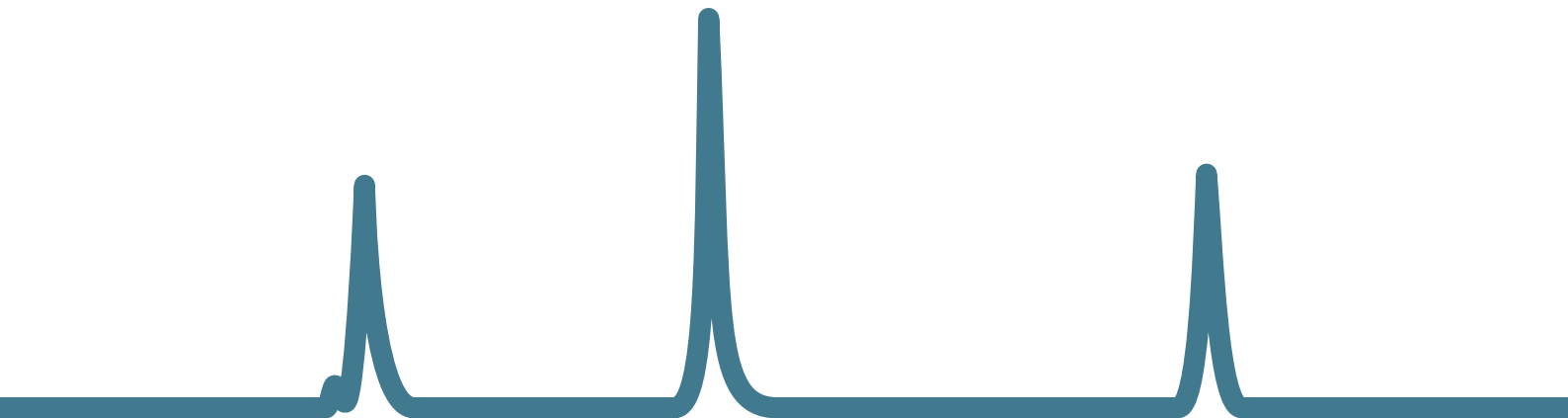
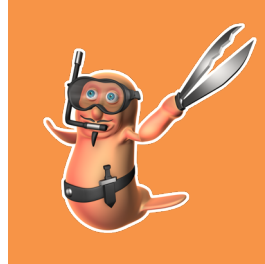
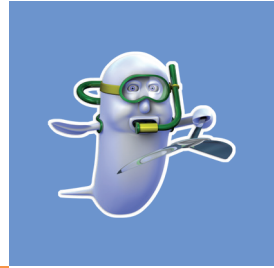
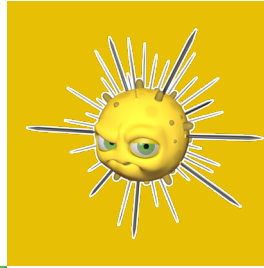
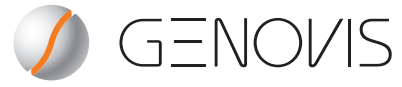


# SmartEnzymes™



**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™**

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**GENOVIS**

**FabRICATOR®**

FabRICATOR®-HPLC / Validation Kit / Anti-FabRICATOR® / FragIT™ / FragIT™ kit

8

A cysteine protease that digests IgG and Fc-fusion proteins at a specific site below the hinge

**FabRICATOR®Z**

FragIT™Z / FragIT™Z kit

16

A cysteine protease that digests mouse IgG2a and IgG3 at a specific site below the hinge

**FabALACTICA®**

Immobilized FabALACTICA® / FabALACTICA® Fab kit

18

A cysteine protease that digests human IgG1 at a specific site above the hinge

**GingisKHAN®**

GingisKHAN® Fab kit

22

A cysteine protease that digests human IgG1 above the hinge

**FabULOUS®**

FabULOUS® Fab kit

24

A cysteine protease that digests IgG in the hinge region

**GingisREX®**

26

An arginine-specific protease that digests proteins C-terminally of arginine residues

**OpeRATOR®**

28

An O-glycan-specific protease that digests mucin-type O-glycosylated proteins N-terminally of the O-glycosylation site

**GlycOCATCH®**

30

An enrichment resin for affinity purification of mucin-type O-glycosylated proteins and peptides

**OglyZOR®**

31

An O-glycosidase that hydrolyzes core 1 type O-glycans on native glycoproteins

**SialEXO®**

SialEXO®23 / Immobilized SialEXO®

32

Sialidases for removal of sialic acids from native glycoproteins

**GalactEXO™**

36

Sialidases for removal of sialic acids from native glycoproteins

**GlycINATOR®**

Immobilized GlycINATOR®

38

An endoglycosidase that rapidly hydrolyzes the N-glycan structures of the Fc domain of IgG

**IgGZERO®**

deGlycIT™

40

An endoglycosidase that hydrolyzes biantennary Fc glycans of IgG

**GlyCLICK®**

42

A site-specific conjugation technology for IgG

IgG PROTEASES

PROTEASES

O-GLYCANS

EXOGLYCOSIDASES

IgG GLYCOSIDASES

CONJUGATION

**FabRICATOR®**

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

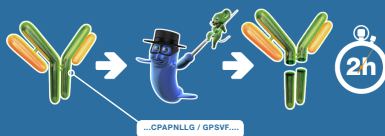
- ▶ Arginine-specific protease
- ▶ Digests proteins and peptides C-terminally of arginine residues
- ▶ 60 min reaction
- ▶ Active in 6 M urea and 0.1% SDS

**FragIT™**

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

**FabRICATOR® Z**

- ▶ Specific - one precise digestion site below the hinge of mouse IgG2a and IgG3
- ▶ Generates F(ab')<sub>2</sub> 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)

**FragIT™ Z**

- ▶ Immobilized FabRICATOR Z enzyme on agarose beads
- ▶ Generates F(ab')<sub>2</sub> 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')<sub>2</sub> 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

## GlycINATOR®



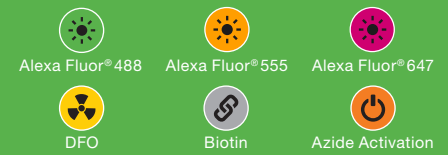
- ▶ Hydrolyzes the N-glycan structure of the IgG Fc domain
- ▶ 30 min reaction
- ▶ Requires native IgG fold
- ▶ Hydrolyzes all Fc glycoforms of IgG

## 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

## GlyCLICK®

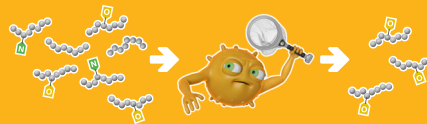


- ▶ Site-specific conjugation of IgG
- ▶ Active on human IgG1-4 and several other species
- ▶ The antibody retains antigen binding
- ▶ Quantitative labeling of 2 labels per antibody

## 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

## 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

## SialEXO®

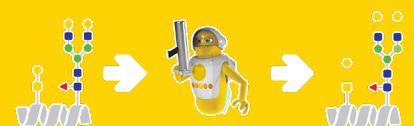


- ▶ SialEXO hydrolyzes all sialic acids and SialEXO 23 specifically hydrolyzes α2-3-linked sialic acids
- ▶ Available immobilized on agarose beads
- ▶ Active on both N- and O-linked glycans
- ▶ 1 - 2 h reaction
- ▶ Active on native glycoproteins

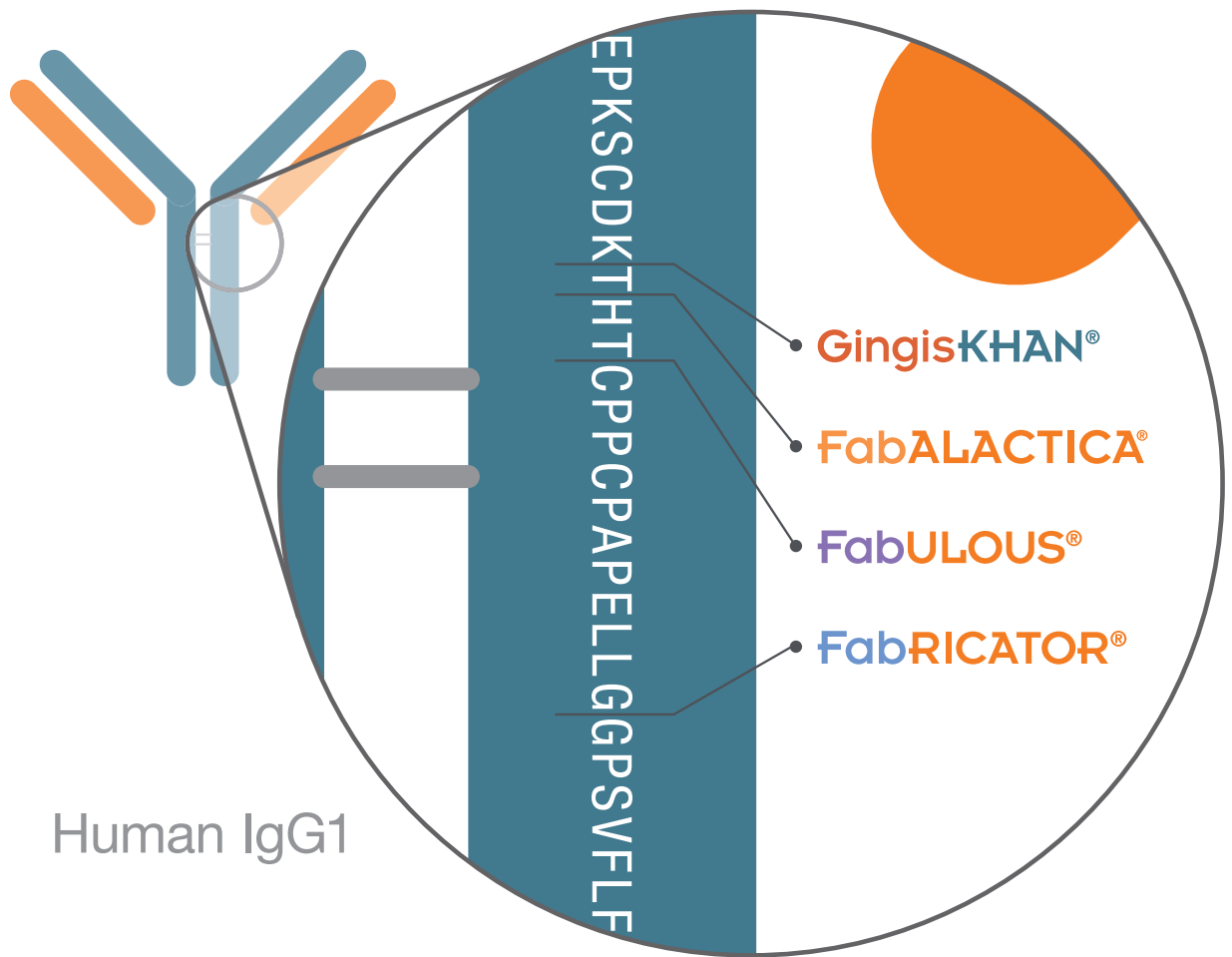
## deGlycIT™

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

## GalactEXO™



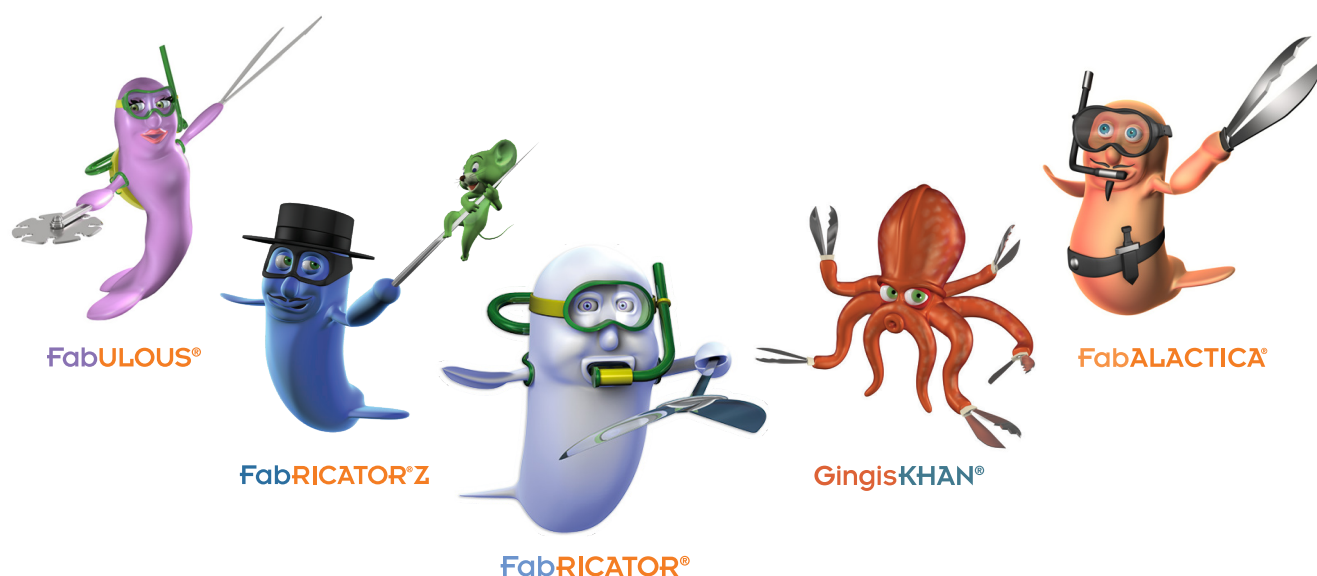
- ▶ Hydrolyzes galactose residues on N- and O-glycosylated proteins
- ▶ Efficient removal of β1-3,4 linked galactose
- ▶ Active on both N- and O-linked glycans
- ▶ 2h reaction
- ▶ For native glycoproteins and free glycans



# 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')<sub>2</sub> 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.

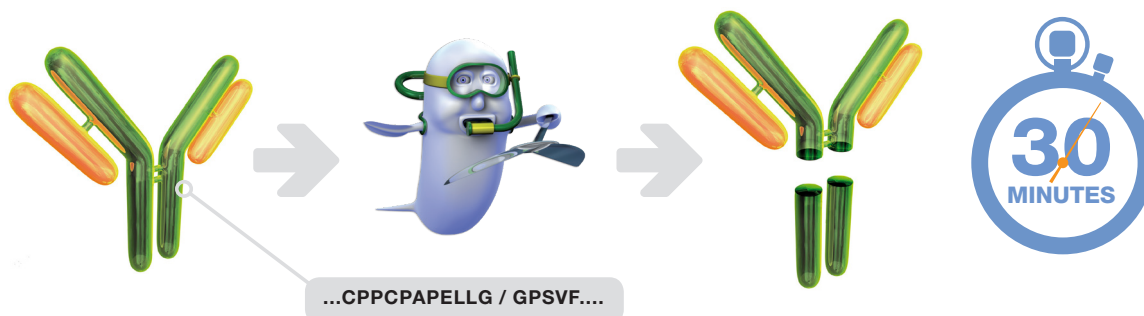
The IgG proteases from Genovis are a group of proteolytic enzymes that digest antibodies from several species and subclasses into subunits. The enzymes FabRICATOR® (IdeS) and FabALACTICA® (IgdE) are specific proteases, digesting IgG at a single site below or above the hinge, respectively. Other proteases 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
<b>IgG species and subclasses</b>	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
<b>Digestion site (human IgG1)</b>	LLG / GPS	DKT / HTC	CDK / THT	THT / CPP	LLG / GPS
<b>Above / below hinge (human IgG1)</b>	Below	Above	Above	Above	Below
<b>Reaction requirements</b>	Physiological buffers	Physiological buffers	2 mM cysteine	Reducing conditions	Physiological buffers
<b>Reaction time</b>	30 min	O/N	1 h	1 h	2 h
<b>pH</b>	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.

