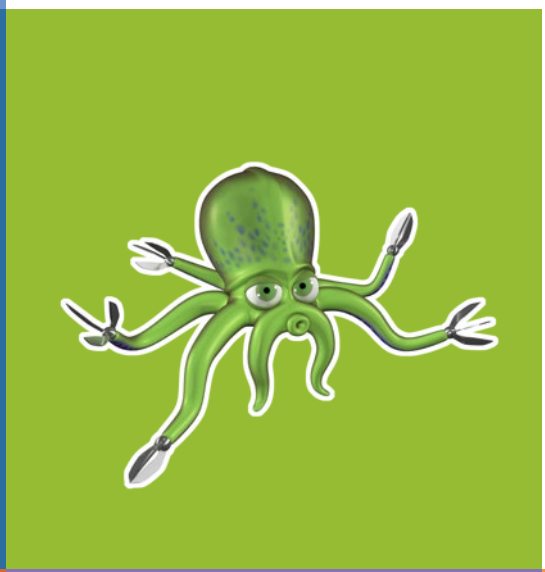
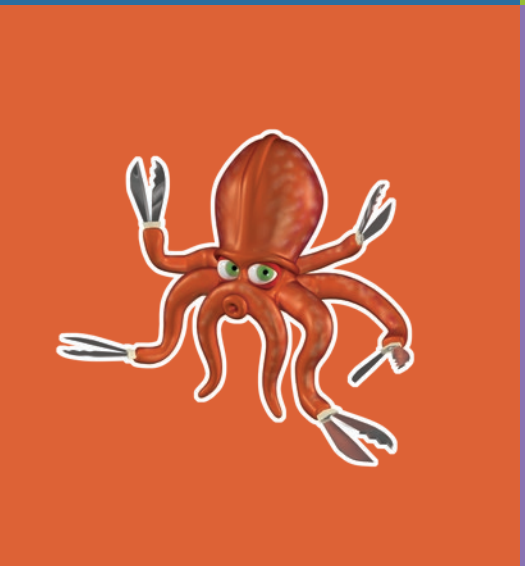
SmartEnzymes™

GENOVIS





Smart Enzymes

FabRICATOR® (IdeS) 3

A cysteine protease that digests IgG and Fc-fusions at one specific single site just below the hinge region

FabRICATOR®Z (IdeZ) 12

A cysteine protease that digests mouse IgG2a at one specific site just below the hinge, creating F(ab')2 fragments

FabALACTICA™ (IgdE) 14

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

GingisKHAN® (Kgp) 16

A cysteine protease that digests human IgG1 at one single amino acid position, just above the hinge

FabULOUS® (SpeB) 20

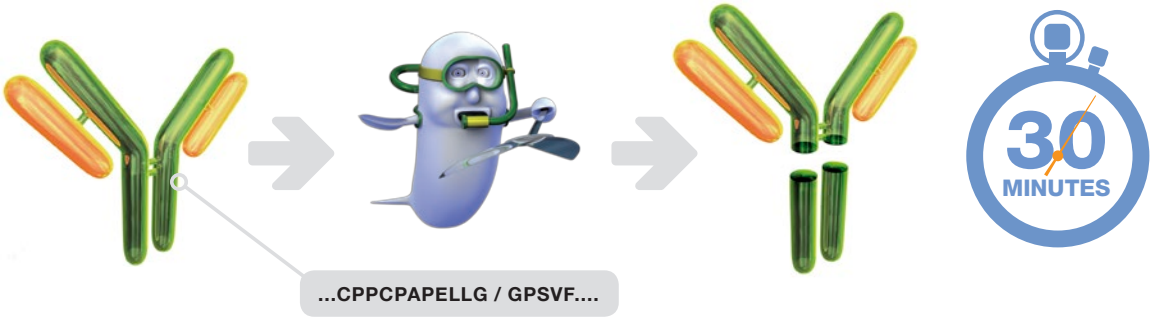
A cysteine protease that digests IgG in the upper hinge region

GingisREX® (Rgp) 24

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

FabRICATOR®

Antibody Subunit Generation



FabRICATOR (IdeS) is a unique cysteine protease that digests IgG at one single amino acid position, just below the hinge.

The enzyme is specific and selective, with no other known substrate besides IgG. A digestion of IgG with FabRICATOR generates a homogenous pool of precise $F(ab')_2$ and Fc fragments. There is no overdigestion or further degradation of the fragments typically seen with other proteolytic enzymes. The reaction is performed at neutral pH without addition of cofactors or reducing agents.

FabRICATOR is easy to use and no optimization of the reaction conditions is needed. A 30 minute digestion protocol is generally applicable. Sample preparation-induced modifications in the antibody characterization are minimized due to mild reaction conditions and a rapid digestion protocol.

IgG Digested by FabRICATOR

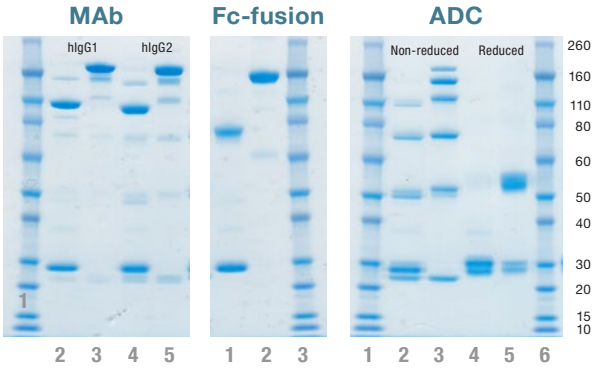
Human IgG	Mouse IgG2a*
Humanized IgG	Mouse IgG3
Chimeric IgG	Monkey IgG
Fc-fusion proteins	Rabbit IgG
ADC	Sheep IgG

** Special incubation conditions required.*

- **Specific – one precise digestion site**
- **Rapid – a 30 minute protocol**
- **Works immediately out of the box**

FabRICATOR Digestion

The picture below illustrates the digestion of monoclonal antibodies (MAbs), Fc-fusion proteins and antibody-drug conjugates (ADCs) with FabRICATOR, at 37°C for 30 min.



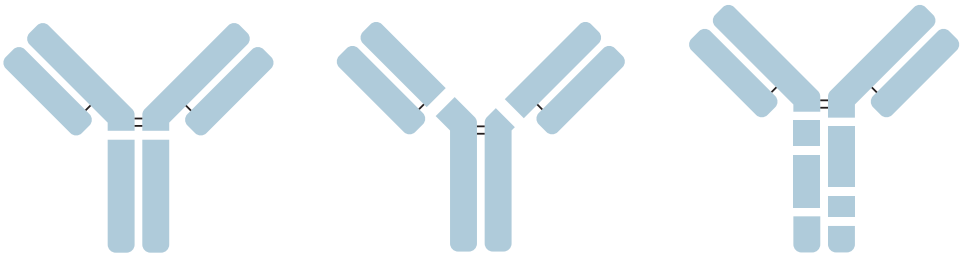
1 MW marker
2 hIgG1 (Avastin®) **FabRICATOR**
3 hIgG1(Avastin®)
4 hIgG2 (Vectibix®) **FabRICATOR**
5 hIgG2 (Vectibix®)

1 Fc-fusion (Enbrel®) **FabRICATOR**
2 Fc-fusion (Enbrel®)
3 MW marker

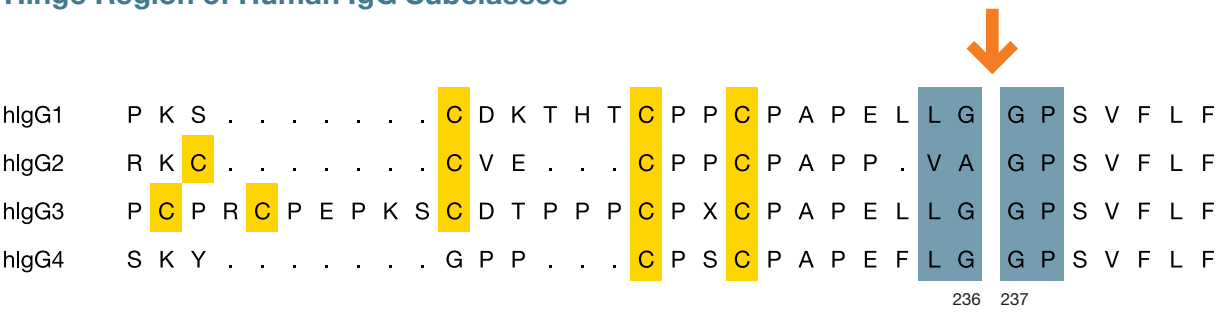
1 MW marker
2 ADC (Adcetris®) **FabRICATOR** non-reduced
3 ADC (Adcetris®) non-reduced
4 ADC (Adcetris®) **FabRICATOR** reduced
5 ADC (Adcetris®) reduced
6 MW marker

Comparison to Other Common Enzymes

FabRICATOR is the most cost-effective method to generate antibody subunits with high sensitivity and yield. Compared to other competing technologies, it is a robust method, requiring no optimization and less hands-on time.

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

Hinge Region of Human IgG Subclasses



The arrow indicates the FabRICATOR digestion site, just below the hinge.

