FabRICATOR®

SmartEnzymes™

GENOVIS

FabRICATOR®Z

FabULOUS®

GingisKHAN™

Smart Enzymes

FabRICATOR® (IdeS) 3

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

FabRICATOR®Z (IdeZ) 12

A cysteine protease that is very efficient in digesting mouse IgG2a at one specific site just below the hinge creating F(ab')2 fragments

GingisKHAN™ (KGP) 14

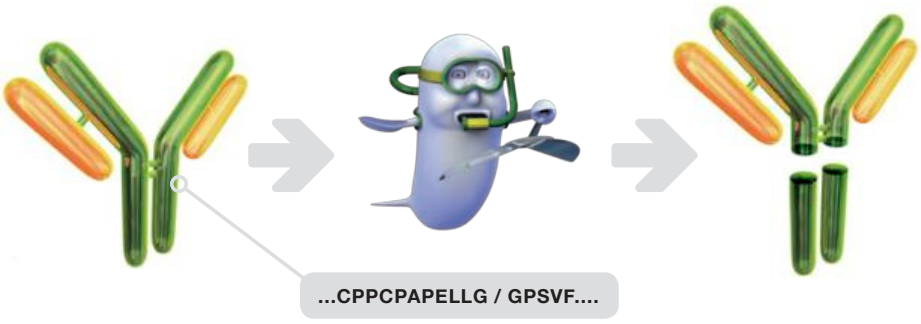
A unique cysteine protease that cleaves HUMAN IgG1 at one single amino acid position just above the hinge.

FabULOUS® (SpeB) 16

A cysteine protease that cleaves IgGs in upper hinge region

FabRICATOR®

Antibody Subunit Fragmentation



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

The enzyme is extremely specific and selective, no other substrate besides IgG is known. Cleavage of IgGs with Fabricator generates a homogenous pool of precise F(ab')$_2$ and Fc fragments and there is no over-digestion or further degradation of the fragments typically associated with other proteolytic enzymes. The reaction is performed at neutral pH without any addition of cofactors and reducing agents.

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

FabRICATOR – IgGs Cleaved

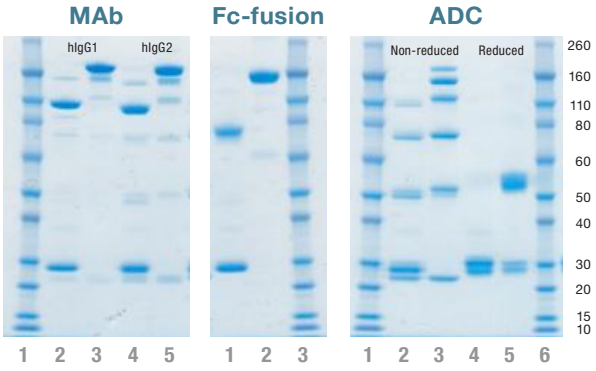
Human IgG	Mouse IgG2a*
Humanized IgG	Mouse IgG3
Chimeric IgG	Monkey IgG
Fc-fusion proteins	Rabbit IgG
ADC	Sheep IgG

** Special incubation conditions required.*

- **Specific - one precise cleavage site**
- **Rapid – 30 minute digestion**
- **Works right out of the box**

FabRICATOR Digestion

Cleavage of monoclonal antibodies, Fc-fusion proteins and antibody drug conjugates with FabRICATOR at 37°C for 30 min.



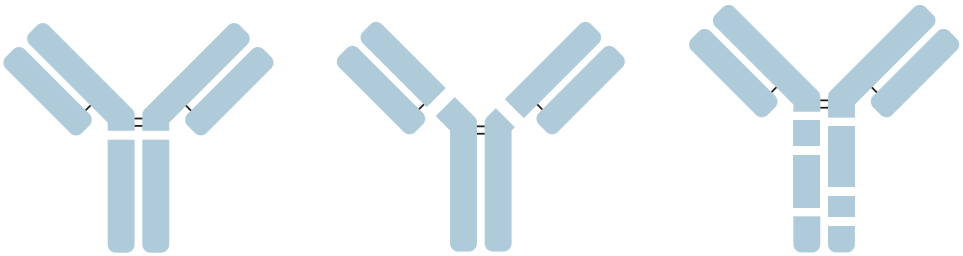
1 MW
2 hIgG1 (Avastin®) **FabRICATOR**
3 hIgG1(Avastin®)
4 hIgG2 (Vectibix®) **FabRICATOR**
5 hIgG2 (Vectibix®)