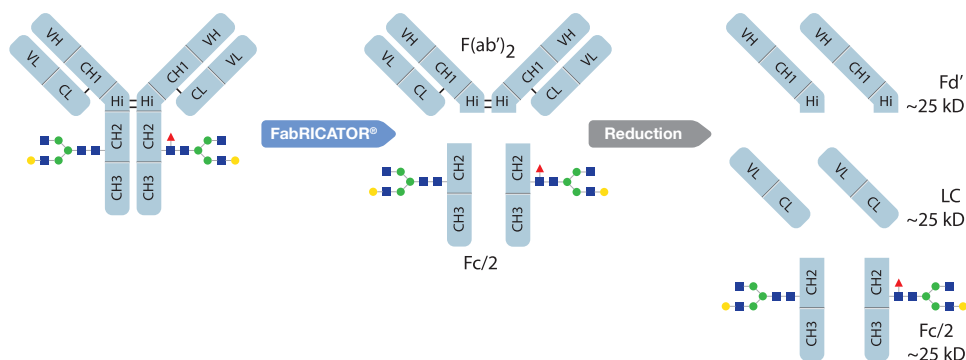
FabRICATOR is an IgG-specific cysteine protease that digests antibodies at a single amino acid site below the hinge region, generating a homogenous pool of F(ab')<sub>2</sub> and Fc/2 fragments within 30 minutes. Neutral pH and no requirements for co-factors make the enzyme easy to use and enable platform 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. 42.

- 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
- CPPAPELLG / 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')<sub>2</sub> 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.

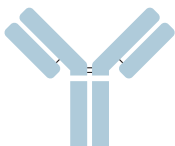
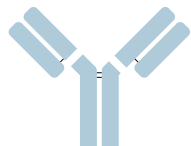
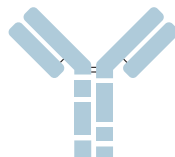


**Figure 1.** FabRICATOR digestion of IgG results in F(ab')<sub>2</sub> and Fc/2 fragments that can be further reduced to antibody subunits.

\* 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).

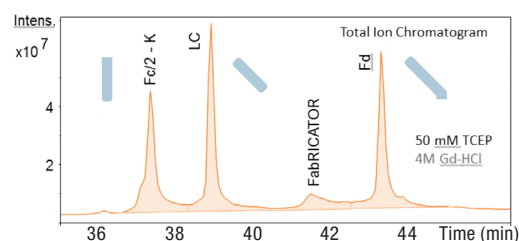


Figure 2. LC-MS on adalimumab

## 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.

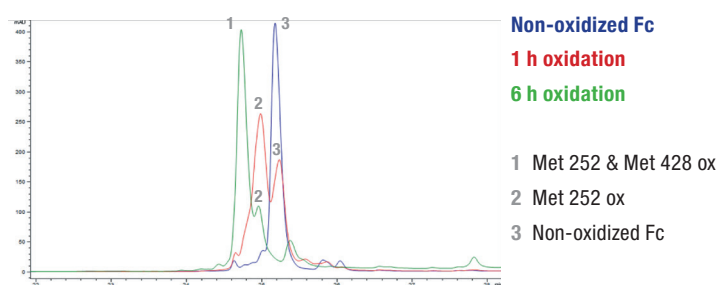


Figure 3. Reversed-phase chromatogram of bevacizumab exposed to oxidizing reaction conditions (0, 1 or 6 hours) before FabRICATOR digestion.

## 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).

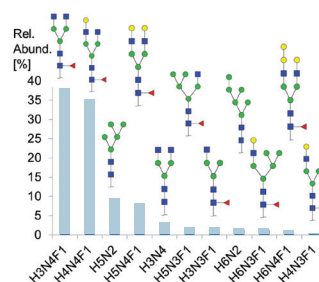


Figure 4. Relative abundance of glycans on the Fc domain of cetuximab. 11 glycans were quantified and no additional digestion or labeling were needed for similarity assessment.

# 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	450	625
A0-FR1-050	FabRICATOR, 5000 units	5 mg IgG	875	935
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	2,255
A0-FR1-008	FabRICATOR, 8x100 units	8 x 100 µg IgG	305	390
A0-FR8-020	FabRICATOR LE (low endotoxin), 2000 units	2 mg IgG	500	680
A0-FR8-050	FabRICATOR LE (low endotoxin), 5000 units	5 mg IgG	965	1,020

## 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	1,355	1,880

## Anti-FabRICATOR™

Anti-FabRICATOR is a goat polyclonal antibody purified on protein G that is used for detection of FabRICATOR with western blot or ELISA.

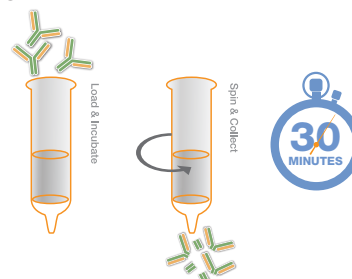


Product ID	Description	Concentration	EUR	USD
A3-AF1-010	Anti-FabRICATOR 0.1 ml	4 mg/ml	260	365

# FragIT™

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

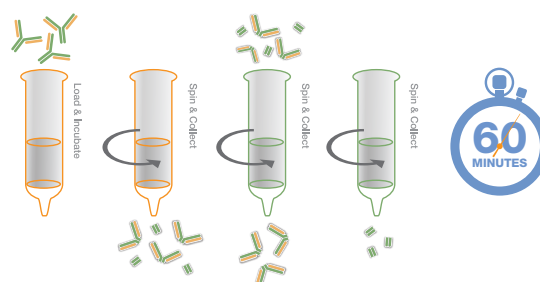


Product ID	Description	Digestion	EUR	USD
A0-FR6-010	FragIT Microspin	2 x 0.5 mg IgG	335	465
A0-FR6-025	FragIT Microspin	5 x 0.5 mg IgG	765	1,060
A0-FR6-050	FragIT Microspin	10 x 0.5 mg IgG	1,270	1,765
A0-FR6-100	FragIT Midispin	1-10 mg IgG	1,020	1,415
A0-FR6-1000	FragIT Maxispin	10-100 mg IgG	3,045	4,255

# FragIT™ kit

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

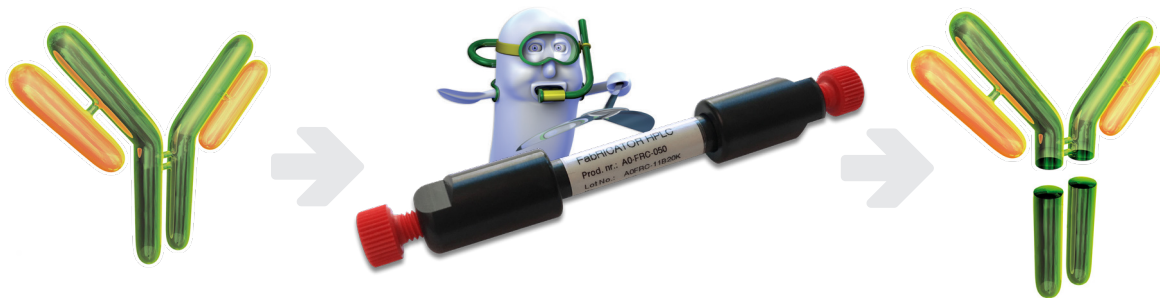
FragIT kit consists of spin columns of FragIT for antibody digestion and spin columns of CaptureSelect™\* Fc resin for affinity binding of the Fc fragments. After digestion, the Fc fragments are captured in the affinity spin column and pure F(ab')<sub>2</sub> fragments are obtained in the flowthrough.



Product ID	Description	Digestion	EUR	USD
A2-FR2-005	FragIT kit, Microspin	0.5 mg IgG	380	520
A2-FR2-025	FragIT kit, Microspin	5 x 0.5 mg IgG	1,020	1,415
A2-FR2-050	FragIT kit, Microspin	10 x 0.5 mg IgG	1,940	2,385
A2-FR2-100	FragIT kit, Midispin	10 mg IgG	1,275	1,775
A2-FR2-1000	FragIT kit, Maxispin	100 mg IgG	3,820	5,340

\*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.

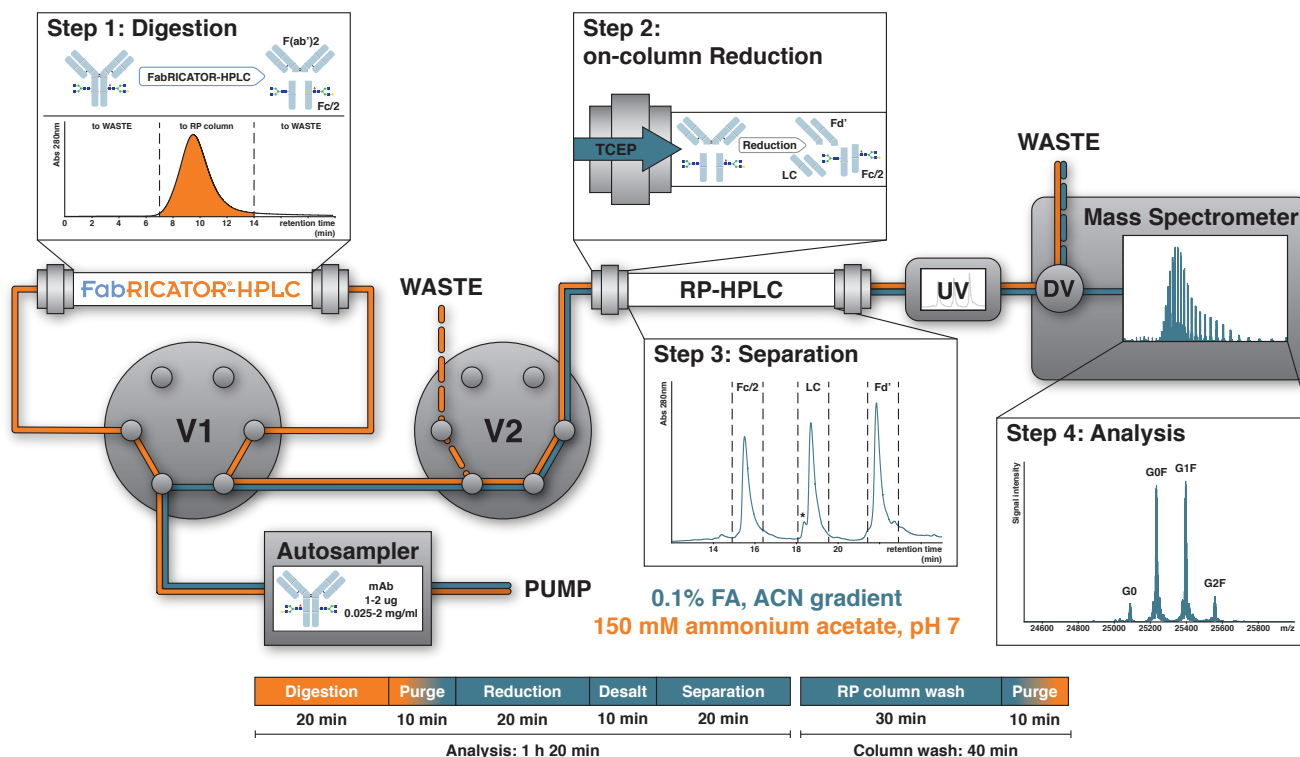
# FabRICATOR®-HPLC



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')<sub>2</sub> 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.



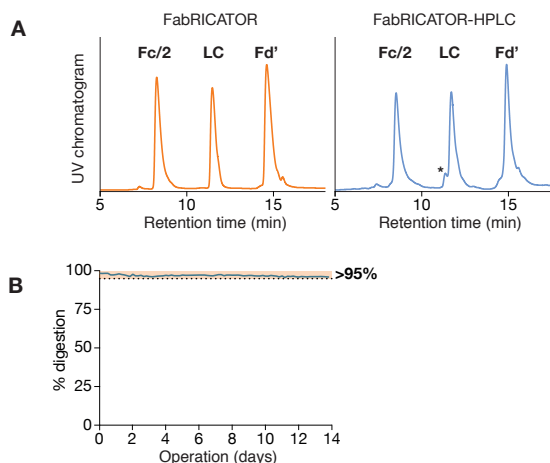
**Figure 1.** Potential set-up for an automated middle-level workflow using FabRICATOR-HPLC. FabRICATOR digests IgGs to F(ab')<sub>2</sub> and Fc, which is well suited for high resolution MS analysis.

## Robust On-column Performance

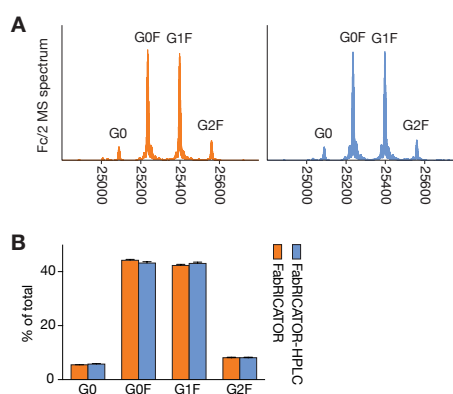
Digestion efficiency is maintained by FabRICATOR-HPLC during continuous operation at 37°C for up to 14 days. In our tests, 2 µg trastuzumab samples were injected every 4h under native conditions in 150 mM ammonium acetate, pH 7 at a flow rate of 25 µl/min. The resulting antibody fragments were reduced 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).

## 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 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.



**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

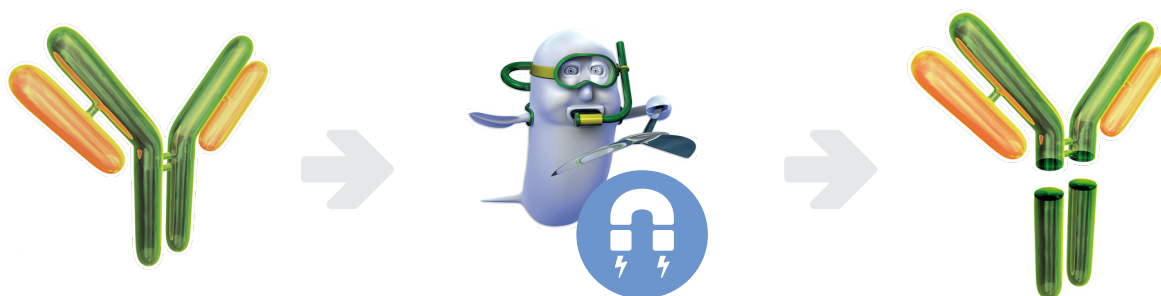
# FabRICATOR®-HPLC

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	1,675	1,885

# FabRICATOR<sup>®</sup> MagIC



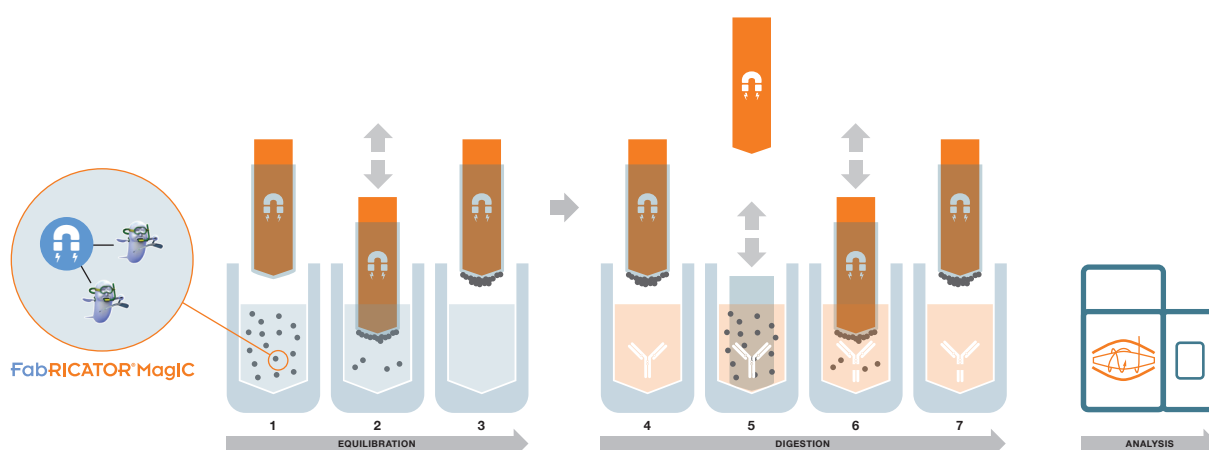
FabRICATOR<sup>®</sup> MagIC enables parallel subunit generation of antibodies for automated middle-level workflows