Compatible Buffers for FabRICATOR Digestion

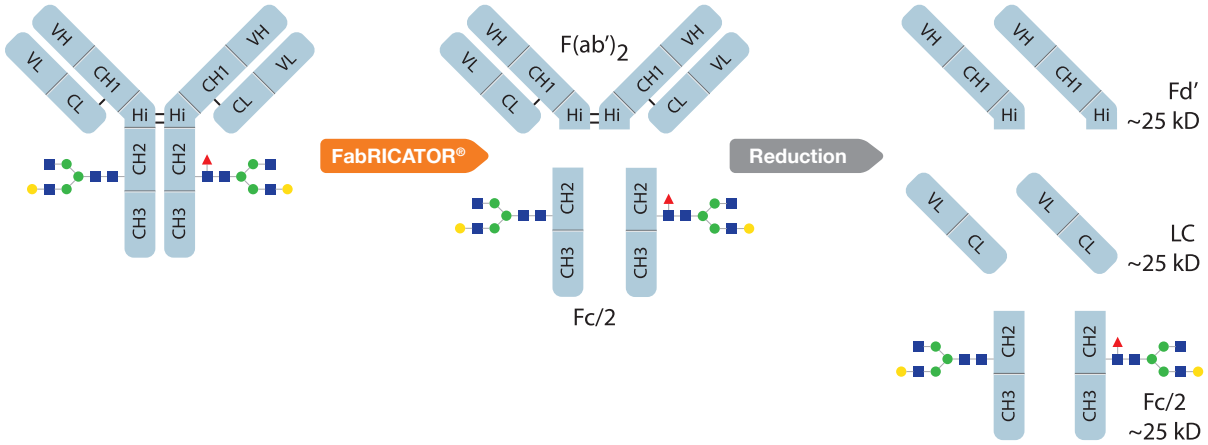
FabRICATOR works in most common buffers at neutral pH. Buffers and media tested for FabRICATOR cleavage activity are listed in the table below. Other buffers and media may also work.

Compatible Reaction Buffers/Media	pH range
Sodium phosphate saline (PBS)	6.0- 8.0
TRIS	7.0-8.0
Ammonium bicarbonate, NH_4HCO_3	6.0-7.0
MES	5.5-6.5
HEPES	7.0-8.0
Sodium acetate, NaAc	6.0

Rapid and Robust Subunit Domain Analysis

LC/MS is a key analytical method for characterization of mAbs, ADCs, Fc-fusion proteins and biosimilars. Precise antibody subunit domains are generated with FabRICATOR enzyme in the sample preparation. By reducing the size of the IgG to subunits of approximately 25 kD each, the resolution is significantly increased, which allows for fast and accurate glycan profiling and determination of different PTMs. Also, conjugation sites and integrity of ADCs can be rapidly established.

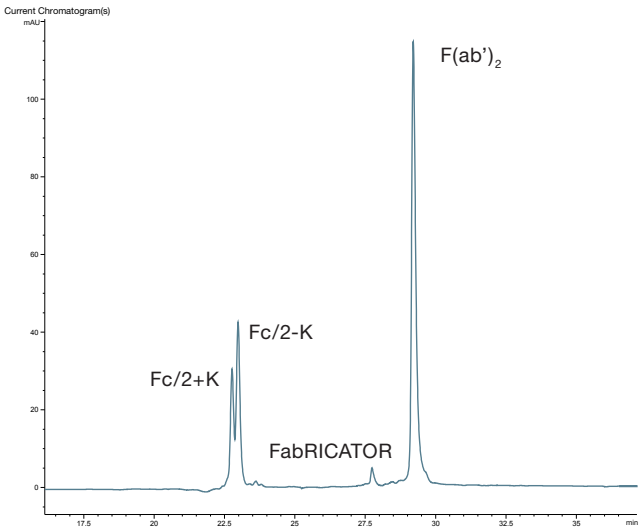
Sample Preparation Workflow



FabRICATOR sample preparation workflow for LC/MS: The IgG is first digested at 37°C for 30 min to generate $F(ab')_2$ and Fc fragments. These are then further reduced and denatured to completely separate the antibody subunit domains.

Determining the Degree of Lysine Clipping

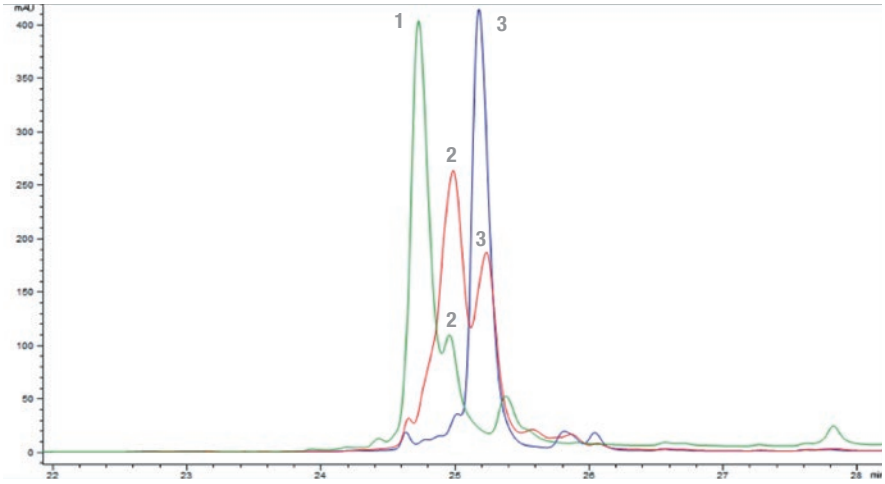
PTMs such as oxidation, pyroglutamation, deamidation and lysine clipping can be rapidly determined by domain-specific characterization of IgG subunits with RP-HPLC. Specific hinge digestion of cetuximab with FabRICATOR generates precise $F(ab')_2$ and Fc fragments. Reversed phase analysis of the antibody subunits resolves Fc/2 with different charges and allows for determination of the degree of lysine clipping.



Lysine clipping - reversed phase chromatogram of cetuximab digested with FabRICATOR.

Determining the Degree of Oxidation

Bevacizumab subjected to oxidizing reaction conditions and subsequent analysis of subunits with RP-HPLC readily establishes the degree of oxidation over time. Specific hinge digestion of bevacizumab with FabRICATOR generates precise $F(ab')_2$ and Fc fragments. As the oxidation proceeds, the amount of oxidized antibody increases, which is seen as a shift in retention time.



Non-oxidized Fc
1 hour oxidation
6 hour oxidation

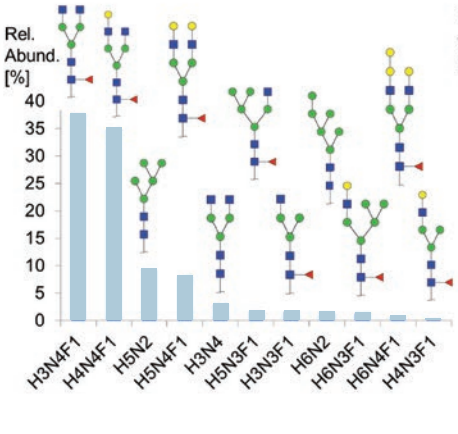
1 Met 252 & Met 428 ox
2 Met 252 ox
3 Non-oxidized Fc

Oxidation - reversed phase chromatogram of bevacizumab Fc exposed to oxidizing reaction conditions.

Quantification of Glycans on Cetuximab

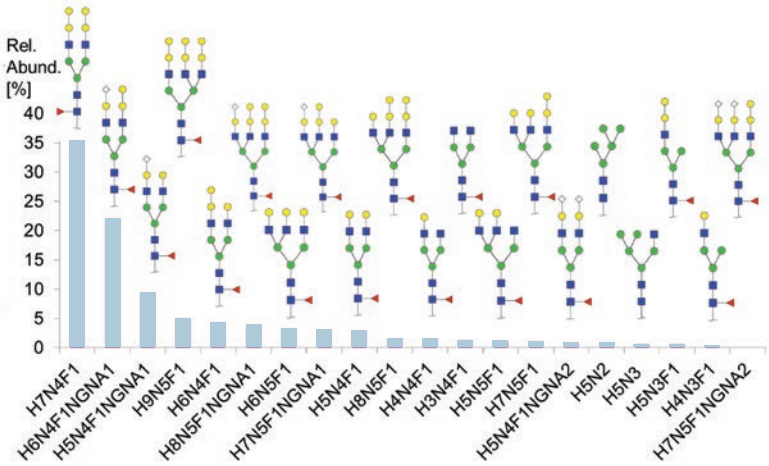
In Ayoub et al., the glycan profile of the individual domains of cetuximab was determined with the subunit domain mapping method and Bruker MaXis UHR-MS. It was shown that cetuximab is glycosylated in both the Fab and the Fc domains. 11 glycans on the Fc and 20 glycans on the Fd were readily quantified. No additional digestion or labeling was needed for similarity assessment (1).

Fc domain



Relative abundance of different glycans on the Fc domain.