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

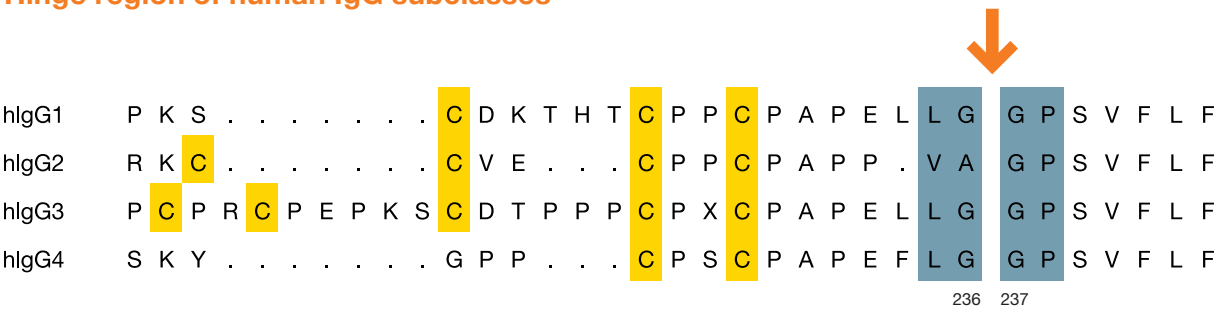
1 MW
2 ADC (Adcetris®) **FabRICATOR** non-reduced
3 ADC (Adcetris®) non-reduced
4 ADC (Adcetris®) **FabRICATOR** reduced
5 ADC (Adcetris®) reduced
6 MW

Comparison to Other Common Enzymes

FabRICATOR is the most cost-effective method to generate antibody subunits with better sensitivity and yield. Compared with 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



FabRICATOR cleavage site just below the hinge region is indicated with an arrow.

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

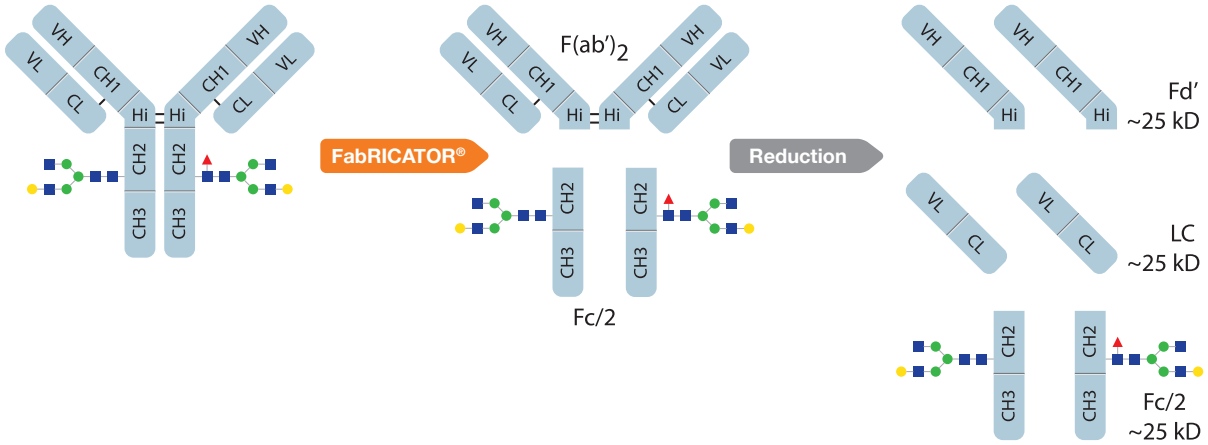
Applications of FabRICATOR - Fast Antibody Characterization

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 IgGs to subunits of approximately 25 kD, the resolution is significantly increased which allows for fast and accurate glycan profiling and determination of different PTMs. Furthermore, conjugation sites and integrity of ADCs can be rapidly established.