FabRICATOR MagIC contains FabRICATOR (IdeS) enzyme immobilized on magnetic agarose beads for digestion of antibodies into of F(ab')<sub>2</sub> 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 routine 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

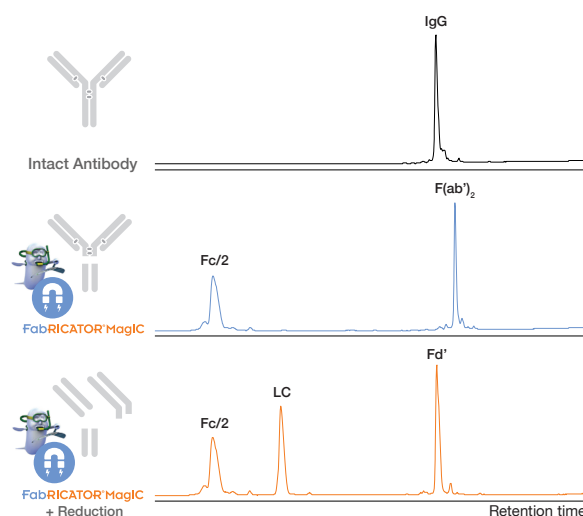


**Figure 1.** FabRICATOR MagIC workflow on Thermo Scientific KingFisher™ Purification System. IgG, FabRICATOR MagIC and PBS are dispensed into a 96-deepwell plate according to instructions for the KingFisher workflow. FabRICATOR MagIC beads are transferred (1) to wells containing PBS for equilibration (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')<sub>2</sub> and Fc are left in the sample wells (7). The trademark KingFisher is the property of Thermo Fisher Scientific.

## Automated Middle-Level Workflow

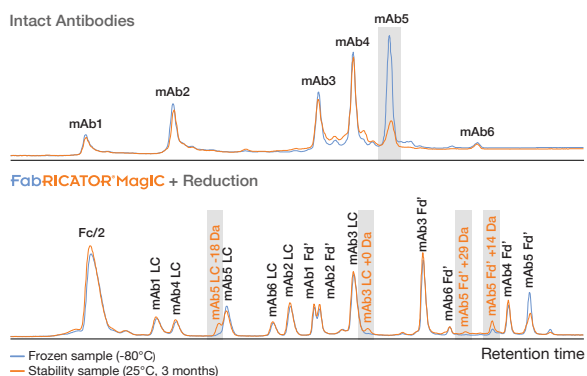
FabRICATOR MagIC generates intact F(ab')<sub>2</sub> and Fc/2 subunits and the disulfide bonds can easily be reduced using 5 mM TCEP in a one-pot reaction. The generated Fc/2, LC and Fd' fragments allow detailed analysis and monitoring of different antibodies using middle-level workflows resulting in 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).



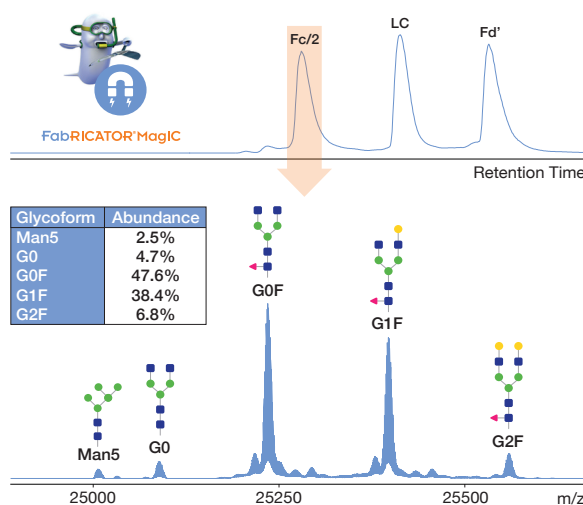
## Fast Automated Monitoring of CQAs

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 degradation 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).

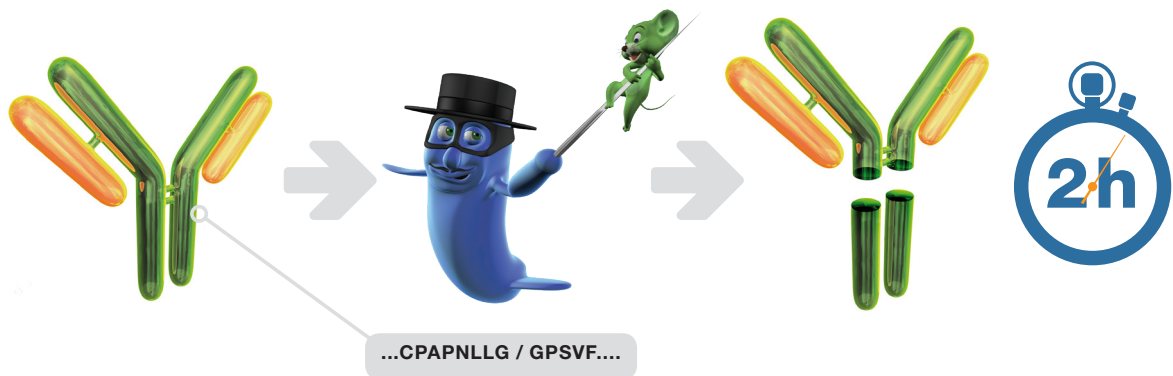


**Figure 4.** Analysis of Fc glycosylation by middle-level LC-MS using FabRICATOR MagIC (top) and quantification of glycoforms from a deconvoluted mass spectrum of the Fc/2 fragment (bottom).

# FabRICATOR® MagIC

	Product ID	Description	EUR	USD
	A0-FRM-024	FabRICATOR MagIC 2 mL 24 samples	550	595
	A0-FRM-096	FabRICATOR MagIC 4 x 2 mL 96 samples	1,875	1,990

# FabRICATOR<sup>®</sup>Z



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

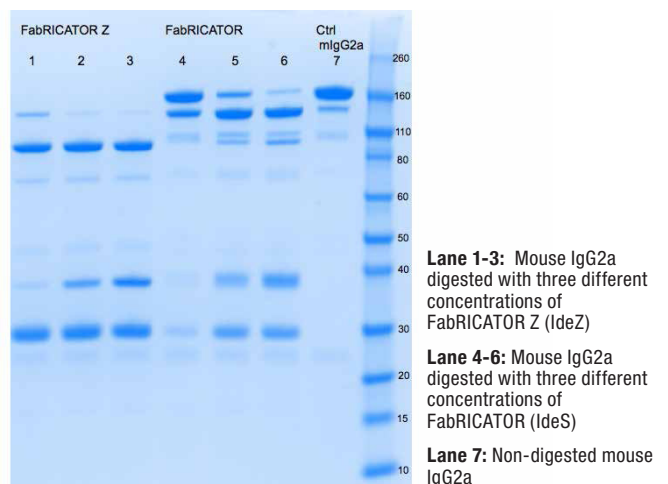
FabRICATOR Z digests mouse IgG2a and IgG3 and generates a homogenous pool of F(ab')<sub>2</sub> and Fc/2 fragments. Some mouse IgG2a that FabRICATOR fails to digest, are readily digested by FabRICATOR Z, but longer incubation times may be required. There is no risk of overdigestion because of the high specificity of the enzyme.

## 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.

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

- 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)



# FabRICATOR<sup>®</sup>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.



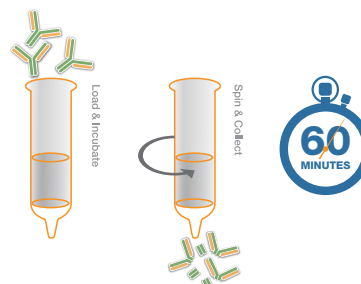
Product ID	Description	Digestion	EUR	USD
A0-FRZ-020	FabRICATOR Z, 2000 units	2 mg IgG	450	625



# FragIT™ Z

FragIT™ Z (immobilized FabRICATOR® Z) digests mouse IgG2a and IgG3 and generates F(ab')<sub>2</sub> 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')<sub>2</sub> and Fc/2 fragments with no enzyme in the final preparation.

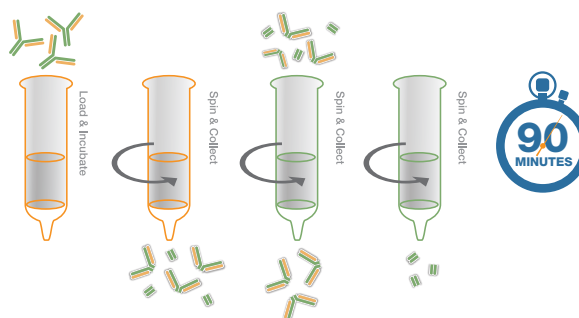


Product ID	Description	Digestion	EUR	USD
A0-FZ6-010	FragIT Z Mircospin	2 x 0.5 mg IgG	335	465
A0-FZ6-025	FragIT Z Mircospin	5 x 0.5 mg IgG	765	1,060
A0-FZ6-050	FragIT Z Mircospin	10 x 0.5 mg IgG	1,270	1,765

# FragIT™ Z kit

FragIT™ Z kit easily generates and purifies F(ab')<sub>2</sub> 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')<sub>2</sub> fragments are obtained in the flowthrough.



Product ID	Description	Digestion	EUR	USD
A2-FZ2-005	FragIT Z kit	0.5 mg IgG	380	520
A2-FZ2-025	FragIT Z kit	5 x 0.5 mg IgG	1,020	1,415

\*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. 42), and for subunit workflows on antibodies with mutated hinge regions.

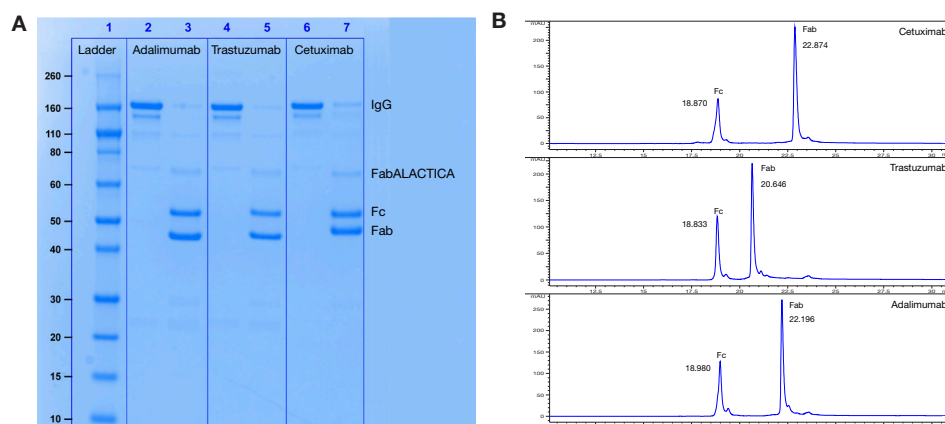
- Human IgG1
- Overnight (O/N) reaction
- No need for reducing agents or co-factors
- KSCDKT / HTCPPCP (above the hinge)

Enzyme	FabALACTICA	GingisKHAN	Papain	Lys-C
<b>Digestion site</b>				
<b>Specificity</b>	IgG specific/ One digestion site	One digestion site	Unspecific/ Several digestion sites	Unspecific/ Several digestion sites
<b>Selectivity</b>	Human IgG1	Human IgG1	Several proteins	Several proteins
<b>Reducing conditions</b>	No	Yes, 2mM cysteine	Yes	No
<b>Reaction time</b>	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.

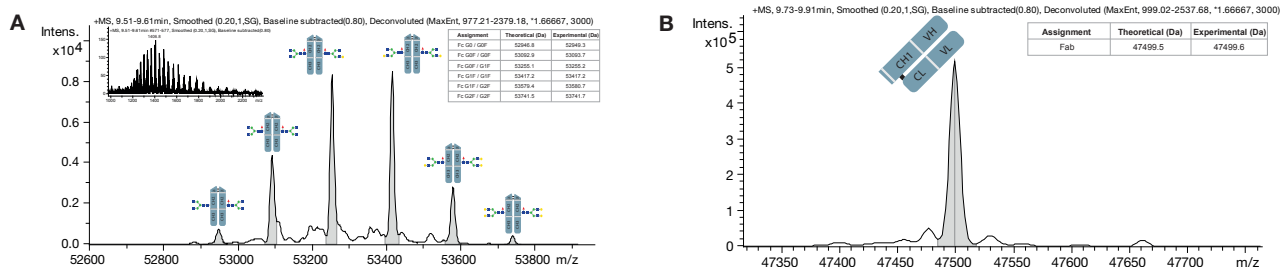


**Figure 1.** Digestion of cetuximab, trastuzumab, and adalimumab using FabALACTICA O/N at 37°C. **a)** Non-reduced SDS-PAGE. **b)** Separation of intact Fc and Fab fragments on RP-HPLC.

## 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.

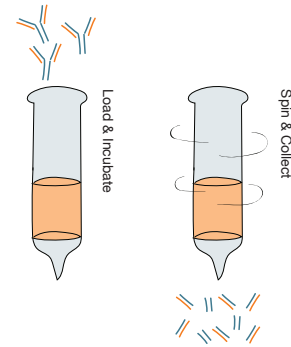


Product ID	Description	Digestion	EUR	USD
A0-AG1-020	FabALACTICA, 2000 units	2 mg hIgG1	535	655

# 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.

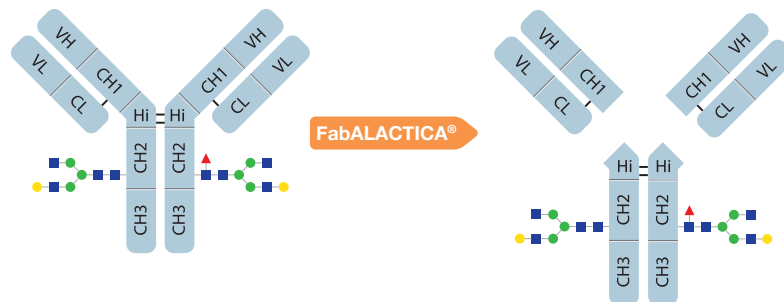


Figure 1. The FabALACTICA sample preparation workflow.

