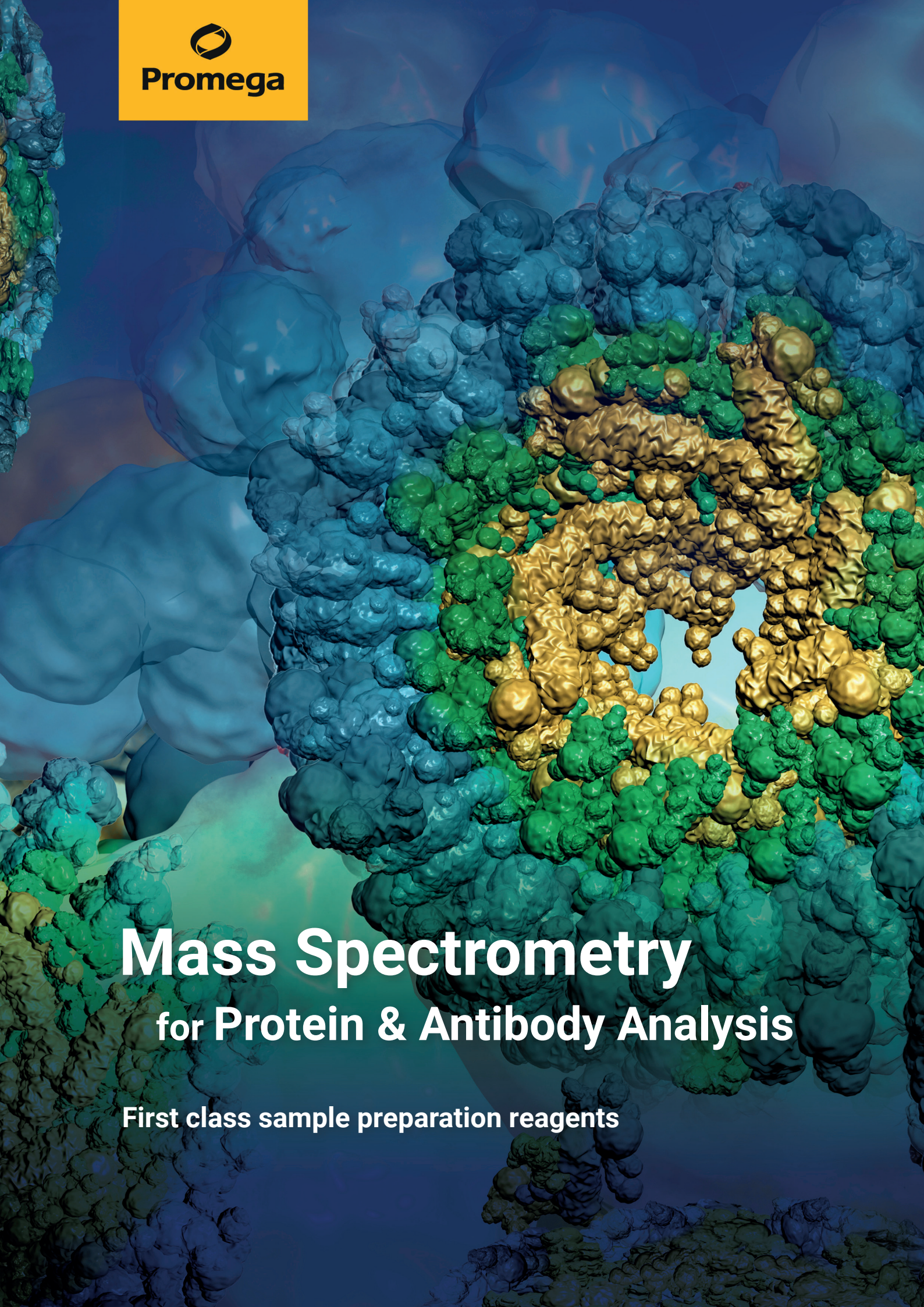
Promega

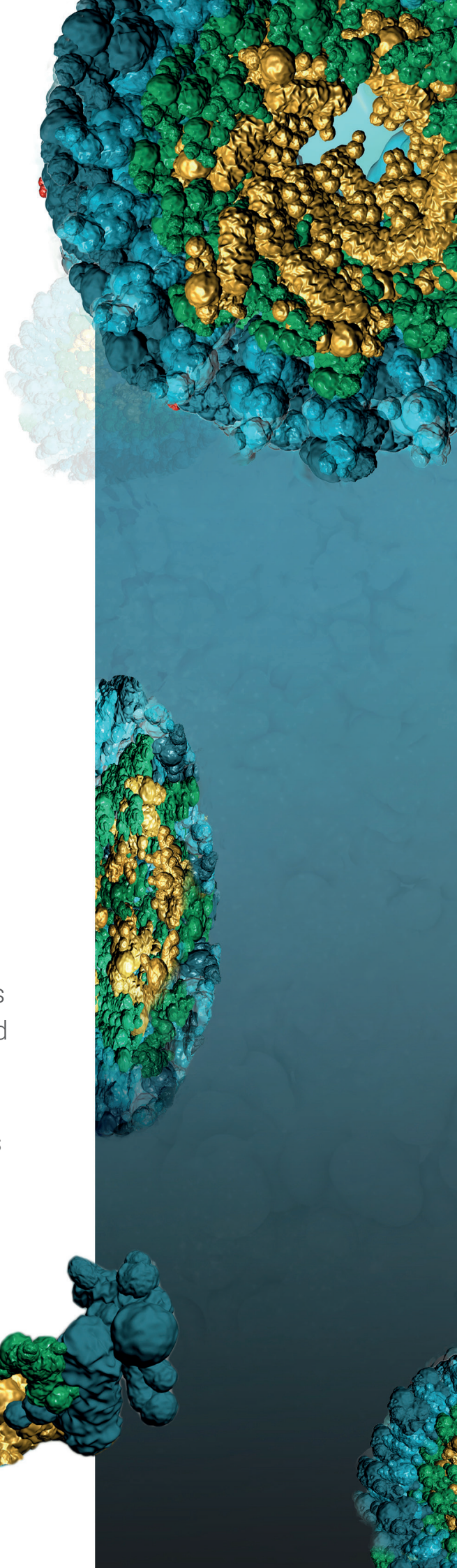


Mass Spectrometry

for Protein & Antibody Analysis

First class sample preparation reagents

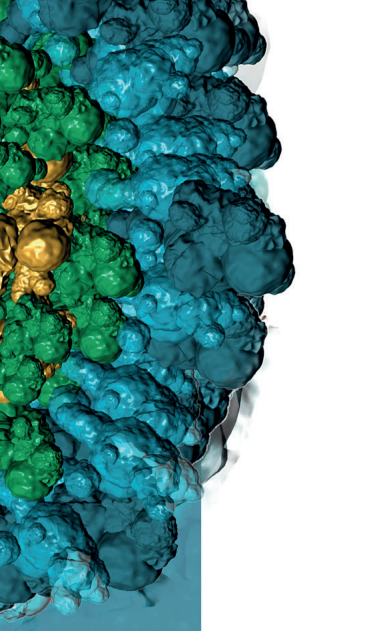
Protein Characterization by Mass Spectrometry

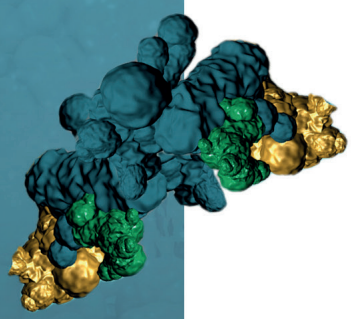


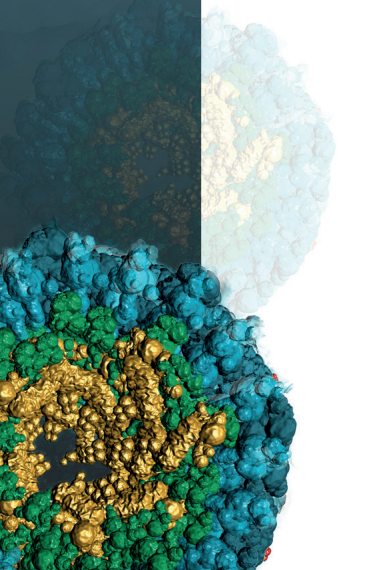
Mass spectrometry (MS) has become a powerful tool in proteomics for proteome-wide analysis and characterization of proteins from a variety of organisms and cell types. Recent advances in mass spectrometry provide tools for:

- protein identification
- protein characterization
- relative and absolute quantitation
- study of post-translational modification
- protein:protein interaction studies.