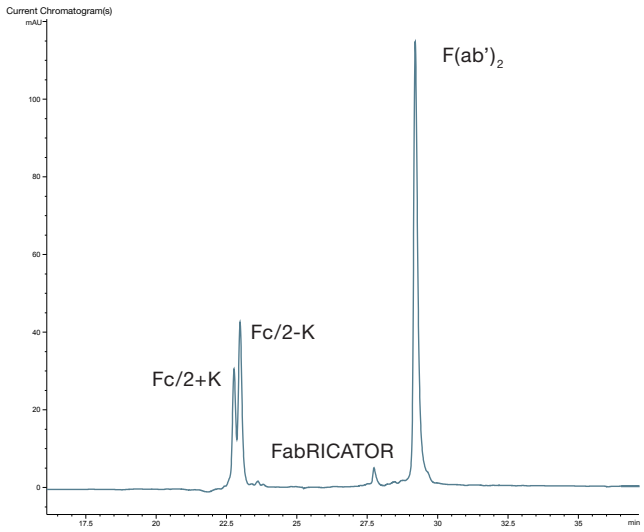
Sample Preparation Workflow



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

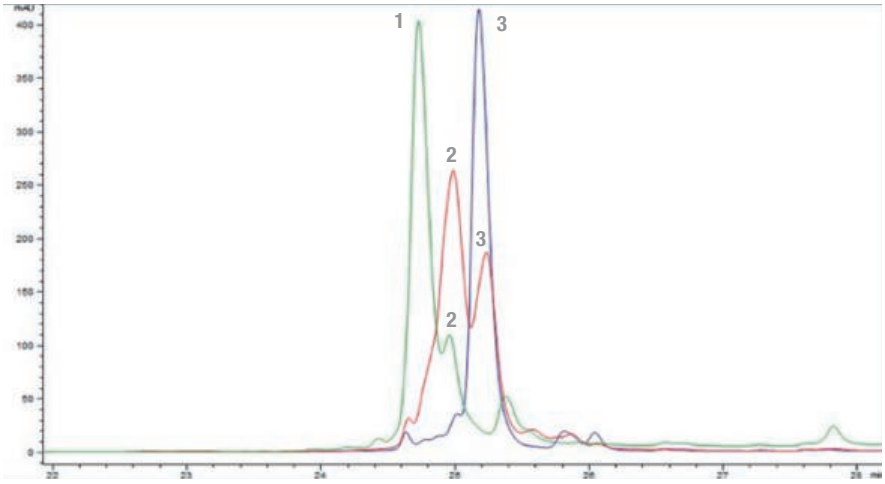
PTMs such as oxidation, pyroglutamation, deamidation and lysine clipping can be rapidly determined by domain specific characterization of IgG subunits with RP/HPLC. Specific hinge cleavage of Cetuximab with FabRICATOR generates precise $F(ab')_2$ and Fc fragments. Reversed phase analysis of the antibody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



Lysine clipping - Reversed phase chromatogram of Cetuximab cleaved 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 cleavage 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.



Unoxidized Fc
1 hour oxidation
6 hour oxidation

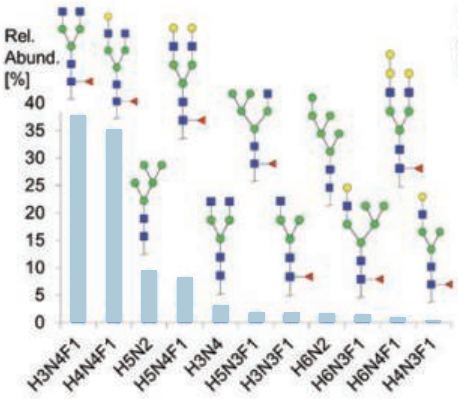
1 Met 252 & Met 428 ox
2 Met 252 ox
3 Unoxidized Fc

Oxidation - Reversed phase chromatogram of Bevacizumab Fc exposed to oxidizing reaction conditions.

Quantification of Glycans on Cetuximab

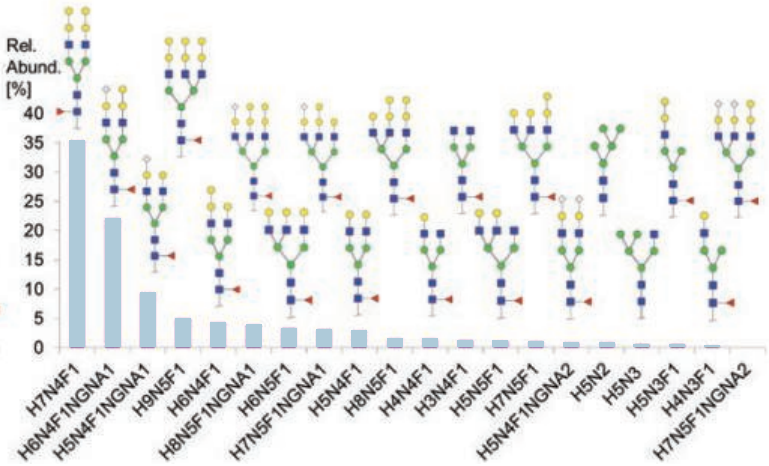
The glycan profile of the individual domains of cetuximab was determined with the subunit domain mapping method and Bruker MaXis UHR-MS by Ayoub et al. (1). Cetuximab is glycosylated in both the Fab and Fc domains. 11 glycans on Fc and 20 glycans on Fd were readily quantified and no additional digestion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

Fc domain



Relative abundance of different glycans on Fc domain.