# Immobilized FabALACTICA®

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

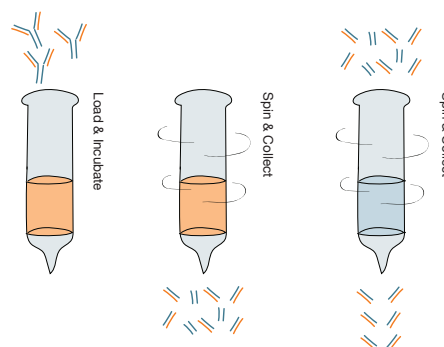


Product ID	Description	Digestion	EUR	USD
A0-AG6-010	Immobilized FabALACTICA Microspin	2 x 0.5 mg hIgG1	365	510
A0-AG6-050	Immobilized FabALACTICA Microspin	10 x 0.5 mg hIgG1	1,310	1,835
A0-AG6-100	Immobilized FabALACTICA Midispin	5-10 mg hIgG1	1,100	1,520
A0-AG6-1000	Immobilized FabALACTICA Maxispin	10-100 mg hIgG1	3,305	4,600

# FabALACTICA<sup>®</sup> Fab kit

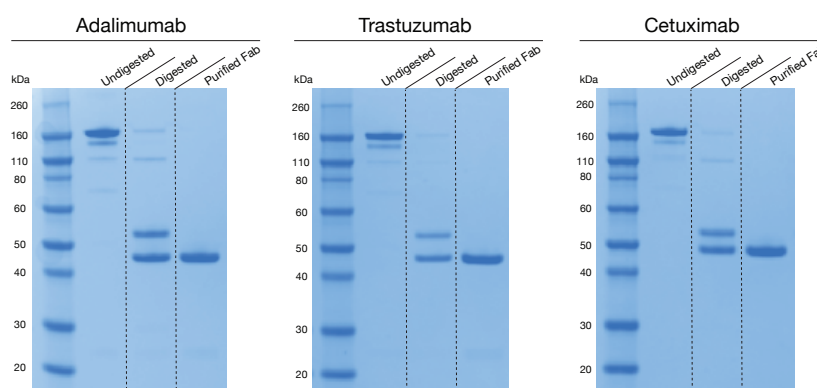
FabALACTICA<sup>®</sup> Fab kit easily generates and purifies intact Fab fragments from human IgG1.

The FabALACTICA Fab kit consists of spin columns of Immobilized FabALACTICA for antibody digestion, and spin columns of CaptureSelect<sup>™</sup>\* 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<sup>™</sup>\* 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<sup>®</sup> Fab kit

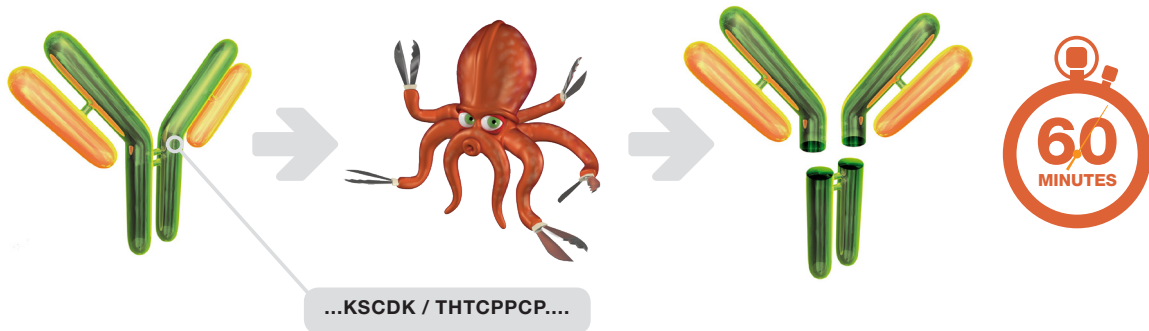
The FabALACTICA Fab kit consists of Immobilized FabALACTICA spin columns and CaptureSelect<sup>™</sup>\* Fc affinity spin columns for easy generation and purification of Fab fragments from human IgG1 antibodies.



Product ID	Description	Digestion & Purification	EUR	USD
A2-AFK-005	FabALACTICA Fab kit	0.5 mg hIgG1	415	575
A2-AFK-025	FabALACTICA Fab kit	5 x 0.5 mg hIgG1	1,100	1,520
A2-AFK-100	FabALACTICA Fab kit	10 mg hIgG1	1,360	1,890
A2-AFK-1000	FabALACTICA Fab kit	100 mg hIgG1	4,145	5,825

\*Thermo Scientific<sup>™</sup> CaptureSelect<sup>™</sup> resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Scientific Inc. and its subsidiaries.

# GingisKHAN®

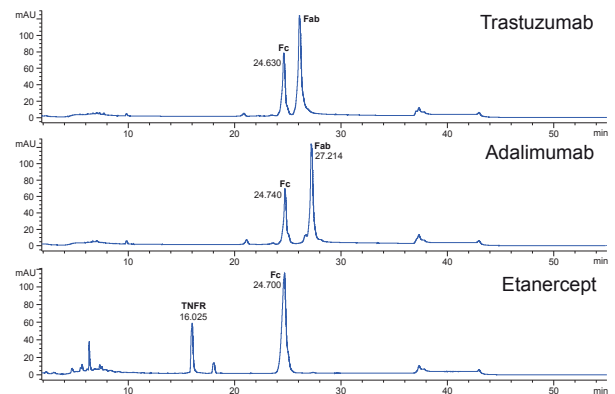


GingisKHAN® (Kgp) is a cysteine protease that digests human IgG1 at a specific site above the hinge.

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. 42.

- 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.



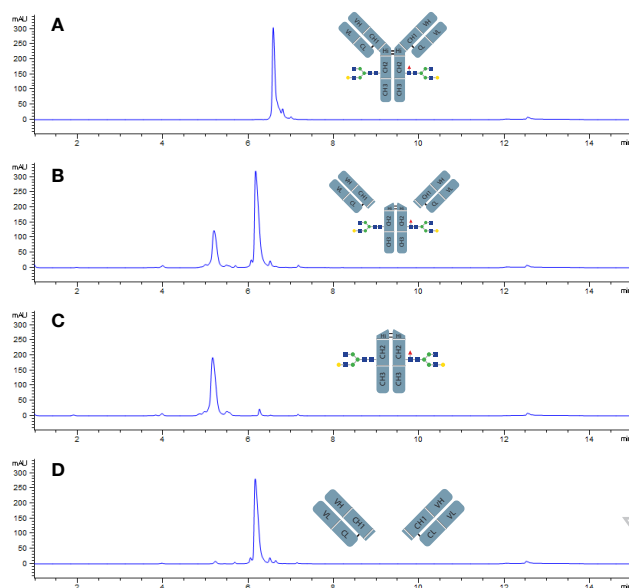
Product ID	Description	Digestion	EUR	USD
B0-GKH-020	GingisKHAN, 2000 units	2 mg hlgG1	450	625

# GingisKHAN<sup>®</sup> Fab kit

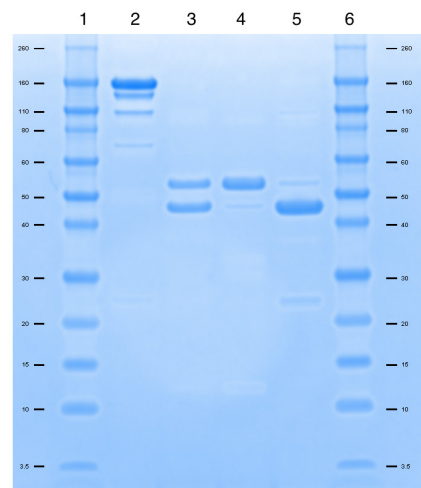
GingisKHAN<sup>®</sup> Fab kit generates and purifies Fab fragments from human IgG1.

The GingisKHAN Fab kit consists of lyophilized GingisKHAN enzyme for antibody digestion, and spin columns of CaptureSelect<sup>™</sup>\* 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<sup>®</sup> Fab kit

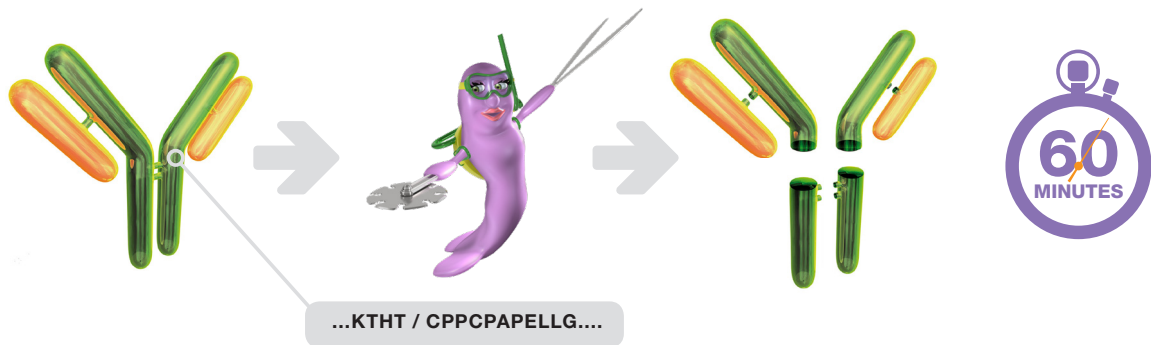
GingisKHAN Fab kit consists of 2000 units of the GingisKHAN enzyme, 5 x lyophilized reducing agent and 4 x CaptureSelect<sup>™</sup>\* CH1 affinity spin columns for generation and purification of Fab fragments from human IgG1 antibodies.



Product ID	Description	Digestion	EUR	USD
B0-GFK-020	GingisKHAN Fab kit, 2000 units	2 mg hIgG1	1,020	1,095

\* Thermo Scientific<sup>™</sup> CaptureSelect<sup>™</sup> resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Scientific Inc. and its subsidiaries.

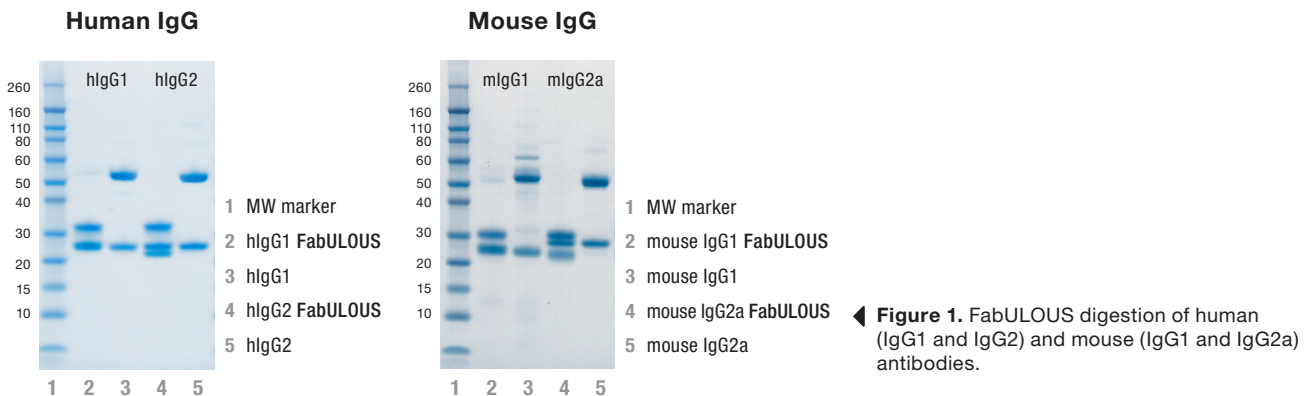
# FabULOUS®



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. 42.

- 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)



## 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	450	625



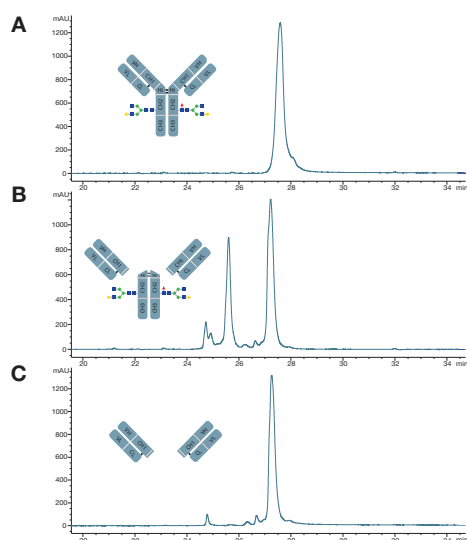
# FabULOUS<sup>®</sup> Fab kit

FabULOUS<sup>®</sup> Fab kit generates and purifies Fab fragments from mouse IgG.

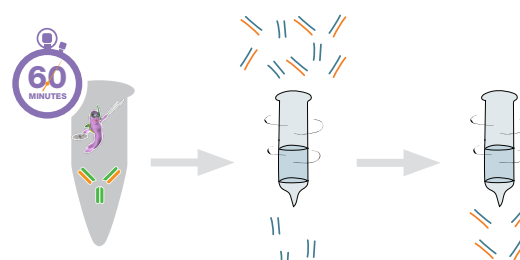
The FabULOUS Fab kit is designed to generate and purify Fab fragments from mouse IgG. The FabULOUS Fab kit consists of lyophilized FabULOUS enzyme for antibody digestion and CaptureSelect<sup>™</sup> LC-Kappa (mur) affinity spin columns for easy purification of the prepared Fab fragments from mouse IgG. CaptureSelect<sup>™</sup> LC-Lambda (mouse) affinity columns are available upon request.

The antibody is digested within 60 minutes using the

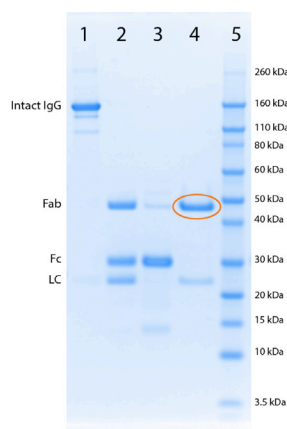
lyophilized FabULOUS enzyme, and the prepared Fab fragments bind to the CaptureSelect<sup>™</sup> affinity spin columns and are easily eluted (Fig. 3). The prepared Fab fragments from mouse IgG1 are demonstrated in Fig. 2 and 4 and can be used in affinity studies, studies of Fab glycosylation and structural 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<sup>™</sup> 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<sup>™</sup> column. Lane 4: Eluted Fab fragments. Lane 5: MW marker.

## FabULOUS<sup>®</sup> Fab kit

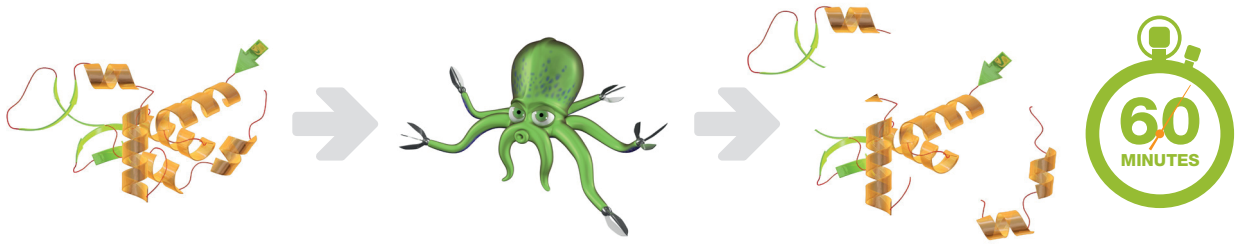
FabULOUS Fab kit consists of 2000 units of the FabULOUS enzyme and 4 x CaptureSelect<sup>™</sup> LC-kappa (mur) affinity spin columns for generation and purification of Fab fragments from mouse IgG.



Product ID	Description	Digestion	EUR	USD
A1-PFK-020	FabULOUS Fab kit mouse, 2000 units	2 mg mIgG	765	825





\*Thermo Scientific<sup>™</sup> CaptureSelect<sup>™</sup> resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Fisher and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

# GingisREX®

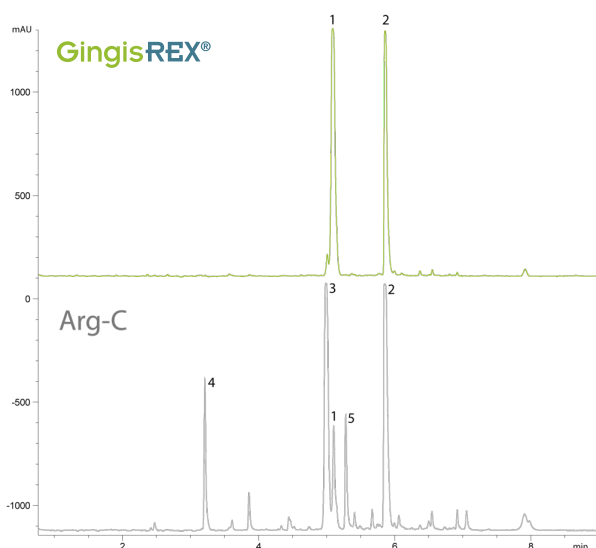


GingisREX® (RgpB) is a protease that digests proteins C-terminally of arginine residues.