Fd domain



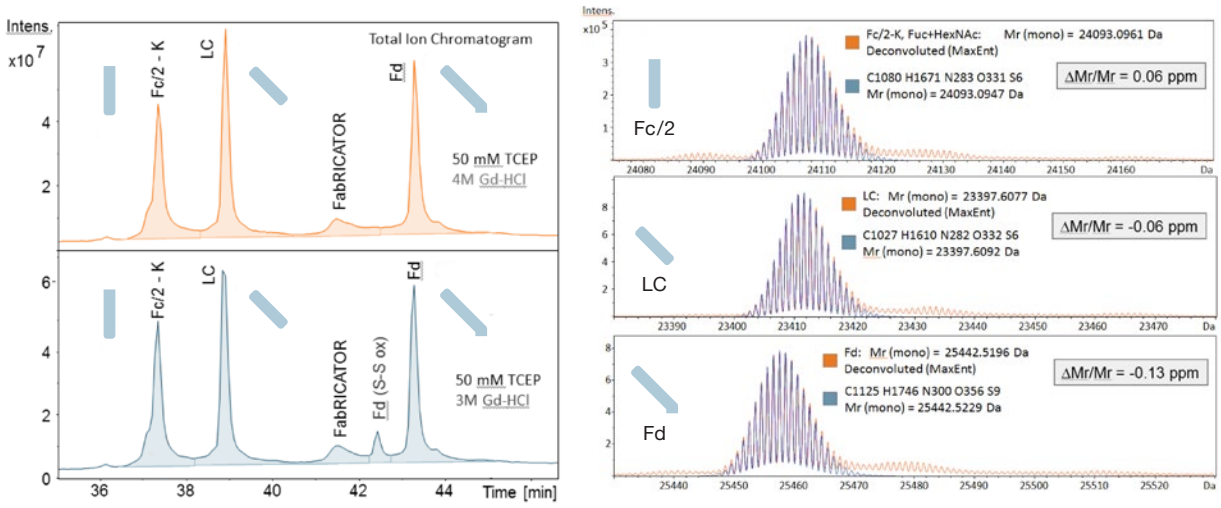
Relative abundance of different glycans on the Fd domain.

Reference

1. Ayoub D, Jabs W, Resemann A, Evers W, Evans C, Main L, Baessmann C, Wagner-Rousset E, Suckau D and Beck A. Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle down and bottom-up ESI and MALDI mass spectrometry techniques. MAbs 2013 (5:5) 699-710.

High Resolution LC/MS for Amino Acid Sequence Verification

State-of-the-art mass spectrometers with high resolving power allow for amino acid verification of mAbs. Precise antibody subunit fragments (Fc/2, LC and Fd) generated with FabRICATOR can be mono-isotopically resolved.



LC/MS on adalimumab analyzed by UHR-MS Bruker MaXis 4G. The theoretically calculated peaks are in red and the experimentally measured peaks are overlaid in blue. The values are in very good agreement and the mass error is very low.

Application Areas

The method is applicable in

- Clone selection
- Production monitoring
- Quality control
- Stability testing

For characterization of

- mAbs
- ADCs
- Biosimilars
- Fc-fusion proteins

Selected References

Review

Sjögren J, Olsson F and Beck A. Rapid and improved characterization of therapeutic antibodies and antibody related products using IdeS digestion and subunit analysis. Analyst 2016 (23:141) 3114-3125.

Monoclonal antibodies

Beck A, Wagner-Rousset E, Aoyub D, van Dorsselaer A and Sanglier-Cianférani S. Characterization of therapeutic antibodies and related products. Anal Chem 2013 (85) 715-736.

Ayoub D, Jabs W, Resemann A, Evers W, Evans C, Main L, Baessmann C, Wagner-Rousset E, Suckau D and Beck A. Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle down and bottom-up ESI and MALDI mass spectrometry techniques. MAbs 2013 (5:5) 699-710.

An Y, Zhang Y, Mueller H-M, Shameem M, Chen X. A new tool for monoclonal antibody analysis: Application of IdeS proteolysis in IgG domain specific characterization. MAbs 2014 (6:4).

Fc-fusions

Lynaugh H, Li H and Gong B. Rapid Fc glycosylation analysis of Fc fusions with IdeS and liquid chromatography mass spectrometry. MAbs 2013 (5:5) 641-645.

Beck a, Diemer H, Aoyub D, Debaene F, Wagner-Rousset E, Carapito C, van Dorsselaer A and Sanglier-Cianférani S. Analytical characterization of biosimilar antibodies and Fc-fusion proteins. Trends Anal Chem 2013 (48) 81-94.

ADC

Wagner-Rousset E, Janin-Bussat M, Colas O, Excoffier M, Aoyub D, Haeuw J, Rilatt I, Perez M, Corvaia N and Beck A. Antibody-drug conjugate model fast characterization by LC-MS following IdeS proteolytic digestion. MAbs 2014 (6:1) 1-12.

FabRICATOR®

Lyophilized

High-quality lyophilized FabRICATOR for rapid antibody subunit generation is available in different sizes, for digestion of 8x100 µg (strip), 2 mg or 5 mg IgG. The low endotoxin preparation of FabRICATOR (FabRICATOR LE) is suitable for use in cell/tissue-based assays.

The FabRICATOR 96-well plate allows for rapid antibody subunit generation in a high throughput format. Antibodies or proteins can be added directly to the wells for convenient and fast antibody subunit generation. The plates can be divided into individual wells to process fewer samples.

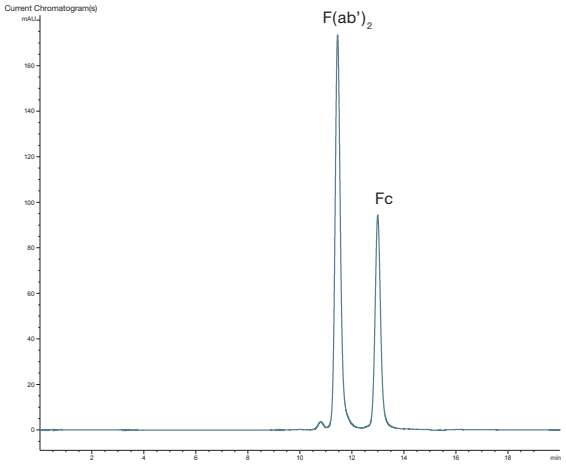
Lyophilized FabRICATOR can be custom-made regarding volumes, packaging and formats. Please contact us for details regarding your specific requirements, info@genovis.com.

	Product ID	Description	Digestion	EUR	USD
	A0-FR1-020	FabRICATOR 2,000 Units	2 mg IgG	410	570
	A0-FR1-050	FabRICATOR 5,000 Units	5 mg IgG	795	850
	A0-FR1-250	FabRICATOR 5 x 5,000 Units	25 mg IgG	3,195	3,495
	A0-FR1-096	FabRICATOR 96x100 Units	96 x 100 µg	1,650	2,115
	A0-FR1-008	FabRICATOR 8x100 Units	8 x 100 µg	280	355
	A0-FR8-020	FabRICATOR LE (low endotoxin) 2,000 Units	2 mg IgG	450	625
	A0-FR8-050	FabRICATOR LE (low endotoxin) 5,000 Units	5 mg IgG	875	925

Validation Kit

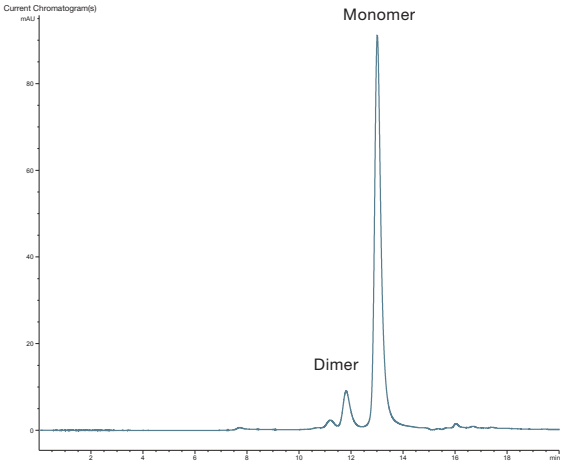
Three different production batches of lyophilized FabRICATOR are included in the FabRICATOR Validation kit for evaluation of FabRICATOR batch-to-batch consistency. The purity and enzyme activity of the three different batches are established with SEC and SDS-PAGE.

Activity



Activity of FabRICATOR is determined by digestion of mAb (Humira) and HPLC-SEC analysis of antibody subunit fragments.

Purity



Purity of FabRICATOR is determined by HPLC-SEC.

	Relative %
Intact/semi-digested ab	< 1 %
$F(ab')_2$	63.3 %
Fc	35.8 %
Summary mAb Digested	99 %

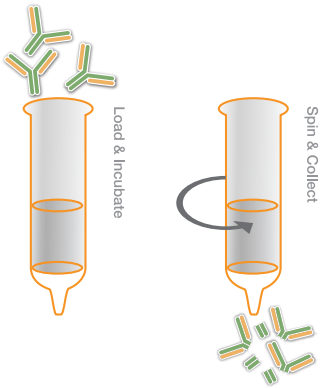
Monomer	86.5 %
Dimer	11.2 %
Aggregates	2.2 %

	Product ID	Description	Digestion	EUR	USD
	A0-FR4-060	FabRICATOR 3 x 2,000 Units	3 x 2 mg	1,230	1,710

FragIT™

Immobilized FabRICATOR

FragIT is a rapid and easy way of creating $F(ab')_2$ and Fc fragments from IgG. FragIT is the FabRICATOR enzyme immobilized on agarose, which allows for antibody subunit generation without FabRICATOR in the final sample preparation. The spin columns are provided prefilled with immobilized FabRICATOR, for digestion of small amounts up to hundreds of mg of antibody or Fc-fusion protein.