Fd domain



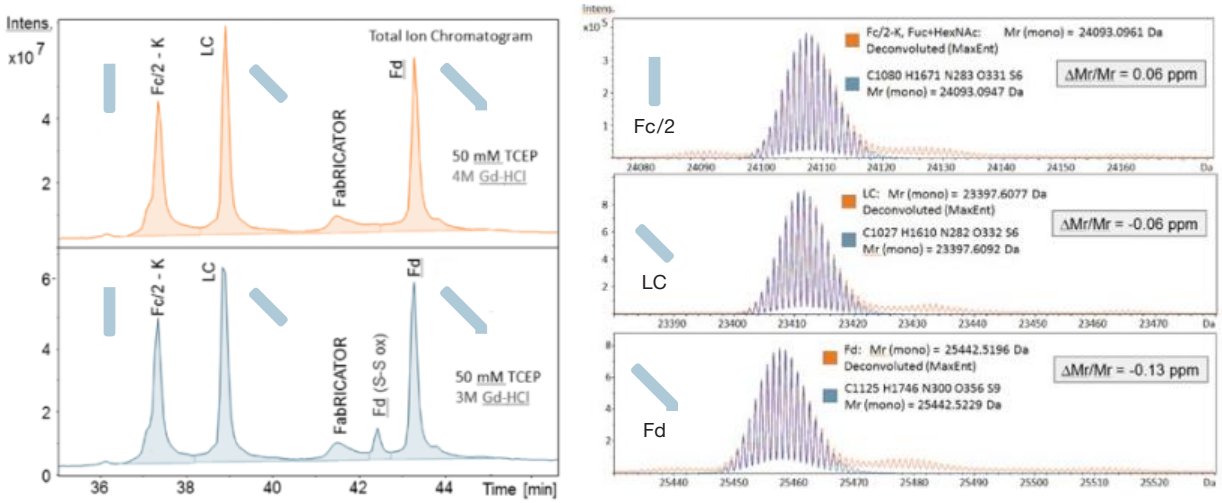
Relative abundance of different glycans on Fd domain.

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**
- **Quality control**
- **Stability**

For characterization of

- **mAbs**
- **ADCs**
- **Biosimilars**
- **Fc-fusion proteins**

Selected References

Monoclonal antibodies

1. 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.
2. 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.
3. 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

4. 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.
5. 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

6. 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 fragmentation is available in different sizes, for cleavage 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 fragmentation in a high throughput format. Antibodies or proteins can be added directly to the wells for convenient and fast antibody subunit generation. Plates can be divided into individual wells to process fewer samples.

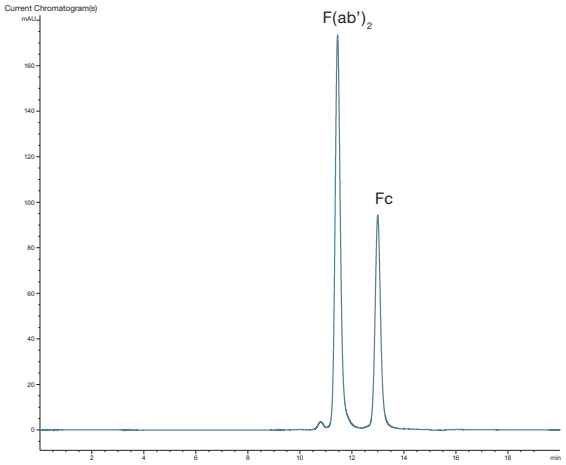
Lyophilized FabRICATOR can be custom-made regarding volumes, packaging and formats. Please contact us for details regarding your specific requirements, info@genvis.com.