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  $\beta$ -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  $\beta$ -chain digested by GingisREX or Arg-C, as indicated in Fig. 1. Green color indicates arginine residues and red color indicates lysine residues.

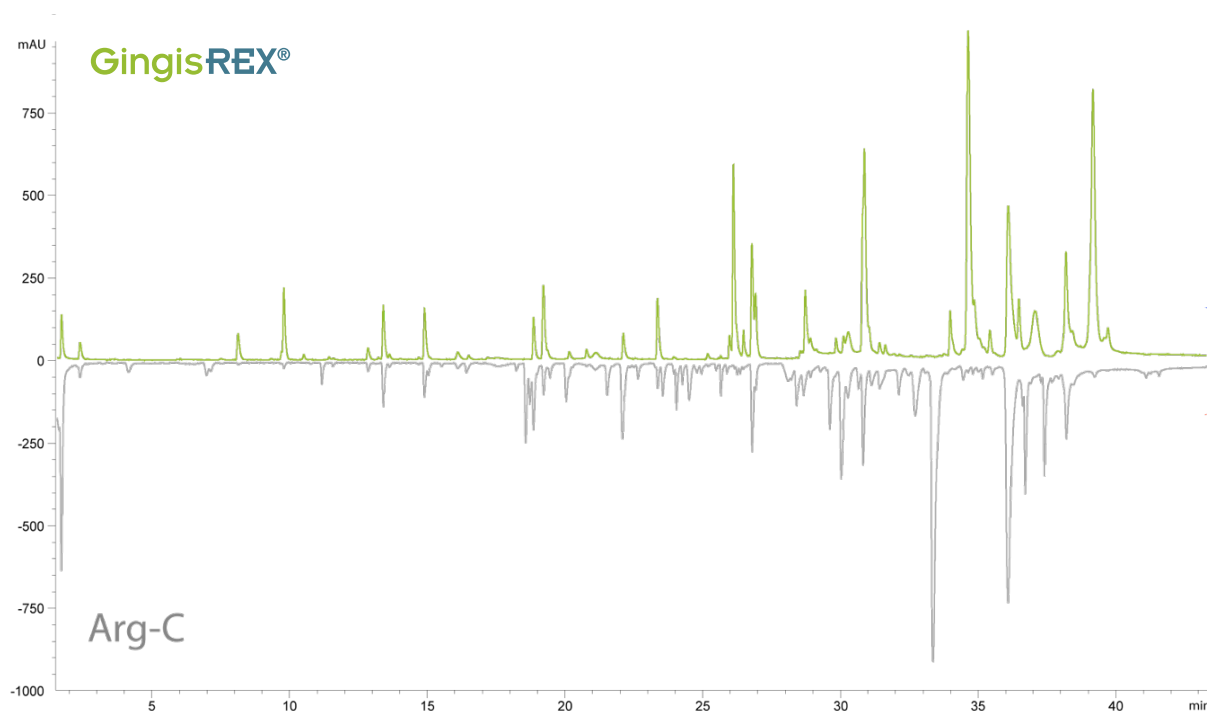
Peptide No.	Amino Acid Sequence
Intact protein	FVNQHLCGSHLVEALYLVCGERGFFYTPKA
1	GFFYTPKA
2	FVNQHLCGSHLVEALYLVCGER
3	GFFYTPK
4	FVNQHLCGSH
5	LVEALYLVCGER + Na

## 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.

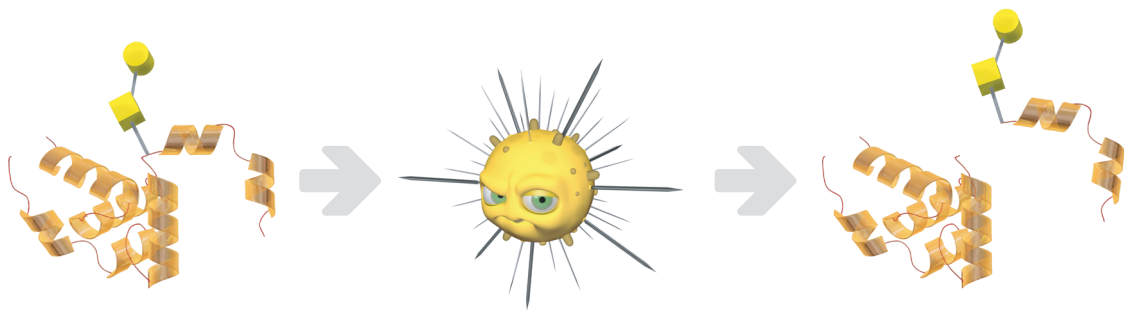
# GingisREX®

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<sup>®</sup>



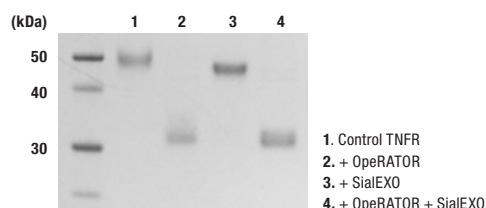
OpeRATOR<sup>®</sup> is an O-glycan-specific protease that digests proteins carrying mucin-type O-glycans N-terminally of Ser and Thr glycosylation sites.

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<sup>®</sup> (p. 30), a sialidase mix for efficient and complete removal of sialic acids, is provided with OpeRATOR.

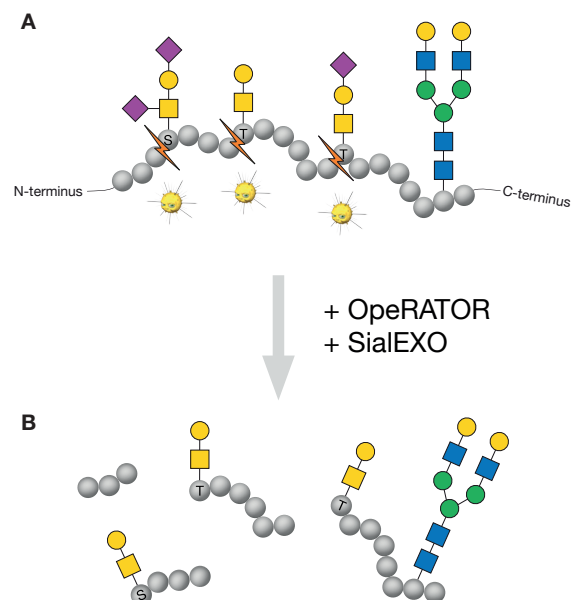
-  Digests native proteins with mucin-type O-glycosylation
-  2 h to overnight (16-18 h) reaction
-  Desialylation using SialEXO<sup>®</sup> (included) increases performance
-  N-terminally of O-glycosylated serine and threonine residues

## 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).



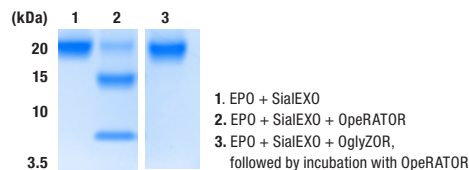
**Figure 1.** The native O-glycosylated TNF receptor (TNFR) was incubated with OpeRATOR with or without the addition of SialEXO, and the reactions were analyzed by SDS-PAGE.



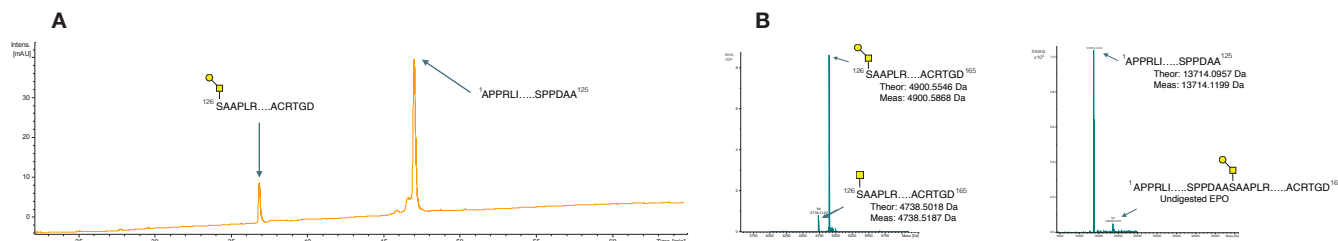
**Figure 2. a)** Schematic overview of OpeRATOR activity. O-glycans are required for OpeRATOR activity and the enzyme is not active at N-glycans. **b)** With OpeRATOR and SialEXO treatment, the O-glycosylated protein is digested into peptides carrying O-glycans. The digestion occurs N-terminally of the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

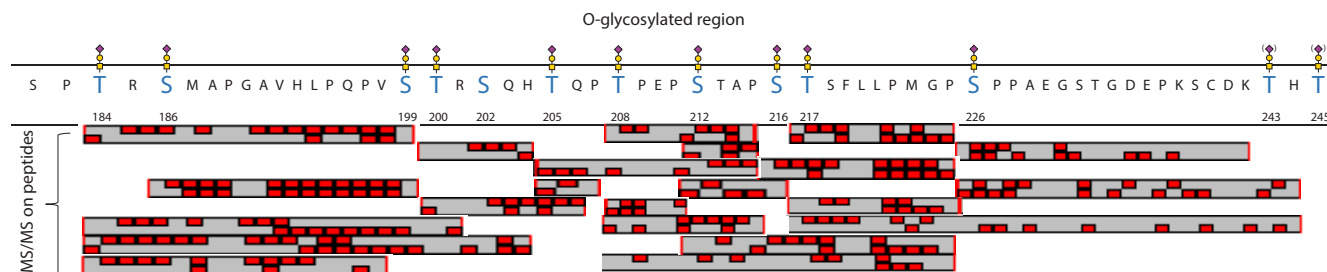


**Figure 3.** N-glycans were removed from EPO using PNGaseF and sialic acids were removed using SialEXO. In parallel, OpeRATOR hydrolyzed the protein N-terminally of the serine O-glycan (core 1) site. After reduction of disulfide bridges with DTT, the resulting two fragments were separated on a RP C4 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.



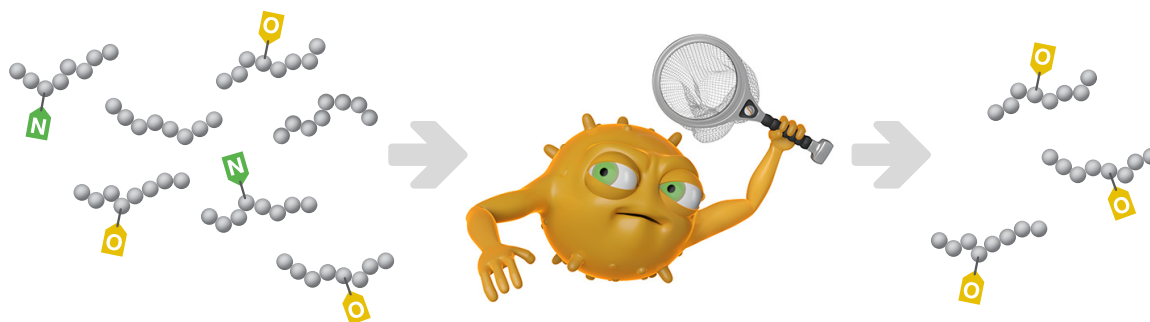
The OpeRATOR enzyme consists of 2000 units for digestion of 2 mg O-glycoprotein. The enzyme is provided together with 2000 units of SialEXO for optional sialic acid removal. Both enzymes are provided as lyophilized powders.



Product ID	Description	Digestion	EUR	USD
G2-OP1-020	OpeRATOR, 2000 units	2 mg O-glycoprotein	880	985





OpeRATOR®

# GlycOCATCH®

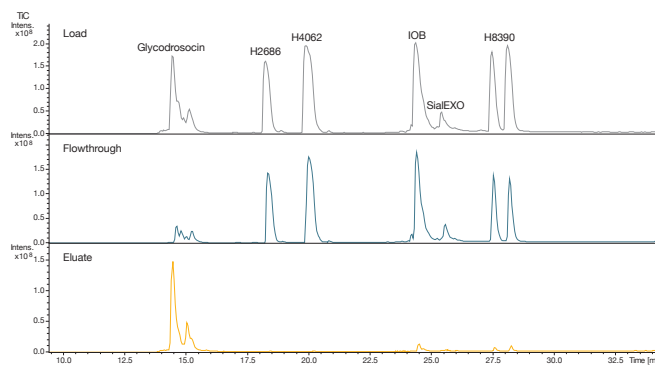


GlycOCATCH® is an enrichment resin for affinity purification of mucin-type O-glycosylated proteins and peptides.

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-

-  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

LC-MS. The data shows selective purification of the glycodrosocin peptide, carrying a core 1 O-glycan (Fig. 1).



**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	605	705

# OglyZOR®







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

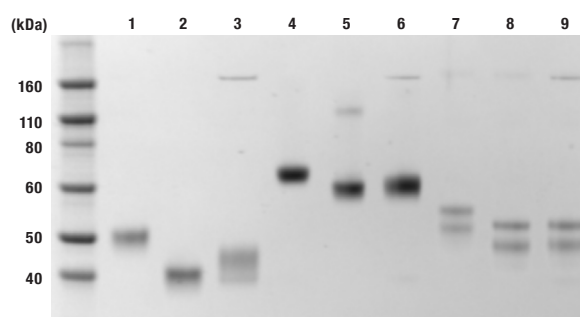
OglyZOR is an O-glycosidase that catalyzes the removal of core 1 and to some extent core 3 type O-linked disaccharides from native glycoproteins. The enzyme does usually not require denaturation of the substrate. The sialic acids of the O-glycans need to be removed for OglyZOR activity. Therefore, the 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.

## 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.

**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.

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



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*)

## 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	765	875





# SialEXO<sup>®</sup>

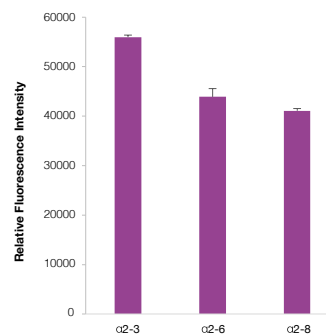


SialEXO<sup>®</sup> is a sialidase mix for complete removal of sialic acids from native glycoproteins.

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  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linkages (Fig. 1). SialEXO can be used to pretreat an O-glycosylated protein prior to digestion with OpeRATOR<sup>®</sup> (p. 26), or prior to deglycosylation with OglyZOR<sup>®</sup> (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 ( $\alpha$ 2-3 bonds), 6'-sialyllactose ( $\alpha$ 2-6 bonds) and colominic acid ( $\alpha$ 2-8 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
-   $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8-linked sialic acids



**Figure 1.** SialEXO activity on sialic acid linkages from the substrates 3'-sialyllactose ( $\alpha$ 2-3 bonds), 6'-sialyllactose ( $\alpha$ 2-6 bonds), and colominic acid ( $\alpha$ 2-8 bonds).

**Table 1.** Summary of key attributes for general  $\alpha$ 2-3, 6, 8 sialidases, including SialEXO.

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

## SialEXO<sup>®</sup>

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	545	655



# SialEXO<sup>®</sup> 23







SialEXO<sup>®</sup> 23 is an  $\alpha$ 2-3-specific sialidase, allowing targeted analysis of  $\alpha$ 2-3 linked sialic acids.