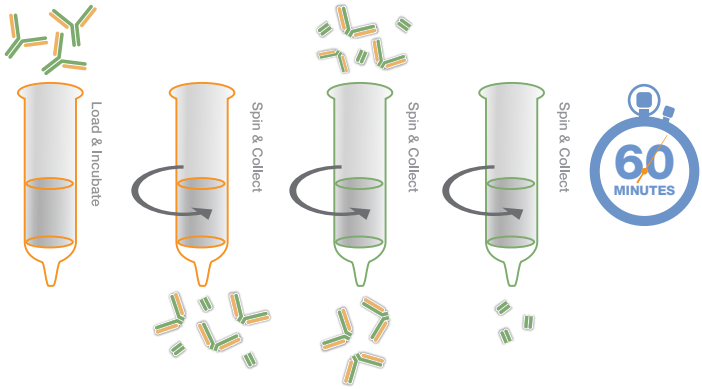


	Product ID	Description	Digestion	EUR	USD
	A0-FR6-010	FragIT Microspin	2 x 0.5 mg	305	425
	A0-FR6-025	FragIT Microspin	5 x 0.5 mg	695	960
	A0-FR6-050	FragIT Microspin	10 x 0.5 mg	1,150	1,605
	A0-FR6-100	FragIT Midispin	1-10 mg	925	1,285
	A0-FR6-1000	FragIT Maxispin	10-100 mg	2,760	3,860

FragIT™ kit

Immobilized FabRICATOR

FragIT kit can be used for rapid preparation and isolation of IgG F(ab')$_2$ and Fc fragments, with a very high yield. It consists of two spin columns – one column for fragmentation (FragIT) and one column for separation and isolation of F(ab')$_2$ and Fc fragments (CaptureSelect™*). The CaptureSelect column has affinity ligands selective for Fc fragments.



*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.

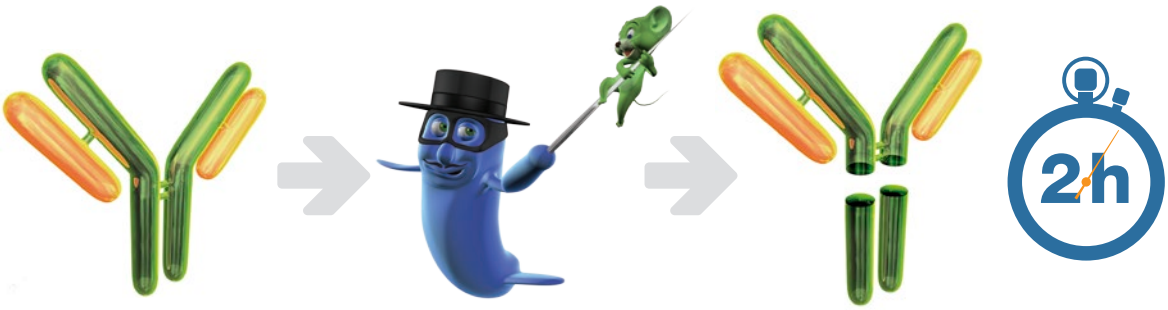
	Product ID	Description	Digestion & Purification	EUR	USD
	A2-FR2-005	FragIT kit	0.5 mg IgG	340	475
	A2-FR2-025	FragIT kit	5 x 0.5 mg IgG	925	1,285
	A2-FR2-100	FragIT kit	10 mg IgG	1,155	1,610
	A2-FR2-1000	FragIT kit	100 mg IgG	3,465	4,845

Anti-FabRICATOR™

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

	Product ID	Description	Concentration	EUR	USD
	A3-AF1-010	Anti-FabRICATOR 0.1 ml	4 mg/ml	240	330

FabRICATOR® Z



...CPAPNLLG / GPSVF...

FabRICATOR Z (IdeZ) is a cysteine protease that is very efficient in digesting mouse IgG2a at one specific site just below the hinge.

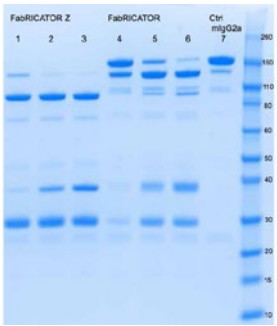
FabRICATOR Z is derived from *Streptococcus equi* ssp. *zooepidemicus.* It digests mouse IgG2a and IgG3, and produces a homogenous pool of $F(ab')_2$ and Fc fragments after only 2 hours of incubation. Some mouse IgG2a that FabRICATOR fails to digest, are readily digested by FabRICATOR Z, but longer incubation times may be required. There is no risk of over-digestion of the fragments because of the high specificity of the enzyme.

FabRICATOR Z – Efficient Digestion of Mouse IgG2a

- More efficient than FabRICATOR on mouse IgG2a
- Works at neutral pH
- No risk of overdigestion

Digestion of Mouse IgG2a using FabRICATOR Z

Three different amounts of FabRICATOR Z (IdeZ) and FabRICATOR (IdeS) were used to digest a mouse IgG2a (see picture to the right). After 2 hours of incubation, it is evident that FabRICATOR Z (IdeZ) readily digests mouse IgG2a, while FabRICATOR only digests a small amount. $F(ab)'_2$ is detected at approximately 110 kDa and Fc fragments at approximately 30 kDa. The enzyme is detected at approximately 37 kDa.



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

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

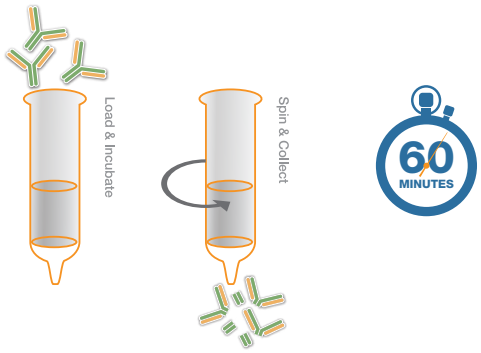
Lane 7: Non-digested mouse IgG2a

	Product ID	Description	Digestion	EUR	USD
	A0-FRZ-020	FabRICATOR Z, 2000 units	2 mg mouse IgG2a	410	570

FragIT™Z

Immobilized FabRICATOR Z

FragIT Z is a rapid and easy way of creating F(ab')$_2$ and Fc fragments from mouse IgG2a. FragIT Z is the FabRICATOR Z enzyme immobilized on agarose, which allows for antibody subunit generation without FabRICATOR Z in the final sample preparation. The spin columns are provided prefilled with immobilized FabRICATOR Z for digestion of 0.5 mg IgG2a.



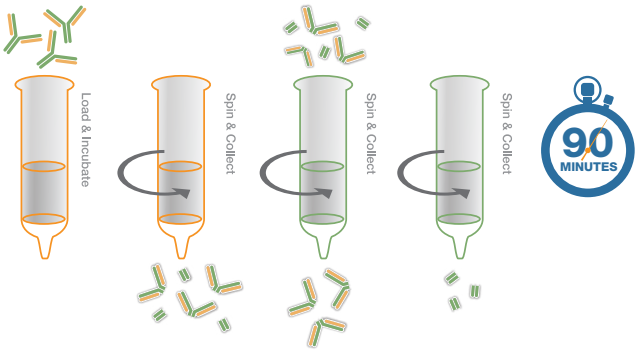
Product ID	Description	Digestion	EUR	USD
A0-FZ6-010	FragIT Z Mircospin	2 x 0.5 mg	305	425
A0-FZ6-025	FragIT Z Mircospin	5 x 0.5 mg	695	960
A0-FZ6-050	FragIT Z Mircospin	10 x 0.5 mg	1,150	1,605

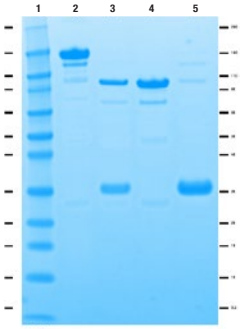
FragIT™Z kit

Immobilized FabRICATOR Z

FragIT Z kit is for a rapid preparation and separation of mouse IgG2a F(ab')$_2$ and Fc fragments, with a very high yield. It consists of two spin columns – one column for fragmentation (FragIT Z) and one column for separation and isolation of F(ab')$_2$ and Fc fragments (CaptureSelect™*). The CaptureSelect column has affinity ligands selective for Fc fragments.

*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.





1. MW marker

2. Mouse IgG2a

3. F(ab')$_2$ and Fc from FragIT Z digested mouse IgG2a

4. F(ab')$_2$ (Flow through from CaptureSelect purification)

5. Fc purified on CaptureSelect

Digestion and Purification of Mouse IgG2a using FragIT Z kit

A mouse IgG2a was digested using FragIT Z kit (see picture to the right). The F(ab')$_2$ was purified and separated from the Fc fragments using the CaptureSelect column, and finally the purified Fc fragments were eluted.

Product ID	Description	Digestion	EUR	USD
A2-FZ6-005	FragIT Z kit	0.5 mg mouse IgG2a	340	475
A2-FZ6-025	FragIT Z kit	5 x 0.5 mg mouse IgG2a	925	1,285

FabALACTICA™

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

FabALACTICA is an IgG1-specific cysteine protease that digests above the hinge and does not require reducing conditions or co-factors. FabALACTICA can be applied to generate intact and homogenous Fab and Fc fragments from human IgG1. The name of the enzyme FabALACTICA is derived from the pathogen *Streptococcus agalactiae*, where it was first discovered (Spoerry et al. 2016). The use of proteases with high specificity for IgG has allowed for subunit profiling of antibody-based therapeutics and the study of key quality attributes using LC/MS.