In these studies, proteins are generally digested with proteases to generate peptides which are subsequently separated, identified and sequenced by liquid chromatography and tandem MS (LC-MS/MS). Promega provides finest proteases and other reagents that are critical for high-quality sample preparation for mass spectrometry.







First class sample preparation reagents

1	Trypsin	4
	Trypsin Platinum, Mass Spectrometry Grade	5
	Trypsin Gold, Mass Spectrometry Grade	7
	Sequencing Grade Modified Trypsin	8
	Immobilized Trypsin	9
	Trypsin/Lys-C Mix, Mass Spectrometry Grade	10
	Rapid Digestion-Trypsin and Rapid Digestion-Trypsin/Lys-C Kits	12
2	Alternative Proteases for Mass Spectrometry	13
	Alternative Proteases	15
	ProAlanase, Mass Spec Grade	17
3	Glycosidases	18
4	Surfactants	21
	ProteaseMAX™ Surfactant, Trypsin Enhancer	22
	SoluMAX™, Mass Spec Compatible Surfactant	24
5	Tools for LC/MS Instrument Performance Monitoring	25
	MS Compatible Human Protein Extract, Digest	26
	MS Compatible Yeast Protein Extract, Digest	27
	6 × 5 LC-MS/MS Peptide Reference Mix	28
6	Antibody Characterization/Fragmentation	30
	AccuMAP™ Low pH Protein Digestion Kit	31
	IdeS and IdeZ Proteases	32
	ISOQUANT® Isoaspartate Detection Kit	33
7	Detailed information on Proteases for MS Sample Preparation	34
8	Practical Information	38
9	Ordering Information	40
10	Custom Services	42

1. Trypsin

OVERVIEW

Trypsin is the most widely used protease in mass spectrometry sample preparation. It is a highly-specific serine protease, which cleaves at the carboxylic side of lysine and arginine residues. Protein digestion with trypsin generates peptides of optimal sizes for mass spectrometry analysis. Tryptic peptides have a strong C-terminal charge, and therefore they can be efficiently ionized.

Trypsin is used for in-gel or in-solution digestion of proteins. Post digestion, the resulting peptides are introduced into the mass spectrometer and identified by *peptide mass fingerprinting or tandem mass spectrometry* (MS/MS). This approach is called bottom-up proteomics and uses identification at the peptide level to detect and characterize proteins.

The stringent specificity of trypsin is essential for characterizing proteins using mass spectrometry. Promega's high quality trypsin is modified to give the highest proteolytic activity and cleavage specificity (**Table 1**).

Table 1. *Trypsin comparison*

	Trypsin Platinum	Trypsin Gold	Sequencing Grade Trypsin	Trypsin/ Lys-C	Rapid Trypsin	Rapid Trypsin/Lys-C	AccuMap™
Cleavage specificity	★	✓	✓	✓	✓	✓	✓
Digestion efficiency	✓	✓	✓	★	✓	★	✓
Autoproteolytic resistance	★	✓	✓	✓	✓	✓	✓
Purity	★	★	✓	✓	✓	✓	✓
Digestion speed					★	★	
Suppression of artificial PTMs							★
Tolerance to inhibiting agents				★		★	
Customization		★	★	★			
Applications	Peptide mapping of biotherapeutic proteins Antibody characterization	Proteomics	In-gel & in-solution digestion Proteomics	Proteomics and quantitative studies Difficult proteins	Proteomics and quantitative studies	Proteomics and quantitative studies	Peptide mapping of biotherapeutic proteins Antibody characterization
Highlights	Free of animal origin contaminations	High purity and activity	Available lyophilized & frozen	Enhanced cleavage efficiency Tolerance to inhibiting agents	Rapid results High throughput/ automation	Rapid results High throughput/ automation	Artifical PTMs suppresion

✓ = *competitive performance* ★ = *superior performance*

Trypsin Platinum, Mass Spectrometry Grade

Recombinant trypsin with outstanding autoproteolytic resistance and cleavage specificity leading to superior activity.