	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	8 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™

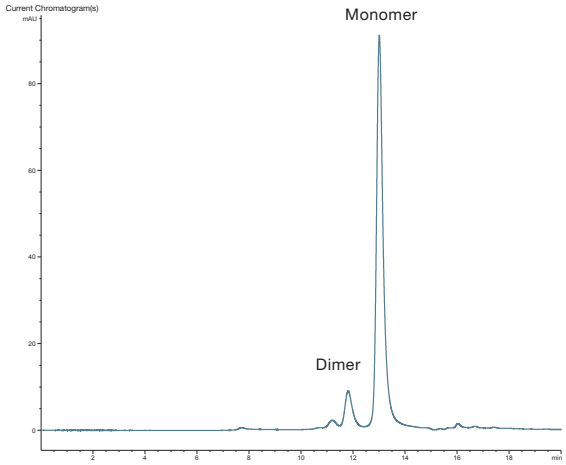
Three different production batches of lyophilized FabRICATOR are included in FabRICATOR validation kit for evaluation of FabRICATOR batch to batch consistency. The purity and enzyme activity of the three different batches are established with SEC and SDS-Page.

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 %

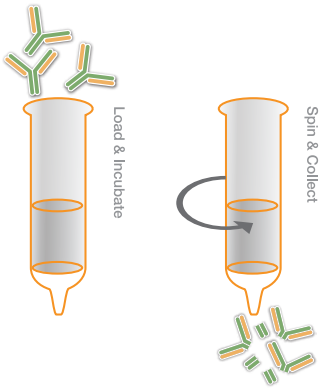
Monomer	86.5 %
Dimer	11.2 %
Aggregates	2.2 %

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

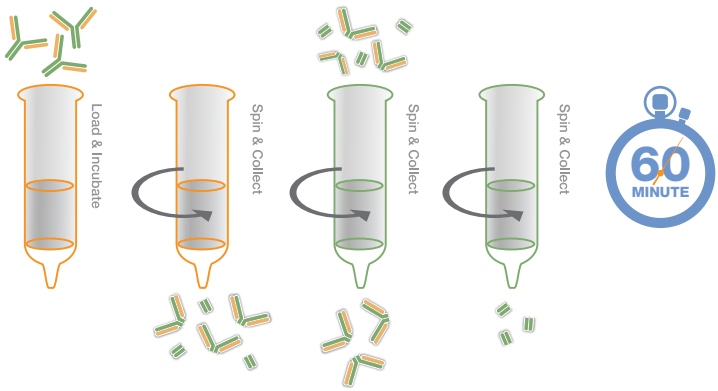


	Product ID	Description	Digestion	EUR	USD
	A0-FR6-010	FragIT Microspin	2 x 0.5 mg	305	425
	A0-FR6-025	FragIT Microspin	5 x 0.5 mg	695	960
	A0-FR6-050	FragIT Microspin	10 x 0.5 mg	1,150	1,605
	A0-FR6-100	FragIT MidiSpin	1-10 mg	925	1,285
	A0-FR6-1000	FragIT MaxiSpin	10-100 mg	2,760	3,860

FragITkit™

Immobilized FabRICATOR

FragITkit is for rapid preparation and isolation of F(ab')$_2$ and Fc fragments from IgG with 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.



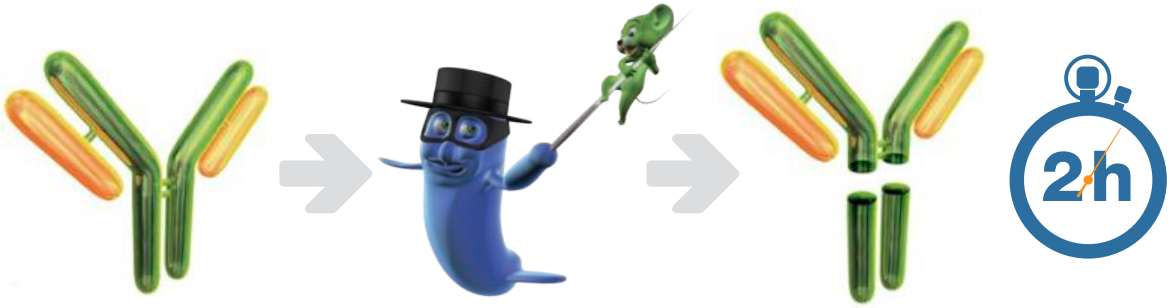
	Product ID	Description	Digestion & Purification	EUR	USD
	A2-FR2-005	FragIT Kit	0.5 mg IgG	340	475
	A2-FR2-025	FragIT Kit	5 x 0.5 mg IgG	925	1,285
	A2-FR2-100	FragIT Kit	10 mg IgG	1,155	1,610
	A2-FR2-1000	FragIT Kit	100 mg IgG	3,465	4,845