Spoerry C, Hessle P, Lewis MJ, Paton L,Woof JM, von Pawel-Rammingen U (2016) Novel IgG-Degrading Enzymes of the IgdE Protease Family Link Substrate Speci city to Host Tropism of Streptococcus Species. PLoS ONE 11(10): e0164809. doi:10.1371/journal.pone.0164809

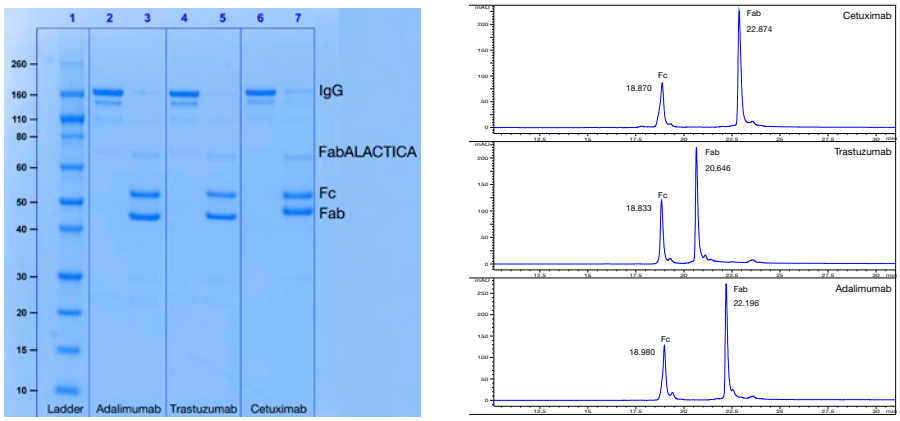
Key Characteristics

- Intact Fab fragments from human IgG1
- High specificity for digestion above the hinge of human IgG1
- Active in pH 6-8
- No need for reducing agents or co-factors
- No overdigestion

Applications of FabALACTICA

- Subunit LC/MS analysis
- Paired glycan analysis
- Characterization of bi- or multispecific antibodies
- Affinity screening
- Studies of higher order structures
- Studies on antibodies with mutated hinges

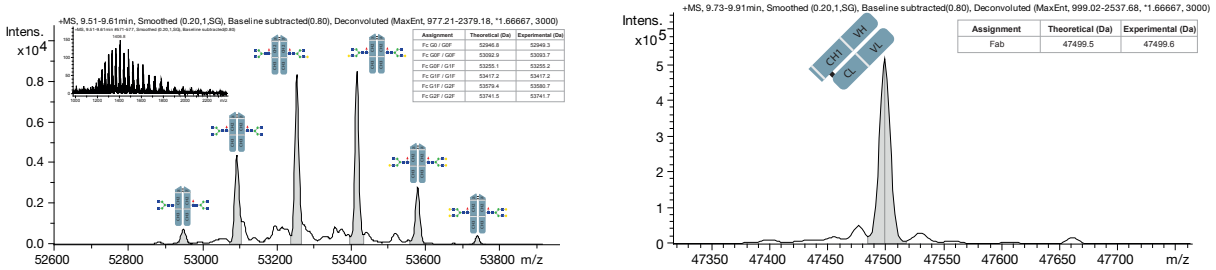
Intact Fc and Fab Fragments from Therapeutic mAbs using FabALACTICA



A B

Figure 1. Digestion of cetuximab, trastuzumab, and adalimumab with FabALACTICA O/N at 37°C. **A)** Non-reduced SDS-PAGE. **B)** Separation of intact Fc and Fab fragments on RP-HPLC. HPLC was performed on Agilent 1260 using Waters Acquity UPLC® BEH300 C4, 1.7 µm, 2.1x100 mm column in an acetonitrile/isopropanol gradient at 60°C.

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



A B

Figure 2. Trastuzumab was digested with 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. The digested sample wasseparated by RP-HPLC (Agilent 1200 system) using a 2.1x7.5 mm Poroshell 300SB-C8 column (Agilent) coupled online to a Q-TOF mass spectrometer (Bruker Maxis Impact). The obtained raw spectra were smoothed, baseline corrected and deconvoluted using the MaxEnt algorithm to determine the masses.

FabALACTICA™

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

	Product ID	Description	Digestion	EUR	USD
	A0-AG1-020	FabALACTICA, 2000 units	2 mg	480	595

GingisKHAN®

GingisKHAN (Kgp) is a unique cysteine protease that digests human IgG1 at one single amino acid position just above the hinge.

The enzyme is very specific and selective. Digestion of human IgG1 with GingisKHAN generates a homogenous pool of precise Fab and Fc fragments (*Figure 1* and *2*). There is no overdigestion or further degradation of the fragments that is typically seen with other proteolytic enzymes. Very mild reducing reaction conditions give intact Fab and Fc fragments.

GingisKHAN is easy to use, no optimization of reaction conditions is needed. A 60 minute digestion protocol is generally applicable. Sample preparation-induced modifications in the antibody characterization are minimized due to mild reaction conditions and a rapid digestion protocol.

GingisKHAN – Rapid Subunit Generation of Human IgG1

- Specific – one precise digestion site in the upper hinge of human IgG1
- Rapid – a 60 minute protocol for complete digestion
- Provided with ready-to-use reaction buffer additive, for convenient and reliable performance

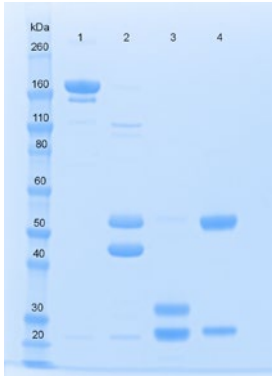


Figure 1. Digestion of trastuzumab with GingisKHAN for 1 hour at 37°C under slightly reducing conditions (2 mM L-Cysteine). From left to right trastuzumab intact (1), trastuzumab GingisKHAN non-reduced (2), trastuzumab GingisKHAN reduced (3), trastuzumab reduced (4).

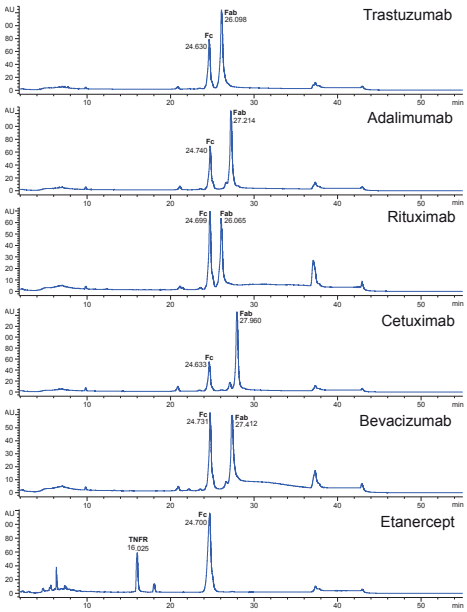
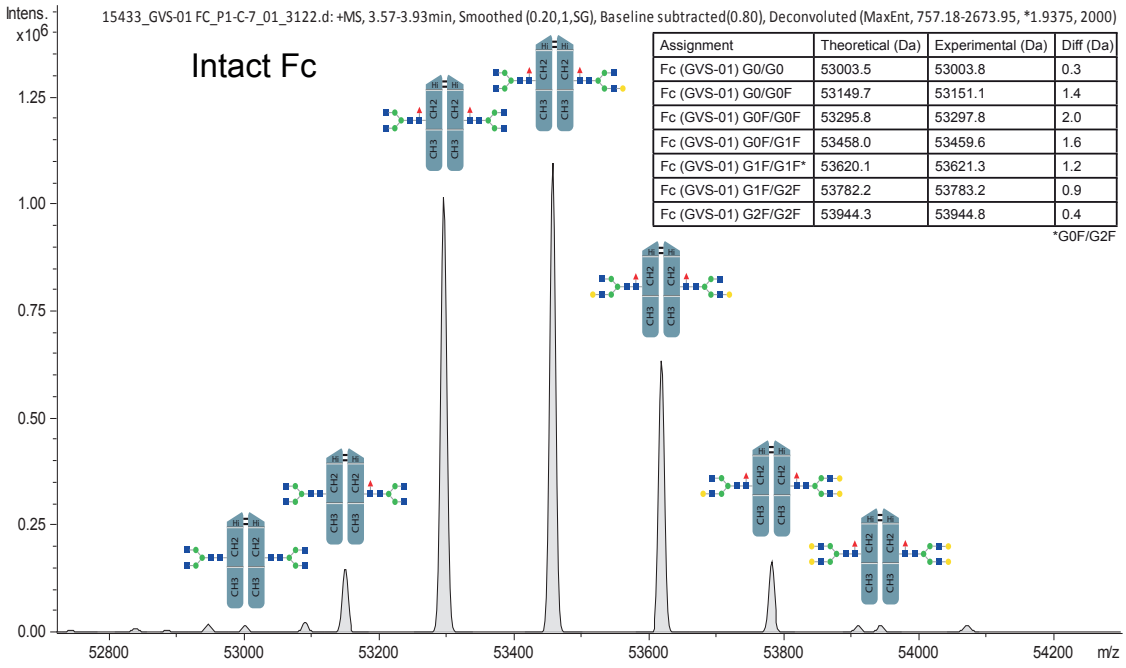


Figure 2. Digestion of five clinically approved human/humanized IgG1 and one Fc-fusion protein by GingisKHAN shows the applicability of the enzyme over a wide variety of different human IgG1. From top to bottom; trastuzumab, adalimumab, rituximab, cetuximab, bevacizumab and etanercept.