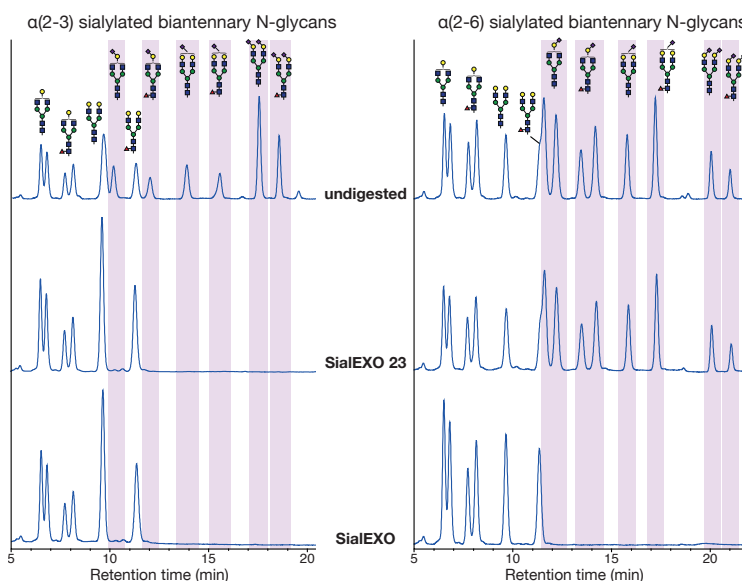
In contrast to SialEXO which is a sialidase mix, SialEXO 23 is an  $\alpha$ 2-3 specific sialidase, allowing targeted analysis of  $\alpha$ 2-3 linked sialic acids only. Efficient  $\alpha$ 2-3 desialylation of O- and N-glycosylated proteins can be achieved within one hour.

Hydrophilic interaction liquid chromatography (HILIC) was used to analyze two glycan libraries; one containing released glycans with  $\alpha$ 2-3-linked sialic acids and the other glycans modified with  $\alpha$ 2-6-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  $\alpha$ 2-3 with SialEXO 23 and of both  $\alpha$ 2-3 and  $\alpha$ 2-6 linked sialic acids by SialEXO.

**Table 2.** Summary of key attributes for specific  $\alpha$ 2-3 sialidases, including SialEXO 23.

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

-  Hydrolyzes sialic acids on N- and O-linked glycans
-  60 min reaction
-  Requires no co-factors
-   $\alpha$ 2-3-linked sialic acids



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

## SialEXO<sup>®</sup> 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	535

# Immobilized SialEXO<sup>®</sup>



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

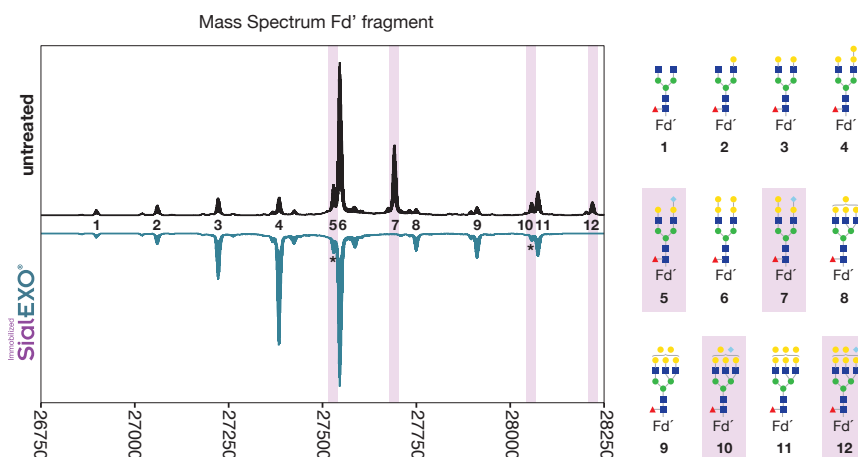
SialEXO hydrolyzes sialic acids on native glycoproteins, and is active on both O- and N-linked glycans. It is a combination of two sialidases acting on  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linkages. Immobilized SialEXO is a resin with a mixture of the two sialidases covalently coupled to agarose 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
-   $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8-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

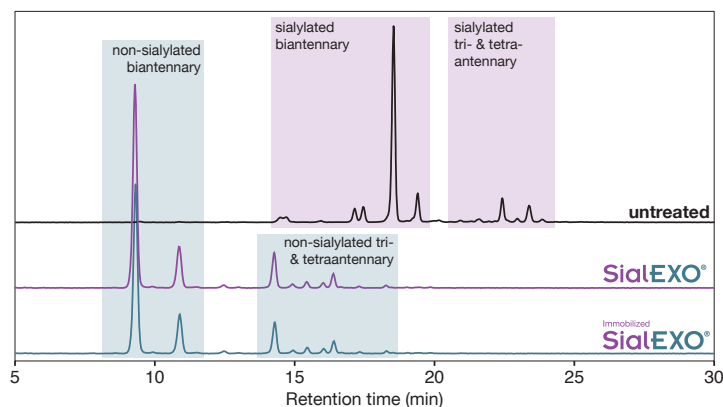
acids can be removed rapidly using Immobilized SialEXO (Fig. 1), demonstrating the utility of this enzyme for analysis of monoclonal antibodies and its activity towards non-human sialic acids.



**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

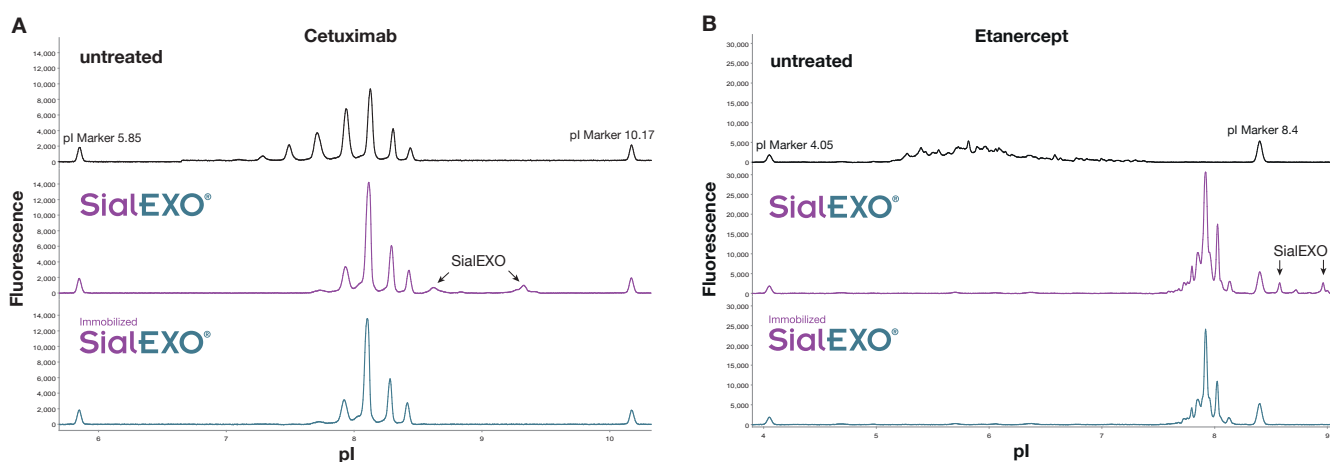
We tested the performance of SialEXO and Immobilized SialEXO on the human C1 inhibitor. This glycoprotein is a challenging substrate with 6 N- and up to 26 O-glycans, modified with both  $\alpha$ 2,3 and  $\alpha$ 2,6-linked sialic acid. Analysis of released N-glycans demonstrates a complete desialylation 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.

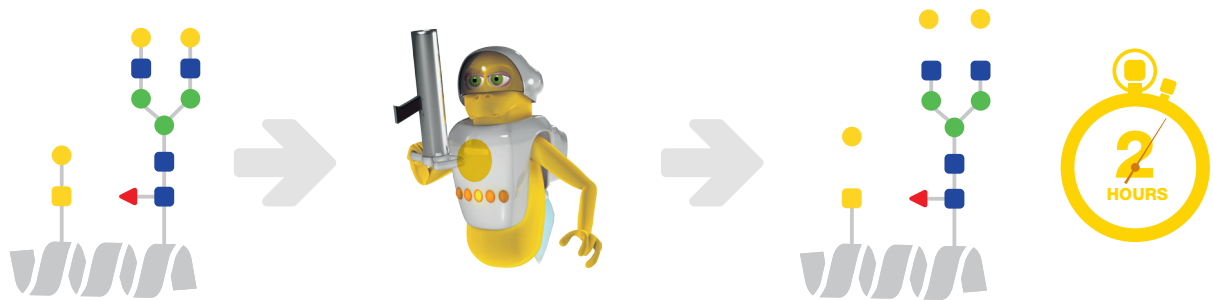
Immobilized  
**SialEXO**<sup>®</sup>

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	365	445
G1-SM6-025	Immobilized SialEXO Microspin	5 x 0.5 mg	775	995
G1-SM6-050	Immobilized SialEXO Microspin	10 x 0.5 mg	1,295	1,745

# GalactEXO™



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

GalactEXO is a  $\beta$ -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  $\beta$ 1-3 and  $\beta$ 1-4 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  $\beta$ 1-3 and  $\beta$ 1-4 linked galactose are removed within 1 h of incubation. GalactEXO can be applied to obtain antibodies with homogenous G0 glycosylation profiles.

-  Hydrolyzes galactose residues on *N*- and *O*-glycosylated proteins
-   $\beta$ 1-3 and  $\beta$ 1-4 linked galactose
-  2 h incubation
-  No co-factors required

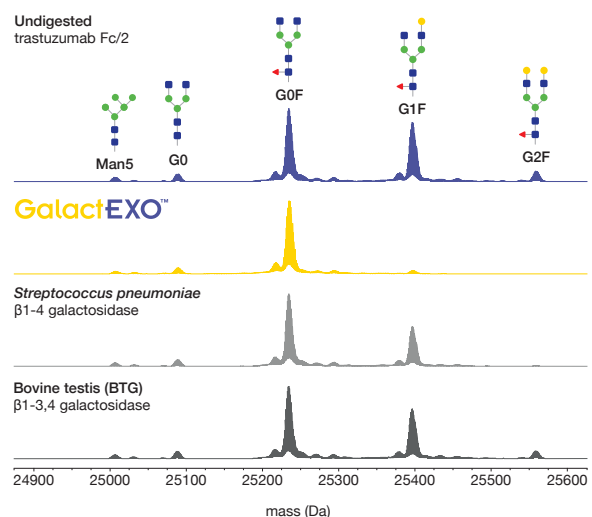
## Key Characteristics

- ▶ Efficient removal of  $\beta$ 1-3,4 linked galactose
- ▶ Activity on both *N*- and *O*-glycan structures
- ▶ For native glycoproteins and free glycans

## Hydrolysis of $\beta$ 1-4 Galactose on a mAb

The  $\beta$ -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.

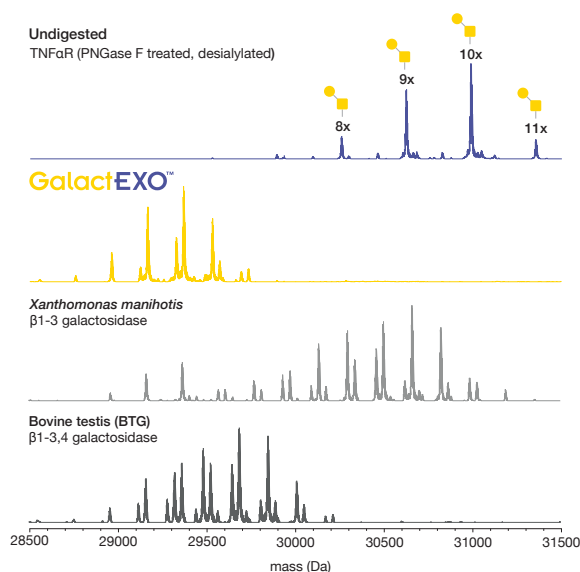
Figure 1. Deconvoluted mass spectra of the Fc/2 of trastuzumab treated with three different  $\beta$ -galactosidases. The antibody was digested with FabRICATOR, separated by reverse phase HPLC (Waters BioResolve RP, 2.1x100 mm) and analyzed by ESI-Q-TOF mass spectrometry (Bruker Impact II).



## Activity on $\beta$ 1-3 Gal from Etanercept

GalactEXO also shows exogalactosidase activity for  $\beta$ 1-3 galactose, present on O-glycosylated biopharmaceuticals. Etanercept was incubated with GalactEXO or galactosidases from other sources and the TNFaR 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 TNFaR 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<sup>®</sup> sialidase and GalactEXO galactosidase for 1 h and analyzed for exoglycosidase activities (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.

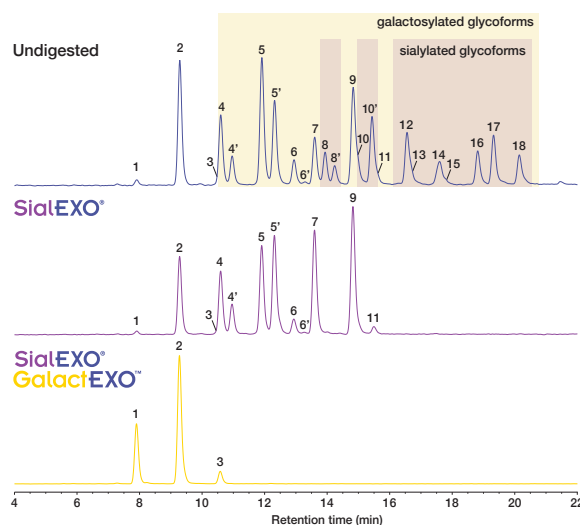
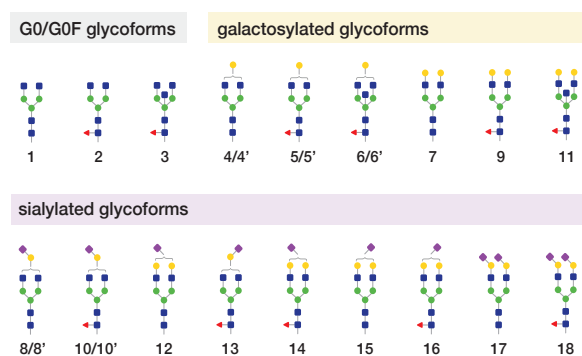

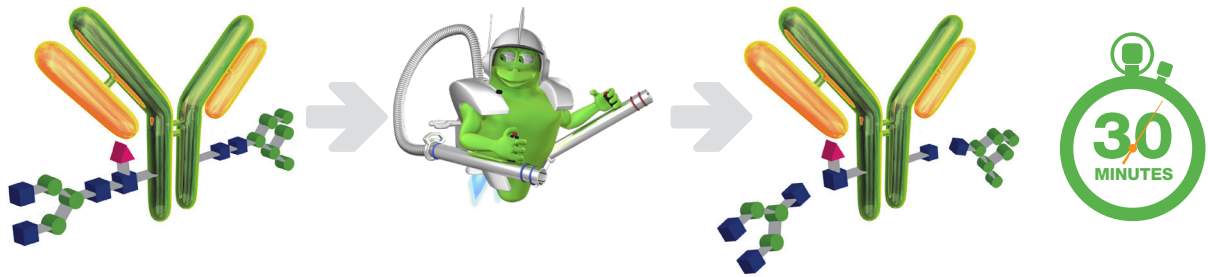


Figure 3. HILIC-FLD UHPLC chromatograms of a 2-AB labeled glycan library analyzed undigested (top), after treatment with SialEXO (middle) or both SialEXO and GalactEXO (bottom). Analysis was performed on a Thermo Scientific Vanquish Duo UHPLC system equipped with a Thermo Scientific Accurcore 150 Amide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

# GalactEXO™





	Product ID	Description	EUR	USD
	G1-GM1-020	GalactEXO, 2000 units	750	845





# 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
<b>Digestion site</b>				
<b>Works on native IgG</b>	Yes	Yes	Yes/No	Yes/No
<b>pH optimum</b>	7.4	7.4	7.5	5-6
<b>Reaction time</b>	30 min	30 min	6-24 h	1-16 h
<b>IgG specific</b>	Yes*	Yes	No	No
<b>Glycoform specificity</b>	All Fc glycoforms	Complex Fc glycoforms, 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.

