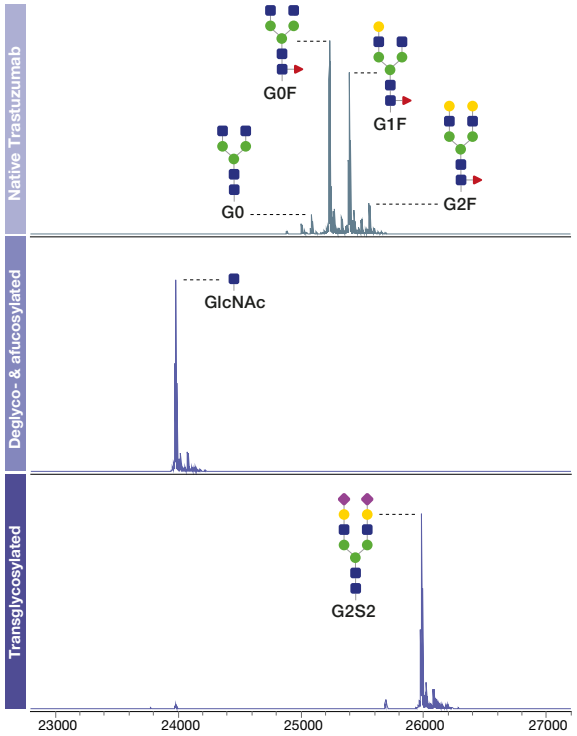


Fig. 2. Deconvoluted mass spectra of the Fc/2 fragment of native (top), deglycosylated and defucosylated (middle), and transglycosylated (bottom) trastuzumab display the N-glycan profile throughout the TransGLYCIT workflow. The mAb was digested with FabRICATOR and the subunits analysed with LC-MS using reversed phase chromatography (Agilent 1290; Waters BioResolve RP mAb 2.1 x 50 mm) and ESI-Q-TOF mass spectrometry (Bruker Impact II).

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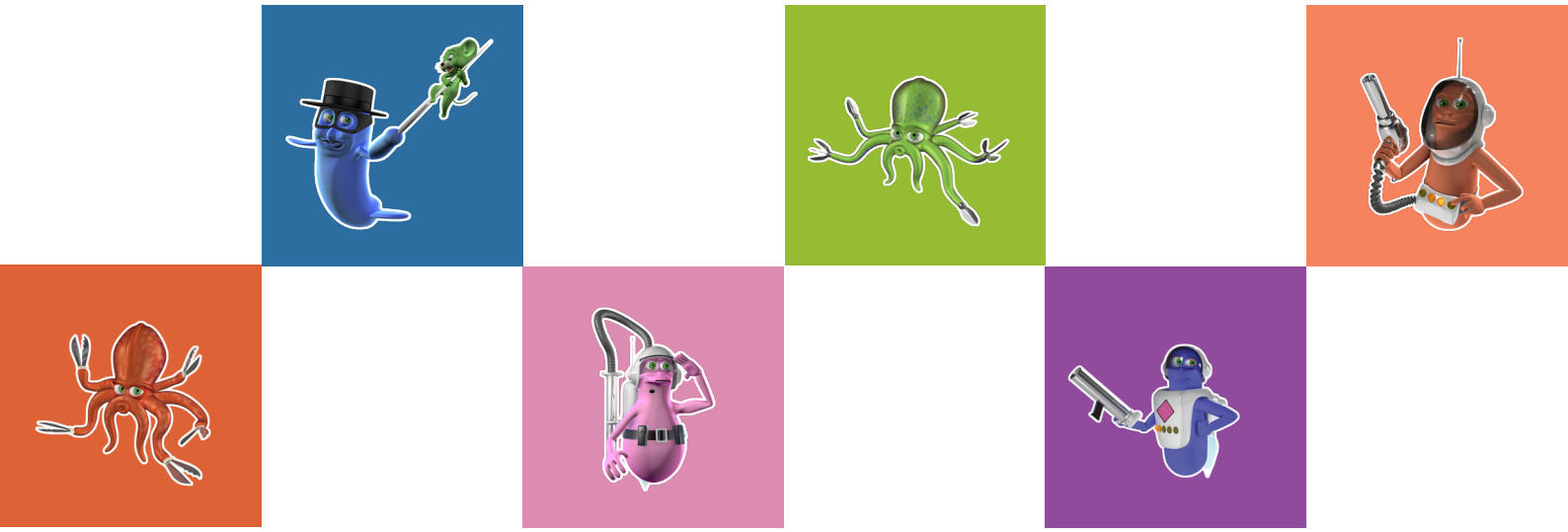

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