GingisKHAN for Characterization of Monclonal Antibody-Based Biotheraputics using MS

- Rapid Fc glycan analysis
- Light and heavy chain pairing of bispecific antibodies
- General PTM identification



Assignment	Theoretical (Da)	Experimental (Da)	Diff (Da)
Fc (GVS-01) G0/G0	53003.5	53003.8	0.3
Fc (GVS-01) G0/G0F	53149.7	53151.1	1.4
Fc (GVS-01) G0F/G0F	53295.8	53297.8	2.0
Fc (GVS-01) G0F/G1F	53458.0	53459.6	1.6
Fc (GVS-01) G1F/G1F*	53620.1	53621.3	1.2
Fc (GVS-01) G1F/G2F	53782.2	53783.2	0.9
Fc (GVS-01) G2F/G2F	53944.3	53944.8	0.4

*G0F/G2F

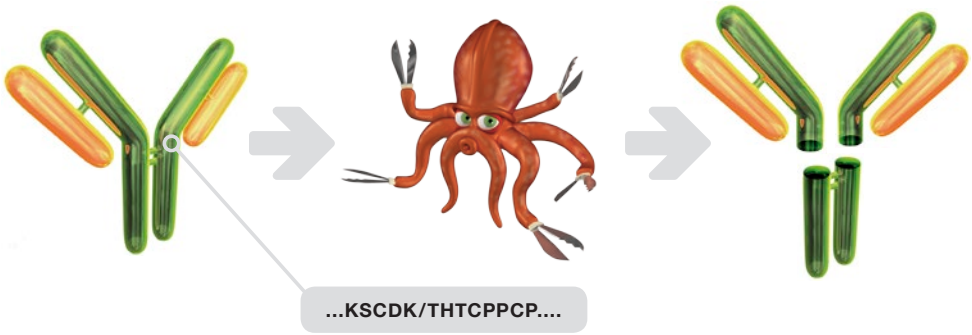
Fig 3. Identification of major glycoforms on intact Fc with LC/MS. The intact Fc of trastuzumab was generated with GingisKHAN.

GingisKHAN®

GingisKHAN digests human IgG1 at one specific site just above the hinge. The enzyme is provided as a lyophilized powder together with 5 vials of lyophilized GingisKHAN reducing agent, for convenient and reliable performance.

	Product ID	Description	Digestion	EUR	USD
	B0-GKH-020	GingisKHAN, 2,000 units	2 mg hIgG1	410	570

GingisKHAN® Fab kit



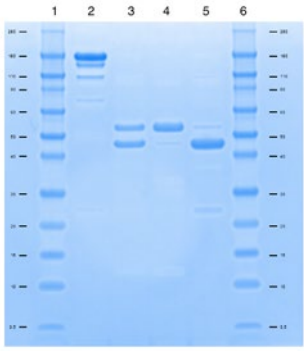
GingisKHAN Fab kit is used for rapid and easy preparation and purification of Fab fragments from human IgG1.

The GingisKHAN (Kgp) enzyme digests human IgG1 above the hinge region, generating intact Fab and Fc fragments. Mild reducing conditions (2 mM cysteine) are used to obtain intact Fab and Fc fragments.

The spin columns, with CaptureSelect™ * CH1 resin, specifically binds the CH1 domain of the intact Fab fragments. This allows for separation of the intact Fab from the Fc and GingisKHAN in the reaction mixture. The Fab fragments are eluted and ready to use.

GingisKHAN Fab kit – Intact Fab Fragments from Human IgG1

- Rapid – a 90 minute protocol for complete digestion and purification
- High yield – typically 90% recovery of Fab fragments
- Provided with ready-to-use reaction buffer additive, for convenient and reliable performance



SDS-PAGE analysis of purified Fab fragments from mono-clonal hIgG1 Herceptin using GingisKHAN FabKit. Lane 1 and 6; MW marker, Lane 2; intact human IgG1, Lane 3: Fab and Fc fragments after GingisKHAN digestion, Lane 4: flow-through Fc fragments and GingisKHAN, Lane 5: eluted Fab fragments.

Applications of GingisKHAN Fab kit

- Preparation of intact Fab fragments
- Study monovalent binding
- Light and heavy chain pairing of bispecific antibodies
- Paired glycan analysis of the intact Fc fragment

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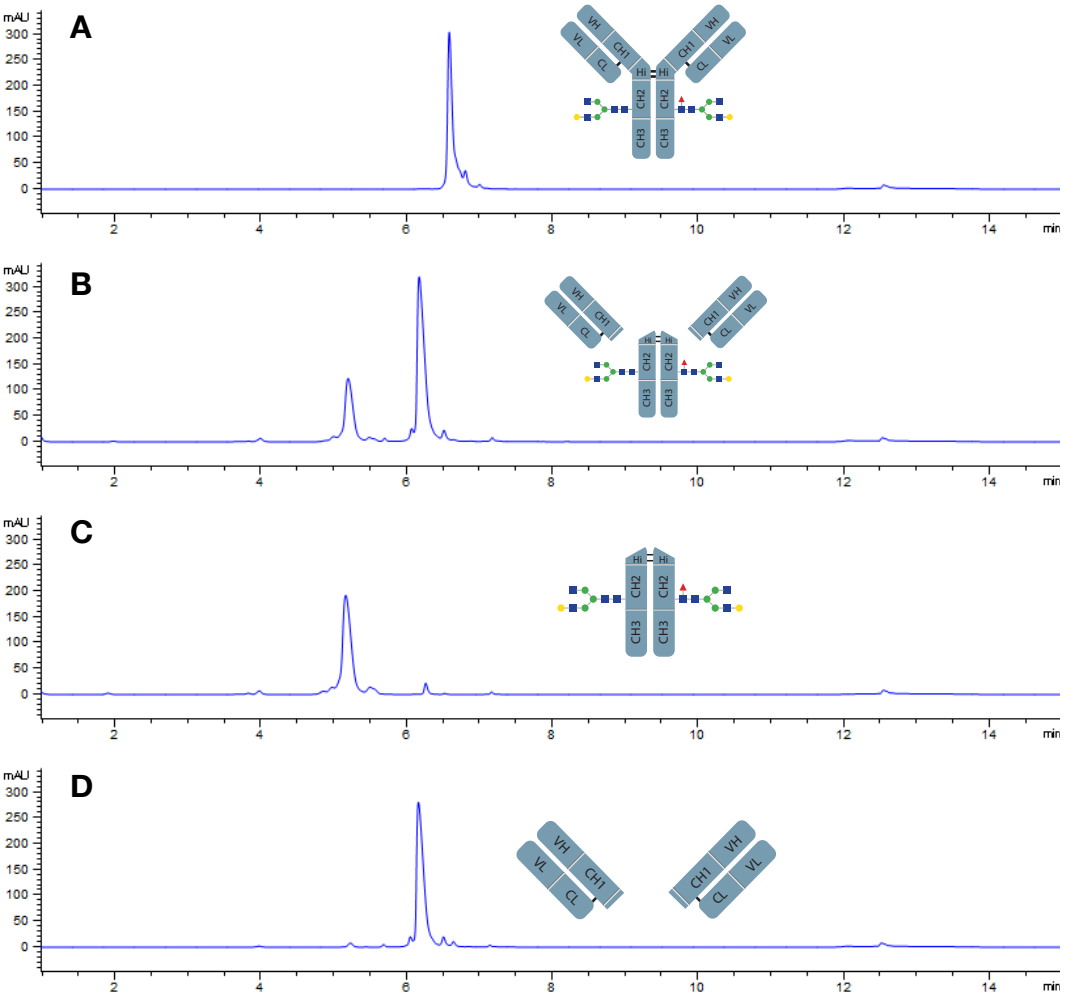


Digestion and purification of Fab fragments using GingisKHAN FabKit. **A)** Intact monoclonal IgG1 antibody. **B)** Analysis of antibody fragments after GingisKHAN digestion. **C)** The digestion mixture was added to the CH1-specific CaptureSelect™ column, and the flow-through Fc fragment is shown. **D)** Elution of the purified Fab fragments. Digestion and purification were analyzed on RP-UHPLC (Agilent 1260) using Supelco C4 bioshell A4.

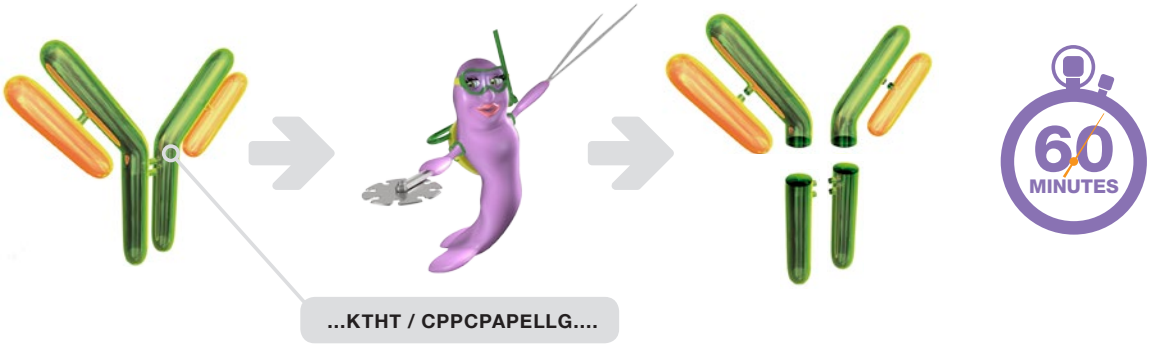
GingisKHAN® Fab kit

GingisKHAN Fab kit is used for rapid and easy preparation and purification of Fab fragments from human IgG1 antibodies. The kit consists of three components; lyophilized GingisKHAN enzyme, reducing agent and affinity spin columns for purification of intact Fab fragments (CaptureSelect™ CH1).