Anti-FabRICATOR™

Anti-FabRICATOR is 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



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

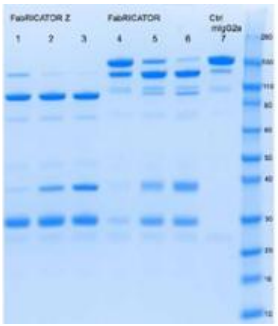
FabRICATOR Z derived from *Streptococcus equi* ssp. *Zooepidemicus* digests mouse IgG2a and IgG3 and produces a homogenous pool of $F(ab')_2$ and Fc fragments after 2 hours incubation. Some mouse IgG2a which FabRICATOR fails to digest are readily digested by FabRICATOR Z but longer incubation times may be required. However, there is no risk of further digestion of the fragments due to 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 over digestion

Digestion of Mouse IgG2a using FabRICATOR Z

Three different amounts of FabRICATOR Z (IdeZ) and FabRICATOR (IdeS) were used to digest a mouse IgG2a. After 2 hours incubation its evident that FabRICATOR Z (IdeZ) readily cleave mouse IgG2a while FabRICATOR only digest 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.



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

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

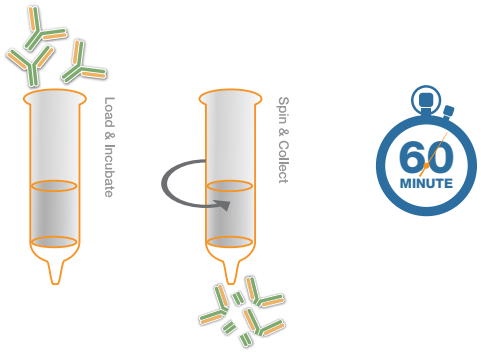
Line 7: Undigested mouse IgG2a

	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 an rapid and easy way of creating F(ab')$_2$ and Fc fragments from mouse IgGa. FragIT Z is FabRICATOR Z enzyme immobilized on agarose, allowing for antibody subunit fragmentation without FabRICATOR Z in the final sample preparation. The spin columns come prefilled with immobilized FabRICATOR Z for digestion of 0.5 mg IgG2a in each column.

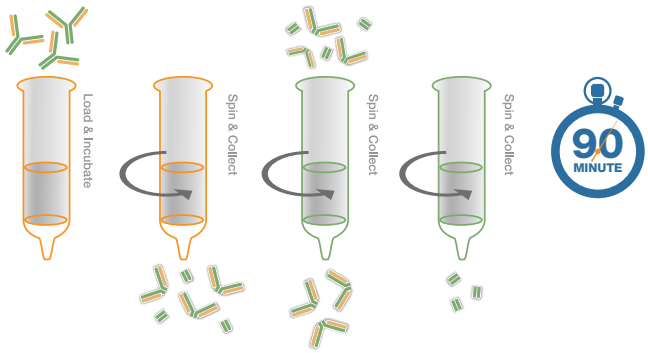


Product ID	Description	Digestion	EUR	USD
A0-FZ6-010	FragIT Z micorspin	2 x 0.5 mg	305	425
A0-FZ6-025	FragIT Z micorspin	5 x 0.5 mg	695	960
A0-FZ6-050	FragIT Z micorspin	10 x 0.5 mg	1,150	1,605

FragIT™ Z kit

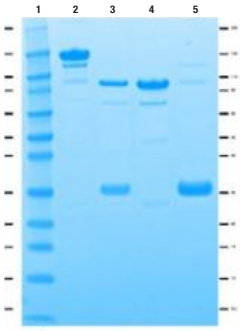
Immobilized FabRICATOR Z

FragIT Z Kit is for rapid preparation and separation of F(ab')$_2$ and Fc fragments from mouse IgG2a with 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.



Digestion and Purification of Mouse IgG2a using FragIT Z Kit

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



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

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

GingisKHAN™

GingisKHAN (KGP) is a unique cysteine protease that cleaves HUMAN IgG1 at one single amino acid position just above the hinge.

The enzyme is extremely specific and selective. Cleavage of human IgG1 with GingisKHAN generates a homogenous pool of precise Fab and Fc fragments. There is no over-digestion or further degradation of the fragments typically associated 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 antibody characterization are minimized due to mild reaction conditions and a rapid digestion protocol.

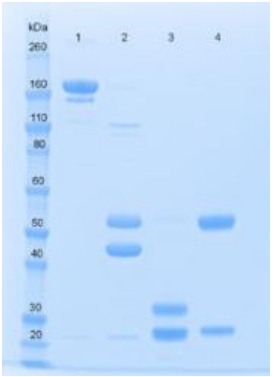


Fig 1.

Fig 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).