Description

Trypsin Platinum is designed for analytical scientists looking for the most accurate and efficient protein characterization with mass spectrometry and RP-HPLC-UV. Elimination of non-specific cleavage activity (**Figure 1**) commonly present in the existing proteomics and MS grade trypsins, superior autoproteolytic resistance (**Figure 2**), and improved proteolytic efficiency makes Trypsin Platinum an ideal tool for the biotherapeutic protein characterization.

Principle

Prior to digestion a protein is denatured, reduced and alkylated. We recommend GuHCl as a protein denaturing agent in the sample preparation procedure. Trypsin Platinum is then added, and a protein is digested overnight at 37°C. To achieve the most efficient digestion, 1:10 Trypsin Platinum:protein ratio is recommended. To digest particularly proteolytically resistant domains or sites, 1:3 ratio can be used. The resulting peptides are desalted and analyzed by mass spectrometry.

Features and Benefits

- **Outstanding Cleavage Specificity:** Free of detectable nonspecific cleavage activity even at 1:1 trypsin:protein ratio and extended digestion period.
- **Superior Autoproteolytic Resistance:** Autoproteolysis is maximally suppressed to maintain high trypsin activity and avoid interference of tryptic autoproteolytic peptides with peptide analysis.
- **Enhanced Proteolytic Activity:** efficient digestion of proteolytically resistant cleavage sites.
- **Free of Contaminating Proteins of Animal Origin.**
- **Superior Purity and Lot-to-Lot Reproducibility:** Tightly regulated production with rigorous quality control assures the highest purity and stability.

Ordering Information

Trypsin Platinum, Mass Spec Grade
(Cat.# VA9000)

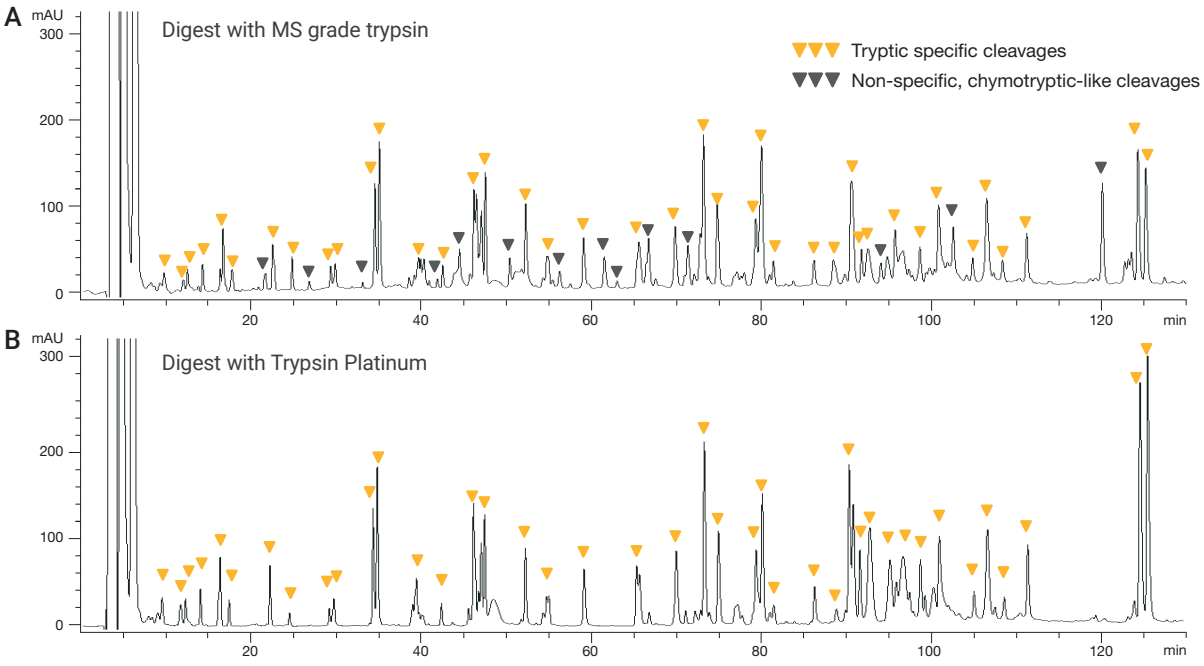


Figure 1. *Trypsin Platinum **(Panel B)** is free of non-specific cleavage activity compared to MS grade trypsin **(Panel A)**. Panitumumab (Vectibix®) antibody used as a model protein substrate was digested with MS grade trypsin and Trypsin Platinum. The digestion reactions used a 1:10 trypsin:protein ratio. The digests were analyzed with RP-HPLC-UV.*