	Product ID	Description	Digestion	EUR	USD
	B0-GFK-020	GingisKHAN Fab kit, 2,000 units	2 mg hIgG1	925	995

FabULOUS®

Antibody Subunit Generation



FabULOUS (SpeB) is a cysteine protease that digests IgG from many different species and subclasses, using a fast and generally applicable protocol.

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 easy protocol is of great benefit, and it saves valuable time since there is no need for optimizing the digestion protocol. Classical cysteine proteases such as papain, ficin and pepsin usually require such optimization.

The enzyme requires reduced conditions for optimal activity, why the reaction is performed in the presence of 1-5 mM DTT or TCEP at neutral pH. Since the reducing agent is present during the reaction, it is likely that interchain thiols will be reduced. The enzyme works in most common buffers.

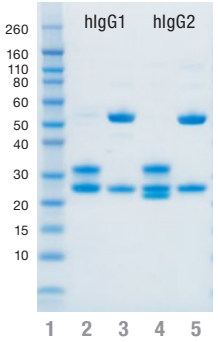
- **Upper hinge digestion of IgG**
- **Works on several IgG species, e.g. human, mouse, rat and goat**
- **A rapid general protocol – 1 hour digestion**

List of IgG / Species Digested by FabULOUS

Human IgG	Mouse IgG
Humanized IgG	Rat IgG
Chimeric IgG	Sheep IgG
ADC	

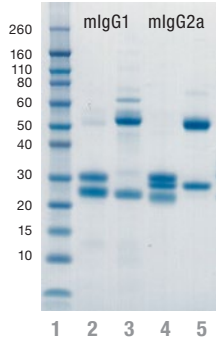
FabULOUS Digestion

Human IgG



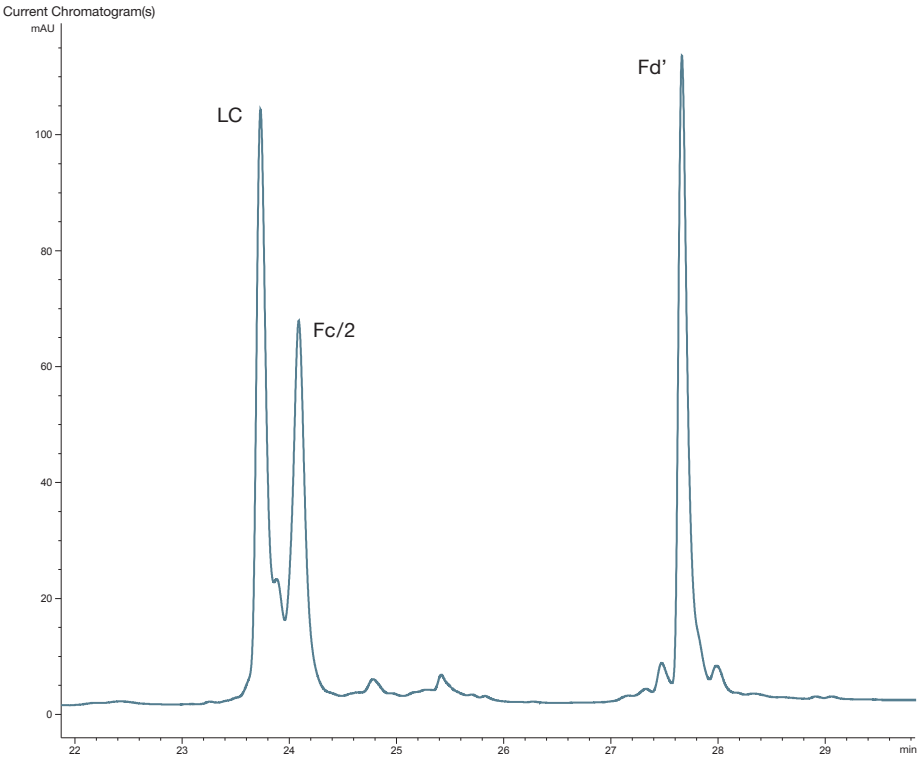
1 MW marker
2 hIgG1 **FabULOUS**
3 hIgG1
4 hIgG2 **FabULOUS**
5 hIgG2

Mouse IgG



1 MW marker
2 mouse IgG1 **FabULOUS**
3 mouse IgG1
4 mouse IgG2a **FabULOUS**
5 mouse IgG2a

FabULOUS Digestion of mAb



The monoclonal antibody Humira® was digested with FabULOUS at 37°C for 1h under reducing conditions. The resulting fragments, LC, Fc/2 and Fd were separated with reversed phase chromatography.

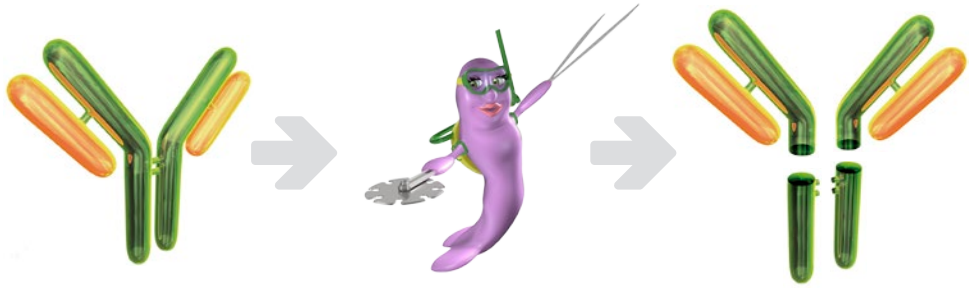
FabULOUS®

Lyophilized

FabULOUS is used for antibody fragmentation in the upper hinge region. The enzyme is available as a lyophilized preparation for digestion of 2 mg IgG.

	Product ID	Description	Digestion	EUR	USD
	A0-PU1-020	FabULOUS 2,000 Units	2 mg IgG	410	570

FabULOUS® Fab kit



FabULOUS® Fab kit is used for rapid and easy purification of Fab fragments from mouse IgG antibodies.

The FabULOUS (SpeB) enzyme digests in the hinge region of IgG from many species, including mouse. The FabULOUS Fab kit is designed for digestion and purification of Fab fragments from mouse IgG antibodies.

The FabULOUS Fab kit consists of 2000 units of the FabULOUS enzyme for digestion of 2 mg of IgG. Included in the kit are CaptureSelect™* LC-Kappa (mur) affinity purification spin columns for easy purification of the prepared Fab fragments from mouse IgG. We also have CaptureSelect™ LC-Lambda (mouse) affinity columns available.

FabULOUS Fab kit – Intact Fab Fragments from Mouse IgG

- Digestion in the hinge with FabULOUS
- Affinity purification of Fab fragments using the light chain
- Preparation of Fab fragments from mouse IgG in less than 2h

Applications of FabULOUS Fab kit

- Preparation of intact Fab fragments from mouse IgG
- Monovalent binding studies
- Elimination of Fc-mediated effector functions
- Reduced unspecific binding from Fc interactions

Affinity Purification of Mouse IgG1 Fab Fragments

Fragmentation and purification of Fab fragments from mouse IgG1 were carried out using the FabULOUS Fab kit. First, the mouse IgG1 antibody was digested at 50 mM cysteine using the FabULOUS enzyme. Then, the Fab fragments were captured on a CaptureSelect™* LC-Kappa (mur) affinity spin column. The flow-through contained the Fc fragments. In an elution step, the intact Fab fragments were purified (shown on the SDS-PAGE analysis in *Figure 1)*. As an orthogonal method, the intact IgG1, the FabULOUS-digested mouse IgG1, and the purified Fab fragments were separated on RP-HPLC, as shown in *Figure 2*.

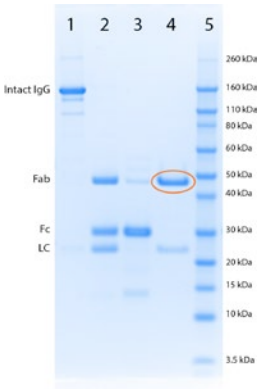


Figure 1. Non-reducing SDS-PAGE analysis of purified Fab fragments from monoclonal mIgG1 using FabULOUS Fab kit. Lane 1; intact mIgG1, Lane 2; digested mIgG1, Lane 3; flow through from CaptureSelect column, Lane 4; eluted Fab fragments, Lane 5; MW marker.