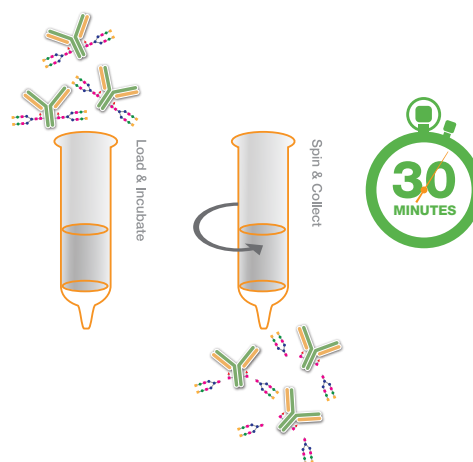


Product ID	Description	Deglycosylation	EUR	USD
A0-GL1-020	GlycINATOR, 2000 units	2 mg IgG	450	625
A0-GL8-020	GlycINATOR LE (low endotoxin), 2000 units	2 mg IgG	495	655

## Immobilized GlycINATOR®

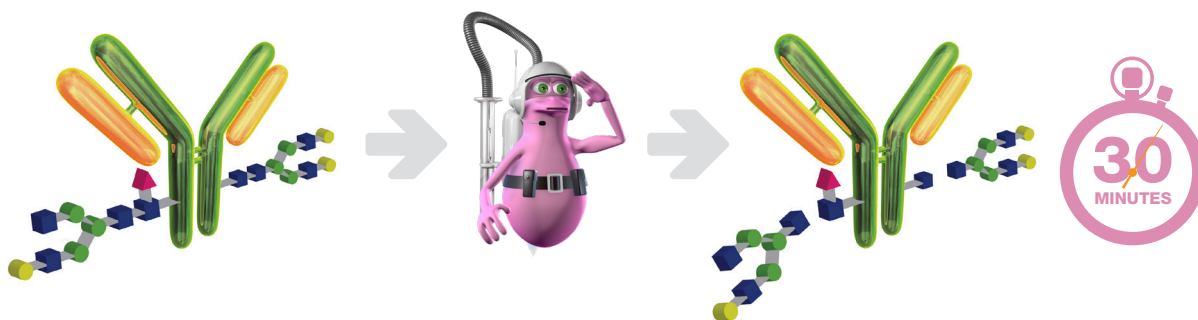
The GlycINATOR enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for deglycosylation of Fc glycans on 0.5 mg up to 100 mg of antibody or Fc-fusion protein.

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	335	465
A0-GL6-025	Immobilized GlycINATOR Microspin	5 x 0.5 mg	765	1,060
A0-GL6-050	Immobilized GlycINATOR Microspin	10 x 0.5 mg	1,270	1,765
A0-GL6-100	Immobilized GlycINATOR Midispin	1-10 mg	1,020	1,415
A0-GL6-1000	Immobilized GlycINATOR Maxispin	10-100 mg	3,045	4,255

# IgGZERO®



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

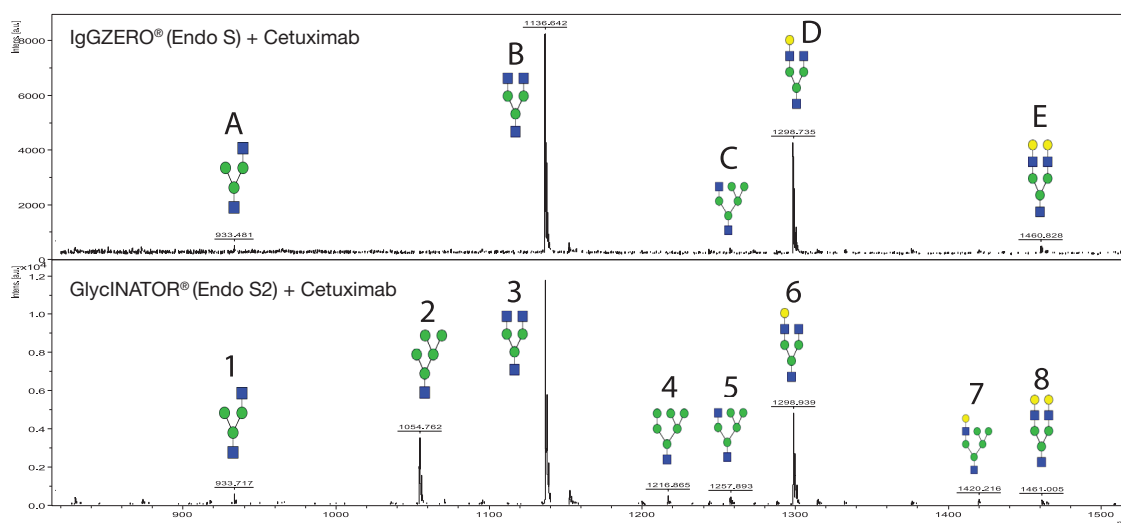
The IgGZERO enzyme hydrolyzes N-glycans specifically at the Fc glycosylation site on IgG. In contrast to GlycINATOR, IgGZERO has limited activity on high-mannose and hybrid-type glycoforms. The hydrolysis of complex glycans is fast and carried out under native and physiological conditions, as the enzyme 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. 42).

-  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 gly-

cans (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.

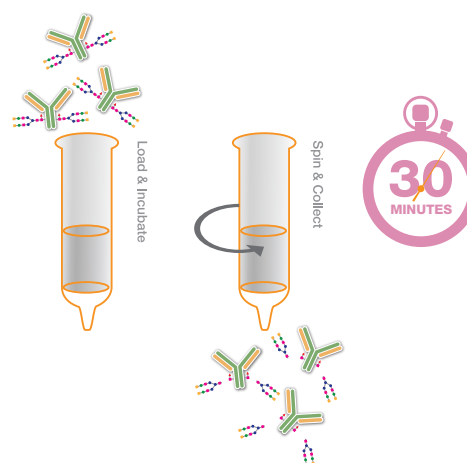


Product ID	Description	Deglycosylation	EUR	USD
A0-IZ1-010	IgGZERO, 1000 units	1 mg IgG	230	320
A0-IZ1-050	IgGZERO, 5000 units	5 mg IgG	915	1,270
A0-IZ8-020	IgGZERO LE (low endotoxin), 2000 units	2 mg IgG	500	655

# deGlycIT™

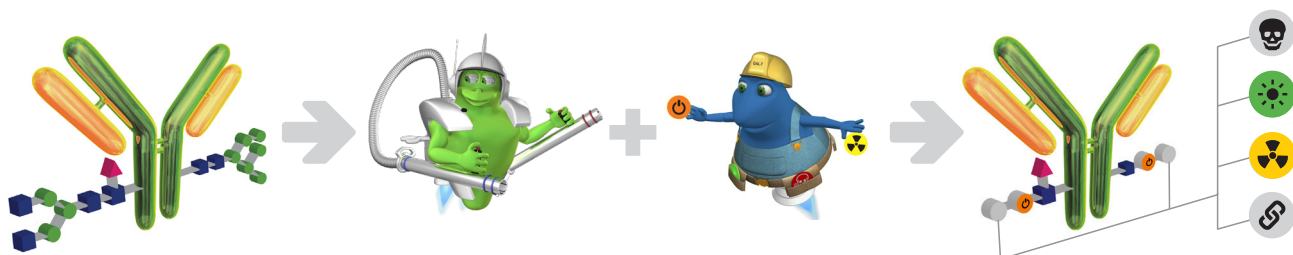
The IgGZERO enzyme is immobilized on agarose beads, and the spin columns are provided with immobilized enzyme for deglycosylation of Fc glycans on 0.5 mg up to 100 mg of antibody or Fc-fusion protein.

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	335	465
A0-IZ6-025	deGlycIT Microspin	5 x 0.5 mg	765	1,060
A0-IZ6-050	deGlycIT Microspin	10 x 0.5 mg	1,270	1,765
A0-IZ6-100	deGlycIT Midispin	1-10 mg	1,020	1,415
A0-IZ6-1000	deGlycIT Maxispin	10-100 mg	3,045	4,255




# 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.

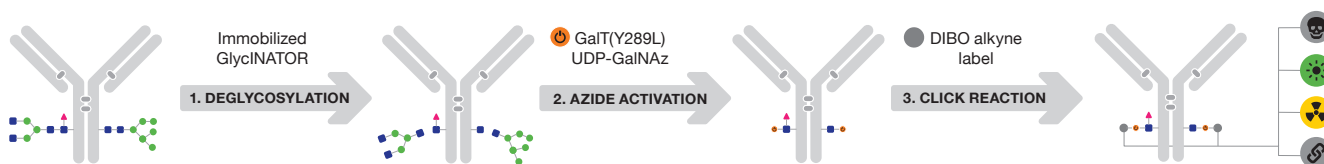
The unique combination of enzymes in GlyCLICK results in a degree of labeling (DOL) of 2 labels per IgG, generating stable and homogenous conjugates suitable for sensitive and versatile applications. The GlyCLICK technology is available in various kit formats to facilitate tailored and site-specific 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  $\beta$ -1,4-Galactosyltransferase, GaT(Y289L), making the antibody azide-activated for conjugation.

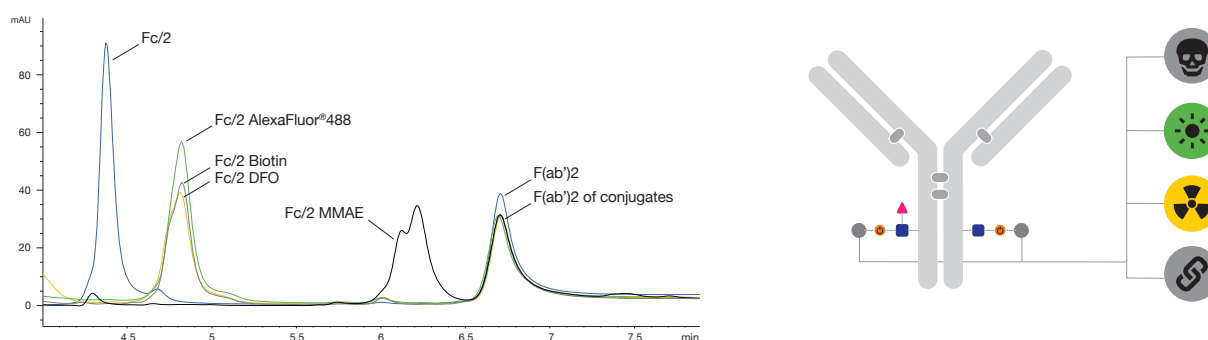
### 3. CLICK reaction

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.

## GlyCLICK Characteristics

### Site-specific and Modular Technology

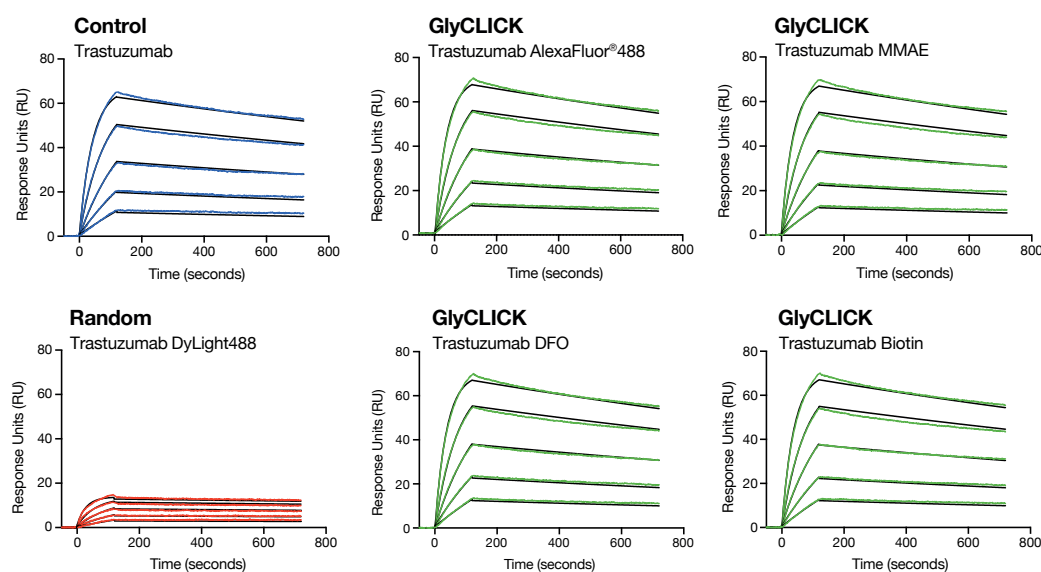
GlyCLICK enables quantitative and complete conjugation with a constant degree of labeling (DOL) of one label per Fc/2. The unique combination of enzymes allows Fc glycan-specific remodeling that restricts labeling to the Fc glycan sites without interference with the antigen binding domain of the antibody. 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  $\mu$ m, 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 impaired.



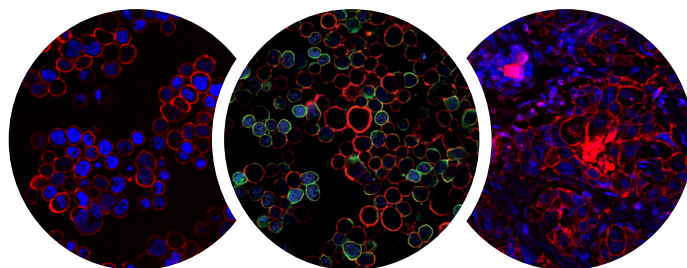
**Figure 3.** Affinity analysis of native and conjugated trastuzumab. Anti-human IgG (Fc) was used as the capturing molecule for trastuzumab, RandomDyLight™488 (DOL=10) conjugates and GlyCLICK conjugates: AlexaFluor488, Biotin, DFO and MMAE (DOL=2).HER2 was injected at a range ensuring sufficient curvature.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

## GlyCLICK Applications

### Immunofluorescent Microscopy

Site-specific 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.

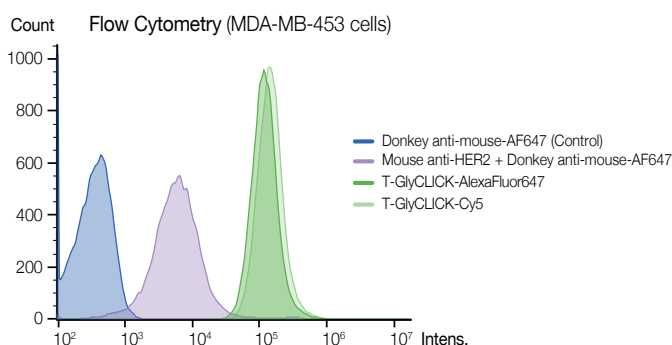
*“This technology benefits the fields of basic research and drug development and may form the basis for exciting diagnostic tools in clinical histopathology.”*



**Figure 4.** HER2(+) cells or tissue. **Left:** PFA-fixed cells incubated with DAPI (0.2  $\mu$ M), GlyCLICK-AlexaFluor647 (1  $\mu$ g/ml). **Middle:** PFA-fixed cells incubated with DAPI (0.2  $\mu$ M), GlyCLICK-AlexaFluor647 (5  $\mu$ g/ml), phalloidinAF488. **Right:** paraffin embedded, BSA blocked tissue incubated with DAPI (0.2  $\mu$ M), GlyCLICK-AlexaFluor647 (10  $\mu$ g/ml).