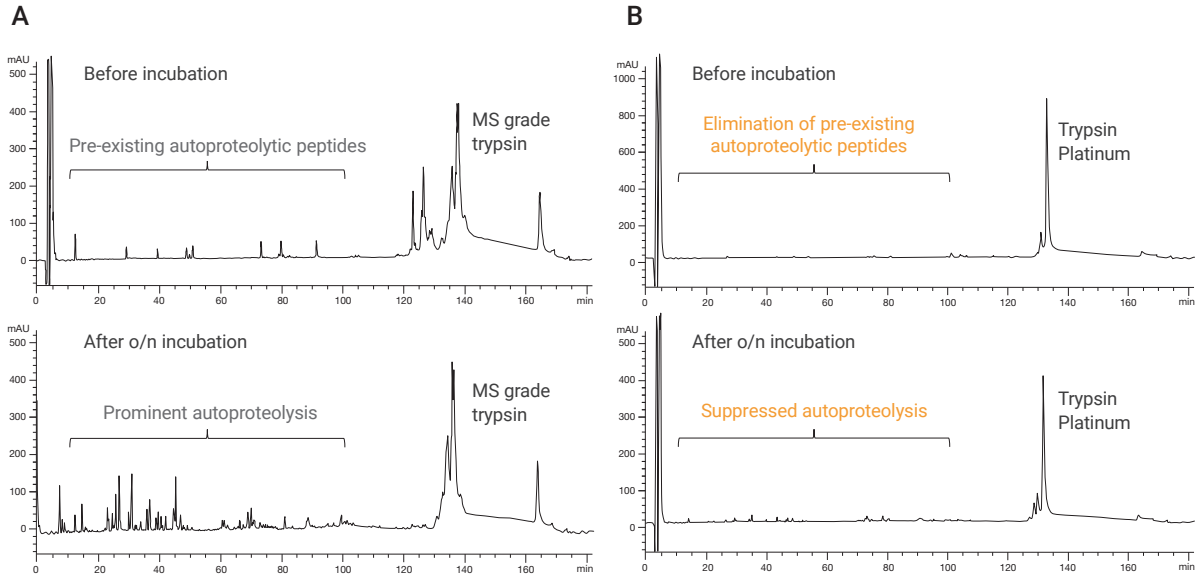


Figure 2. *Comparison of autoproteolytic resistance of MS grade trypsin **(Panel A)** and Trypsin Platinum **(Panel B)** shows strong suppression of autoproteolysis in Trypsin Platinum. Both trypsins were incubated in a digestion buffer 50 mM Tris-HCl, pH 8 at 37°C and analyzed with RP-HPLC-UV.*

Trypsin Gold, Mass Spectrometry Grade

Excellent specificity and resistance to autoproteolysis for in-gel and in-solution digestion.

Description

Trypsin Gold, Mass Spectrometry Grade, is manufactured to provide maximum specificity. Lysine residues in porcine trypsin are modified by reductive methylation, yielding a highly active and stable molecule that is extremely resistant to autolytic digestion. The specificity of the purified trypsin is further improved by TPCK treatment, which inactivates chymotrypsin. Additionally, the high performance of Trypsin Gold is assured by selecting affinity fractions with the highest proteolytic activity.

Principle

For in-gel digestion, protein samples are run on SDS-PAGE. Protein bands are stained and the bands of interest are excised. A buffer containing resuspended trypsin is added and the reaction is incubated overnight at 37°C. The resulting peptides are then extracted from the gel and analyzed by mass spectrometry.

For in-solution digestion, protein samples are denatured, reduced and alkylated. Trypsin is added and the solution is typically incubated for 3–12 hours at 37°C. The resulting peptides are desalted and analyzed by mass spectrometry.