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

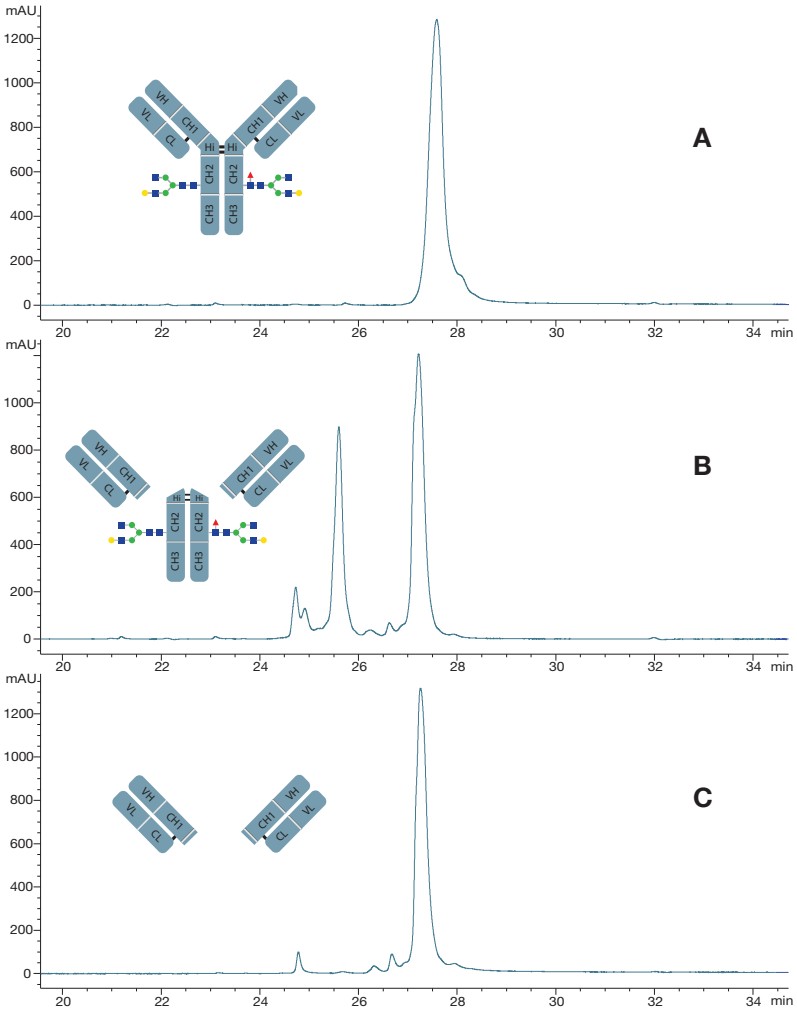


Figure 2. A) Intact monoclonal mouse IgG1 antibody. **B)** Analysis of fragments after FabULOUS Fab kit digestion. **C)** Eluted Fab fragments from the CaptureSelect™ LC-Kappa column. Digestion and purification were analyzed on RP-HPLC (Agilent 1290) using Waters Acquity UPLC® BEH300 C4.

FabULOUS® Fab kit

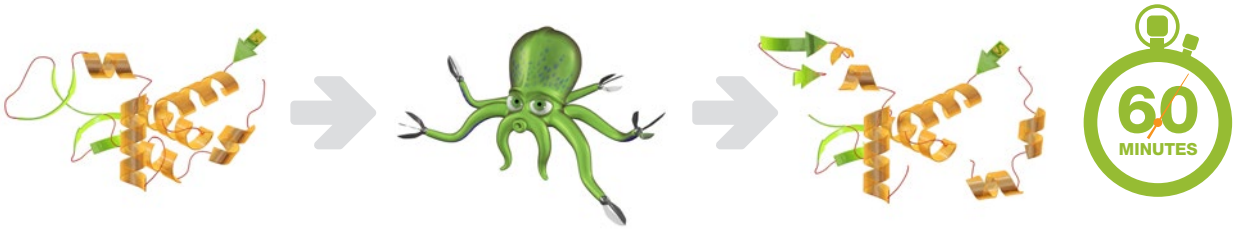
- 2000 units of the FabULOUS enzyme, which digests 2 mg mouse IgG
- 4 x 0.5 mg CaptureSelect™ LC-Kappa (mur) affinity spin columns

FabULOUS Fab kit is used for rapid and easy preparation and purification of Fab fragments from mouse IgG. The kit contains two components; lyophilized FabULOUS enzyme and CaptureSelect™* LC-kappa (mur) affinity spin columns.

	Product ID	Description	Digestion	EUR	USD
	A1-PFK-020	FabULOUS Fab kit mouse, 2000u	2 mg	695	750

GingisREX™

Arginine-Specific Protease



GingisREX (Rgp) protease digests proteins C-terminally of arginine residues. This enzyme can be used for analyzing proteins in mass spectrometry for the use in e.g. peptide mapping, protein fingerprinting and sequence analysis.

GingisREX specifically digests peptides and the C-terminal of proteins into arginine residues. The protease is specific for Arg-X motifs and does not have activity at lysines, as commonly observed using Arg-C (*Figure 1*). The enzymatic activity of GingisREX includes digestion of Arg-Pro linkages, linkages that are difficult to digest with other enzymes. The enzyme is active at a pH ranging from pH 5.5-9.0, and requires denaturing agents such as 6M urea and 0.1% SDS.

GingisREX Enzyme Characteristics

- Specific activity on Arg-X residues
- Superior specificity compared to Arg-C
- Active on Arg-Pro linkages
- Active in 6M urea and at pH 5.5-9.0

GingisREX – Unique Specificity for Arginine Residues

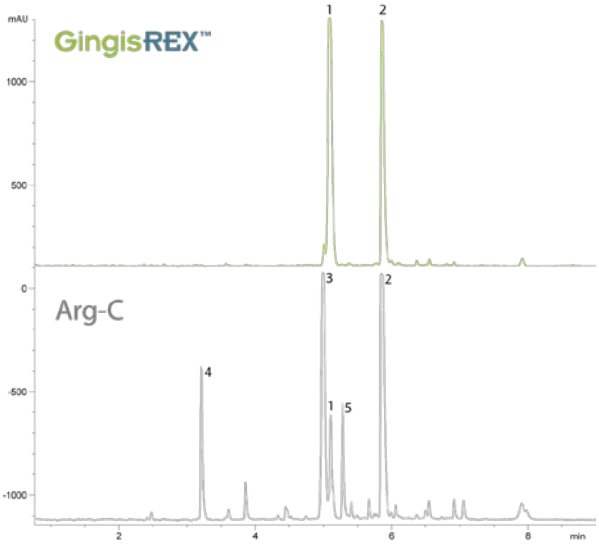


Table 1. Sequences of oxidized insulin β-chain digested by GingisREX or Arg-C, as indicated in *Figure 1*. Peptide number 6 refers to the intact oxidized β-chain of insulin.

Peak No.	Amino Acid Sequence
1	GFFYTPKA
2	FVNQHLCGSHLVEALYLVCGER
3	GFFYTPK
4	FVNQHLCGSH
5	LVEALYLVCGER + Na
6	FVNQHLCGSHLVEALYLVCGERGFFYTPKA

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

Applications of GingisREX

The protease GingisREX generates larger peptides with more charge per peptide, which is beneficial for liquid chromatography and mass spectrometry 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 (PTMs).

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 analysis. As an example, the digestion profiles of trastuzumab (Herceptin®) by GingisREX and Arg-C are presented in *Figure 2*.

Application Areas

- Peptide mapping
- Protein sequence analysis
- Protein fingerprinting
- PTM analysis

Peptide Mapping of Trastuzumab

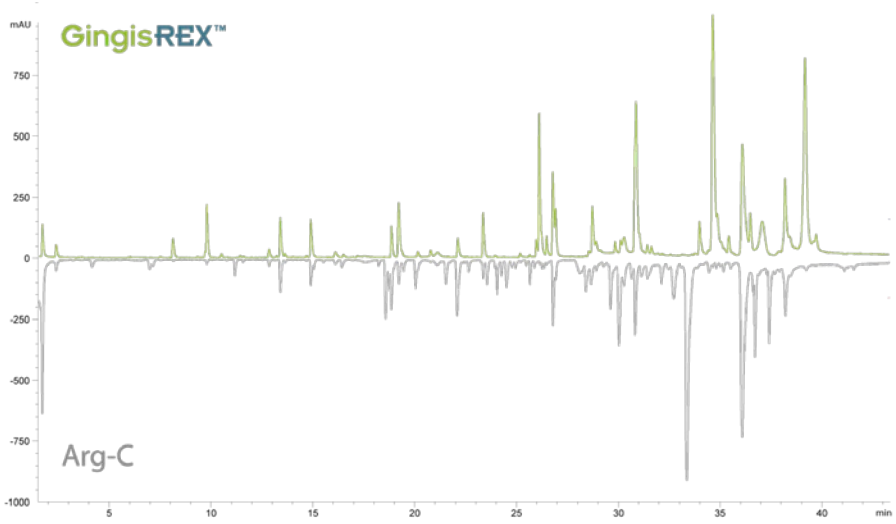


Figure 2. Peptide map of trastuzumab (Herceptin®) after digestion with GingisREX or Arg-C. Trastuzumab was incubated with GingisREX or Arg-C at 1:50 (w/w) enzyme:protein ratio O/N at 37°C at pH 7.4 (GingisKHAN) and pH 7.6 (Arg-C). The reactions were quenched, the peptides alkylated and separated on RP-HPLC.

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 5µg enzyme.

	Product ID	Description	Enzyme:Protein ratio	EUR	USD
	B0-GRX-005	GingisREX 1 x 5 ug enzyme	1:20 - 1:200	445	495

FabRICATOR®
This product is provided under an exclusive world-wide intellectual property license from Hansa Medical AB derived from international publication WO03051914, including granted US Patent No US 7,666,582 and granted European Patent No 1458861. The license encompasses IdeS from Streptococcus pyogenes for biotechnical industrial applications which are neither therapeutic nor diagnostic, other than the following exception which is included within the license: digesting IgG in vitro in clinical samples for diagnostic purposes.

Aspects of FabRICATOR® technology are also encompassed by pending patent applications in the name of Genovis AB, including those derived from international publications WO2015040125 and WO2016046220.

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US & Canada

Genovis Inc.
245 First Street, Suite 1800
Cambridge, MA 02142
USA

Customer service: 001 (617)-444-8421
Order phone (toll free): 001 (855)-782-0084
Order fax: 001 (858)-524-3006
Email: orders.us@genovis.com

EMEA & Asia

Genovis AB
Box 790
SE-220 07 Lund
Sweden

Customer service: 0046 (0)46 10 12 30
Order phone: 0046 (0)46 10 12 30
Order fax: 0046 (0)46 12 80 20
Email: order@genovis.com

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info@genovis.com | www.genovis.com