Fig 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 – Rapid subunit generation of human IgG1

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

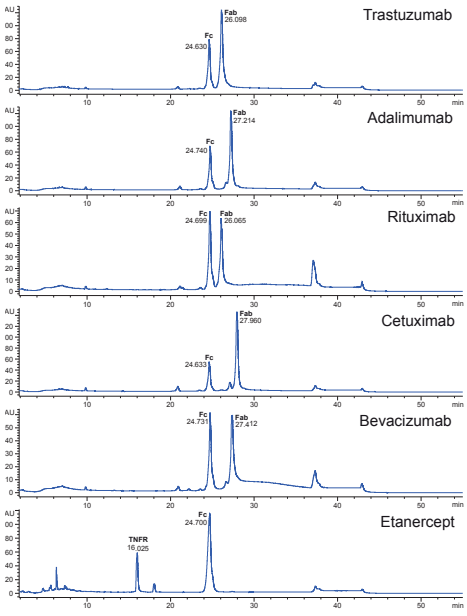
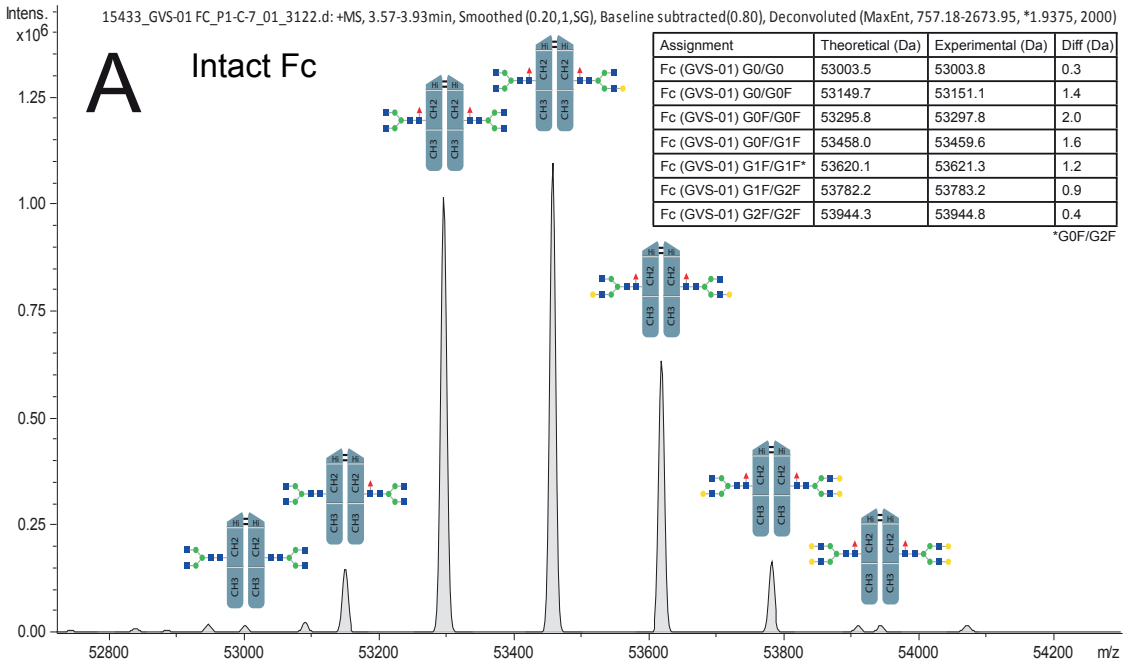


Fig 2.

GingisKHAN (KGP) for Characterization of Monclonal Antibody Based Biotheraputics using MS

- Rapid Fc-glycan analysis
- LC-HC pairing for 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 were generated with GingisKHAN.

PRODUCTS

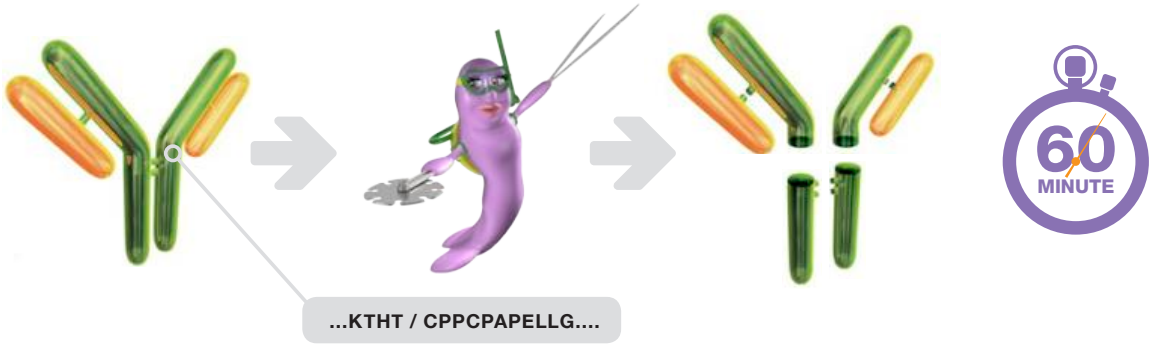
GingisKHAN™

GingisKHAN cleaves 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

FabULOUS® (SpeB)