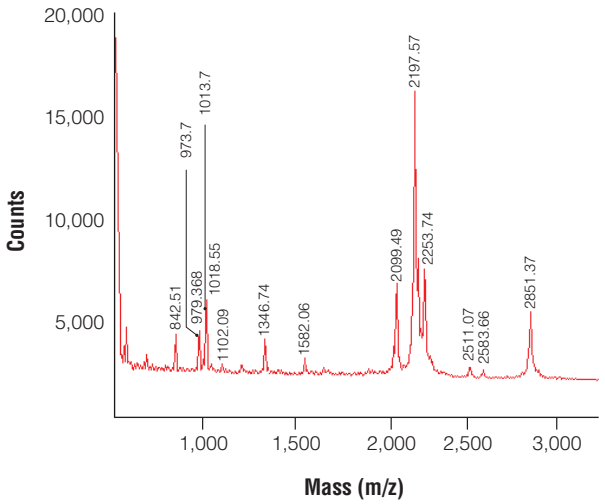


Figure 3. *Mass Spectrum of bovine carbonic anhydrase II digested by Trypsin Gold, Mass Spectrometry Grade. A 500ng sample of carbonic anhydrase II was separated by gel electrophoresis and digested with 500ng of Trypsin Gold, Mass Spectrometry Grade, overnight at 37°C.*

Features and Benefits

- **Application Qualified:** Each lot is qualified by mass spectrometry.
- **Pure:** Trypsin Gold is prepared by TPCK treatment followed by affinity purification.
- **Good Value:** Stable for up to 5 freeze-thaw cycles, thus minimizing reagent waste.
- **Referenced in Thousands of Papers:** Reliable and customer proven.

Ordering Information

Trypsin Gold, Mass Spectrometry Grade
(Cat.# V5280)

Sequencing Grade Modified Trypsin

High specificity and stability with a wide variety of custom manufacturing options.

Description

Sequencing Grade Trypsin has been manufactured to provide excellent specificity. Lysine residues in the porcine trypsin have been modified by reductive methylation, yielding a highly active and stable molecule resistant to autolytic digestion. The specificity of the purified trypsin was further improved by TPCK treatment, which inactivates chymotrypsin. Reliability of Sequencing Grade Trypsin is proven by being referenced in thousands of publications.

Principle

For in-gel digestion, protein samples are run on SDS-PAGE. Protein bands are stained and bands of interest are excised. A buffer containing resuspended trypsin is added and incubated overnight at 37°C. The resulting peptides are then extracted from the gel and analyzed by mass spectrometry.

For in-solution digestion, protein samples are denatured, reduced and alkylated. Trypsin is then added and the solution is incubated for 3–12 hours at 37°C. The resulting peptides are desalted and analyzed by mass spectrometry.

Features and Benefits

- **High Specificity and Purity:** Trypsin is prepared by TPCK treatment followed by affinity purification.
- **High Stability:** Due to reductive methylation of lysine residues.
- **Reliable and Customer-Proven:** Referenced in more than 10,000 publications.
- **Additional Value:** Stability is ensured for up to 5 freeze-thaw cycles, minimizing leftover reagents.
- **Variety of Packaging:** We offer flexibility in packaging depending on experimental design and scope.

References

Flynn, RA. *et al.* (2021) Small RNAs are modified with N-glycans and displayed on the surface of living cells. Cell **184(12)**, 3109–3124.

Schuster, O. *et al.* (2021) Specific and Rapid SARS-CoV-2 Identification Based on LC-MS/MS Analysis. ACS OMEGA **6**, 3525–3534.

Mehta, AK. *et al.* (2020) Targeting immunosuppressive macrophages overcomes PARP inhibitor resistance in BRCA1-associated triple-negative breast cancer. Nature Cancer **2**, 66–82.

Parker, BL. *et al.* (2019) An integrative systems genetic analysis of mammalian lipid metabolism. Nature **567**, 187–193.

Burkhart J. *et al.* (2012) Systematic and quantitative comparison of digest efficiency and specificity reveals the impact of trypsin quality on MS-based proteomics. J. Proteomics **75(4)**, 1454–1462.