### Flow Cytometry

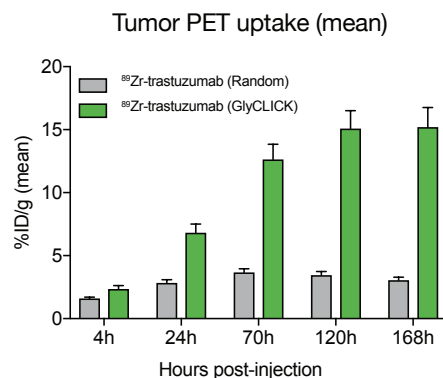
Directly labeled antibodies for primary detection offer advantages in flow cytometry applications but the right combination of antibody and label may not be available. The risk of unspecific binding and cross-reactivity using widely available secondary antibodies often require intricate experimental 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  $\mu$ g/ml) 30 min or mouse primary antibody (3.7  $\mu$ g/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

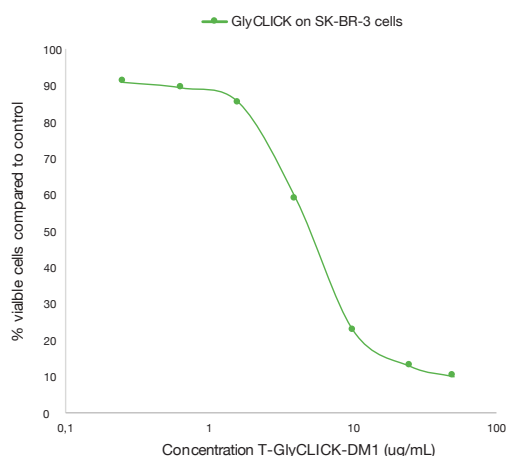
Radioactively labeled antibodies are excellent tracers for immuno imaging *in vivo*. The *in vivo* effect of GlyCLICK conjugated trastuzumab-DFO labeled with  $^{89}\text{Zr}$  was analyzed and compared to random labeling at lysines (DOL=0-6). Tumor-bearing mice injected with GlyCLICK-conjugates show 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







Antibody-drug conjugates (ADCs) are potent therapeutic agents combining the high selectivity of antibodies with controlled dosage of cytotoxic payloads. In contrast to conventional random labeling at amino acid sites, site-specific labeling offers reliable drug-antibody-ratio (DAR) and have been observed to exhibit improved pharmacologic performance *in vivo*. The site-specific GlyCLICK conjugation preserves full antigen-binding capacity and exhibits dose-response cytotoxicity (Fig. 7).



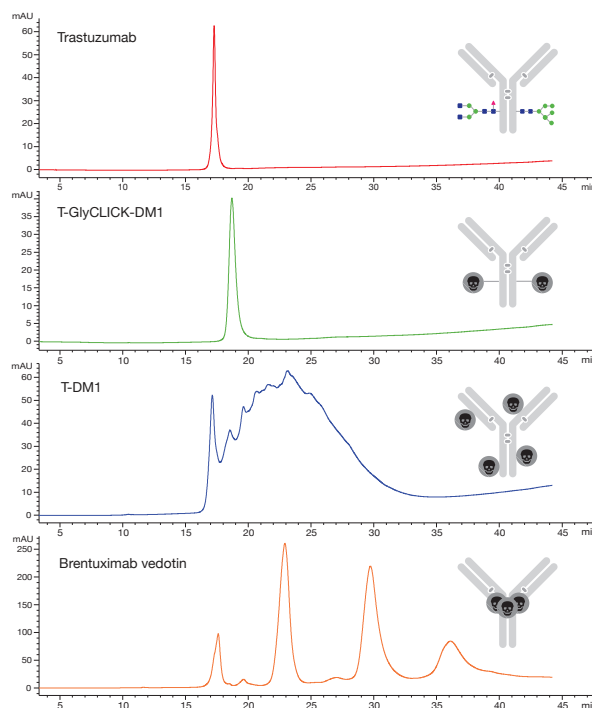
**Figure 7.** Cytotoxicity analysis of HER2(+) cells (SK-BR-3) by DM1-conjugated trastuzumab, DBCO-PEG4-Ahx-DM1, using GlyCLICK and compared to randomly conjugated trastuzumab-DM1. Cell viability was measured using CellTiter-Glo® Luminescent assay (Promega), 5000 cell/well incubated with 0-10 µg/ml GlyCLICK-conjugates at 37°C, 5% CO<sub>2</sub> for 72 h prior to addition.

# 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	985
	L1-F01-200	GlyCLICK Alexa Fluor® 488	Conjugates 1 x 2 mg IgG	1,495	1,545
	L1-F02-025	GlyCLICK Alexa Fluor® 555	Conjugates 250 µg IgG	925	985
	L1-F02-200	GlyCLICK Alexa Fluor® 555	Conjugates 1 x 2 mg IgG	1,625	1,695
	L1-F03-025	GlyCLICK Alexa Fluor® 647	Conjugates 250 µg IgG	925	985
	L1-F03-200	GlyCLICK Alexa Fluor® 647	Conjugates 1 x 2 mg IgG	1,625	1,695
	L1-C01-025	GlyCLICK DFO	Conjugates 250 µg IgG	925	985
	L1-C01-200	GlyCLICK DFO	Conjugates 1 x 2 mg IgG	1,495	1,545
	L1-A01-025	GlyCLICK Biotin	Conjugates 250 µg IgG	925	985
	L1-A01-200	GlyCLICK Biotin	Conjugates 1 x 2 mg IgG	1,495	1,545
	L1-AZ1-025	GlyCLICK Azide Activation	Activates 250 µg IgG	825	875
	L1-AZ1-200	GlyCLICK Azide Activation	Activates 1 x 2 mg IgG	1,250	1,355
	L1-AZ1-100	GlyCLICK Azide Activation	Activates 1 x 10 mg IgG	5,145	6,195

GlyCLICK enables custom conjugation using the warhead of choice for homogenous material with minimal batch-to-batch variation (Fig. 8).



**Figure 8.** HIC separation of GlyCLICK conjugated trastuzumab-DM1, DBCO-PEG4-Ahx-DM1, and randomly conjugated trastuzumab-DM1 on TSKgel@Butyl-NPR column (Tosoh Biosciences) in 25 mM NaP, 1.5 M ammonium sulfate and eluted with decreasing salt gradient (20% isopropanol). DAR variants of T-DM1 were unresolved due to heterogeneity of hydrophobicity in the material.

## Selected References


FabRICATOR®	An, Y. et al., 2014. <b>A new tool for monoclonal antibody analysis: application of IdeS proteolysis in IgG domain-specific characterization.</b> <i>mAbs</i> , 6(4), pp.879–893.
	Ayoub, D. et al., 2013. <b>Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques.</b> <i>mAbs</i> , 5(5), pp.699–710.
	Beck, A., Diemer, H., et al., 2013. <b>Analytical characterization of biosimilar antibodies and Fc-fusion proteins.</b> <i>TrAC Trends in Analytical Chemistry</i> , 48, pp.81–95.
	Beck, A., Wagner-Rousset, E., et al., 2013. <b>Characterization of therapeutic antibodies and related products.</b> <i>Analytical Chemistry</i> , 85(2), pp.715–736.
	Botzanowski, T. et al., 2017. <b>Insights from native mass spectrometry approaches for top- and middle- level characterization of site-specific antibody-drug conjugates.</b> <i>mAbs</i> , 9(5), pp.801–811.
	Faid, V. et al., 2017. <b>Middle-up analysis of monoclonal antibodies after combined IgD and IdeS hinge proteolysis: Investigation of free thiols.</b> <i>Journal of Pharmaceutical and Biomedical Analysis</i> , 149, pp.541–546.
	Lynaugh, H., Li, H. & Gong, B., 2013. <b>Rapid Fc glycosylation analysis of Fc fusions with IdeS and liquid chromatography mass spectrometry.</b> <i>mAbs</i> , 5(5), pp.641–645.
	Sjögren, J., Olsson, F. & Beck, A., 2016. <b>Rapid and improved characterization of therapeutic antibodies and antibody related products using IdeS digestion and subunit analysis.</b> <i>The Analyst</i> , 141(11), pp.3114–3125.
	Sokolowska, I. et al., 2017. <b>Subunit mass analysis for monitoring antibody oxidation.</b> <i>mAbs</i> , 9(3), pp.498–505.
	Wagner-Rousset, E. et al., 2014. <b>Antibody-drug conjugate model fast characterization by LC-MS following IdeS proteolytic digestion.</b> <i>mAbs</i> , 6(1), pp.173–184.
Yang, R. et al., 2017. <b>Rapid assessment of oxidation via middle-down LCMS correlates with methionine side-chain solvent-accessible surface area for 121 clinical stage monoclonal antibodies.</b> <i>mAbs</i> , 9(4), pp.646–653.	
FabALACTICA®	Faid, V. et al., 2017. <b>Middle-up analysis of monoclonal antibodies after combined IgD and IdeS hinge proteolysis: Investigation of free thiols.</b> <i>Journal of Pharmaceutical and Biomedical Analysis</i> , 149, pp.541–546.
	Spoerry C, et al., 2016. <b>Novel IgG-Degrading Enzymes of the IgD Protease Family Link Substrate Specificity to Host Tropism of Streptococcus Species.</b> <i>PLoS ONE</i> 11(10): e0164809. doi:10.1371/journal.pone.0164809
GingisKHAN®	Leblanc, Y. et al., 2017. <b>Charge variants characterization of a monoclonal antibody by ion exchange chromatography coupled on-line to native mass spectrometry: Case study after a long-term storage at +5°C.</b> <i>Journal of Chromatography B</i> , 1048, pp.130–139.
	Moelleken, J. et al., 2017. <b>GingisKHAN™ protease cleavage allows a high-throughput antibody to Fab conversion enabling direct functional assessment during lead identification of human monoclonal and bispecific IgG1 antibodies.</b> <i>mAbs</i> , 131(6), pp.1–12.
	Sjögren, J. et al., 2017. <b>Generating and Purifying Fab Fragments from Human and Mouse IgG Using the Bacterial Enzymes IdeS, SpeB and Kgp.</b> <i>Methods in Molecular Biology</i> , 1535, pp.319–329.
van den Bremer, E.T.J. et al., 2017. <b>Cysteine-SILAC Mass Spectrometry Enabling the Identification and Quantitation of Scrambled Interchain Disulfide Bonds: Preservation of Native Heavy-Light Chain Pairing in Bispecific IgGs Generated by Controlled Fab-arm Exchange.</b> <i>Analytical Chemistry</i> , 89(20), pp.10873–10882.	
FabULOUS®	Mahan, A.E. et al., 2015. <b>A method for high-throughput, sensitive analysis of IgG Fc and Fab glycosylation by capillary electrophoresis.</b> <i>Journal of Immunological Methods</i> , 417, pp.34–44.
	Zhang, Z. et al., 2016. <b>SpeB Proteolysis with Imaged Capillary Isoelectric Focusing for the Characterization of Domain-Specific Charge Heterogeneities of Reference and Biosimilar Rituximab.</b> <i>Journal of Chromatography B</i> , 1020, pp.148–157.
IgGZERO®	Gao, P. et al., 2014. <b>Deglycosylation of mAb by EndoS for Improved Molecular Imaging.</b> <i>Molecular Imaging and Biology</i> , pp.1–9.
	Marcoux, J. et al., 2015. <b>Native mass spectrometry and ion mobility characterization of trastuzumab emtansine, a lysine-linked antibody drug conjugate.</b> <i>Protein science : a publication of the Protein Society</i> , 24(8), pp.1210–1223.
	Sokolowska, I. et al., 2017. <b>Subunit mass analysis for monitoring antibody oxidation.</b> <i>mAbs</i> , 9(3), pp.498–505.
GlycINATOR®	Bobály, B. et al., 2017. <b>Protocols for the analytical characterization of therapeutic monoclonal antibodies. II – Enzymatic and chemical sample preparation.</b> <i>Journal of Chromatography B</i> , 1060, pp.325–335.
	Liu, S. & Zang, L., 2016. <b>Rapid quantitation of monoclonal antibody N-glyco-occupancy and afucosylation using mass spectrometry.</b> <i>Analytical Biochemistry</i> , 509(C), pp.142–145.
	Sjögren, J. et al., 2015. <b>EndoS and EndoS2 hydrolyze Fc-glycans on therapeutic antibodies with different glycoform selectivity and can be used for rapid quantification of high-mannose glycans.</b> <i>Glycobiology</i> , 25(10), pp.1053–1063.
	Upton, R. et al., 2016. <b>Orthogonal Assessment of Biotherapeutic Glycosylation: A Case Study Correlating N-Glycan Core Afucosylation of Herceptin with Mechanism of Action.</b> <i>Analytical Chemistry</i> , 88(20), pp.10259–10265.
Wang, Y. et al., 2017. <b>Monitoring Glycosylation Profile and Protein Titer in Cell Culture Samples Using ZipChip CE-MS.</b> <i>Journal of Analytical &amp; Bioanalytical Techniques</i> , 08(02).	


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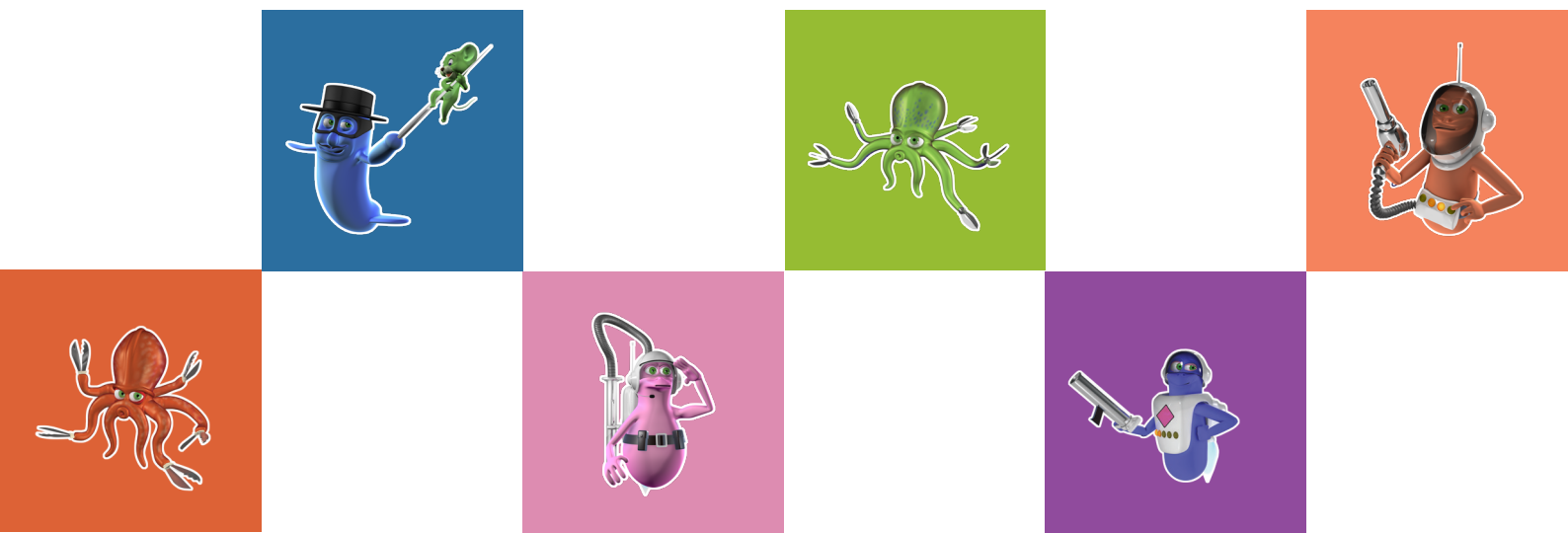
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