Antibody Subunit Fragmentation



Fabulous is a cysteine protease that digests IgG from many different species and subclasses using one fast and generally applicable protocol.

IgGs are cleaved by FabULOUS generating Fab and Fc fragments. The primary cleavage site on human IgG1 is between T225 and C226. One fast and general protocol is of great benefit, saving valuable time, otherwise spent on optimization of a cleavage protocol. This is commonly associated with using classical cysteine proteases such as papain, ficin and pepsin.

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

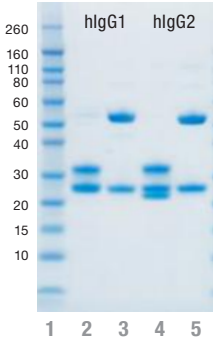
FabULOUS – IgGs / Species Cleaved

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

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

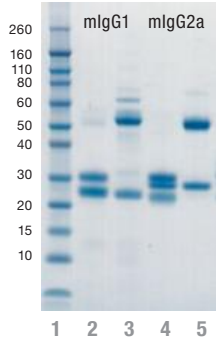
FabULOUS Digestion

Human IgG



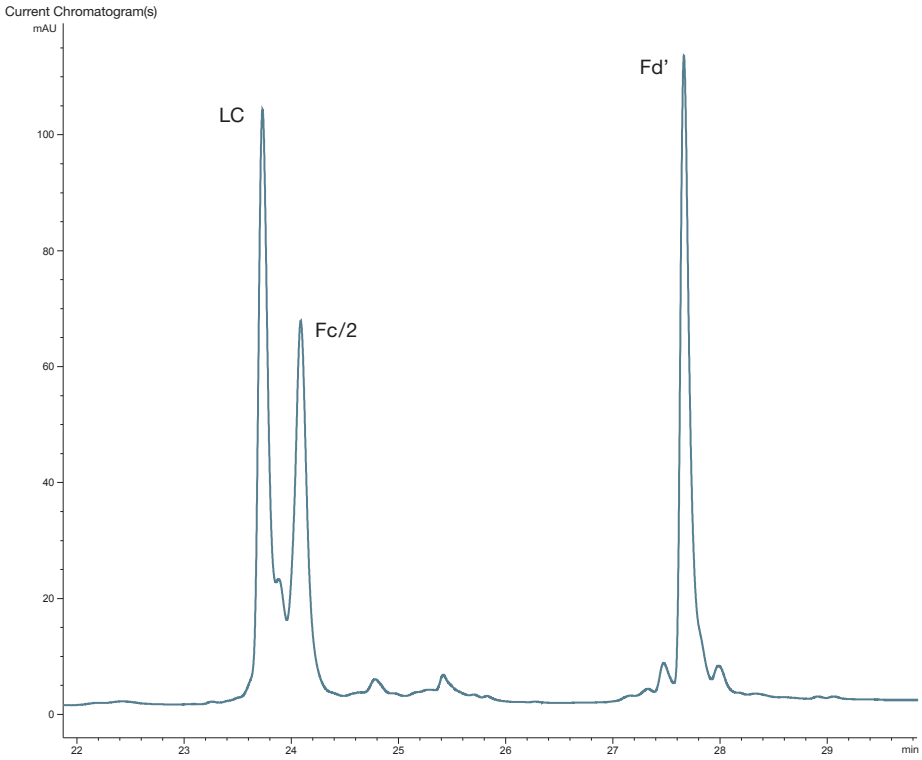
1 MW
2 hIgG1 **Fabulous**
3 hIgG1
4 hIgG2 **Fabulous**
5 hIgG2

Mouse IgG



1 MW
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.

PRODUCTS

FabULOUS®

Lyophilized

FabULOUS is for antibody fragmentation in the upper hinge region. The enzyme comes 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

USA

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Order phone (toll free): (855) 782-0084
Order fax: (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
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