Ordering Information

Sequencing Grade Modified Trypsin (Cat.# V5111, V5117)

Sequencing Grade Modified Trypsin, Frozen (Cat.# V5113)

Immobilized Trypsin

Digestion in as little as 30 minutes with easy removal of trypsin using a spin column.

Description

Trypsin in solution is the most widely used protease for digestion of proteins. However, there are a few limitations in using free trypsin, including:

1) The digestion requires a long time (typically three hours to overnight).
2) High concentrations of trypsin cannot be used because of the generation of dominant trypsin autolytic fragments in the digested samples.
3) Possible interference due to trypsin with downstream sample preparation steps (such as addition of additional proteases).

To overcome these limitations we offer Immobilized Trypsin. It provides a fast and convenient method for protein digestion.

Principle

Immobilized Trypsin reduces digestion time and allows easy removal of trypsin from the digestion reaction (**Figure 4**). Immobilized Trypsin provides the ability to digest 20–500µl of protein in solution simply by adjusting the amount of resin used in the reaction. This flexibility facilitates digestion while decreasing potential trypsin interference in downstream sample analysis. The percentage of digestion and sequence coverage is comparable to overnight digestion with free trypsin. Immobilized Trypsin has been used to study protein expression profiling in serum, microwave-assisted digestion of proteins, phoshopeptide analysis, analysis of membrane proteins and for $^{18}O/^{16}O$ labeling of peptides.

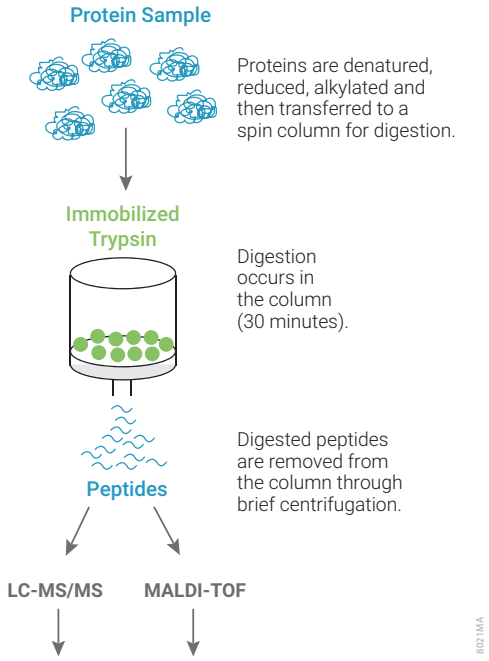


Figure 4. *Overview of the Immobilized Trypsin digestion protocol.*

Features and Benefits

- **Fast:** Digestion can be accomplished in as little as 30 minutes.
- **Scalable:** Easily adjustable protocol to accommodate various protein concentrations.
- **Easy Setup:** Minimal centrifugation steps.

Ordering Information

Immobilized Trypsin (Cat.# V9012, V9013)

Trypsin/Lys-C Mix, Mass Spectrometry Grade

Highest digestion efficiency enables accurate peptide identification and quantitation. Tolerant to trypsin-inhibiting agents.

Description

Trypsin/Lys-C Mix, Mass Spectrometry Grade, is a mixture of Trypsin Gold, Mass Spectrometry Grade, and rLys-C, Mass Spec Grade. The Trypsin/Lys-C Mix is designed to improve digestion of proteins or protein mixtures in solution. Replacing trypsin with Trypsin/Lys-C Mix has multiple benefits for protein analysis, including more accurate mass spectrometry-based protein quantitation and improved analytical reproducibility. Trypsin/Lys-C Mix also provides greater tolerance to trypsin-inhibiting agents, assuring efficient digestion of proteins for which purification is limited or not feasible.

Principle

Typical trypsin reactions do not digest proteins to completion, missing 10–30 % of cleavage sites (**Figure 5**). Incomplete digestion affects protein identification, reproducibility of mass spectrometry analysis and accuracy of protein quantitation. The number of missed cleavage sites may be even higher if the protein is not properly purified or contains protease-inhibiting contaminants. Using the conventional trypsin digestion protocol (i.e., overnight incubation under nondenaturing conditions), Trypsin/Lys-C Mix improves protein digestion by eliminating the majority of missed lysine cleavages. The mixture is stable since trypsin is modified and therefore resistant to digestion by Lys-C.

Features and Benefits

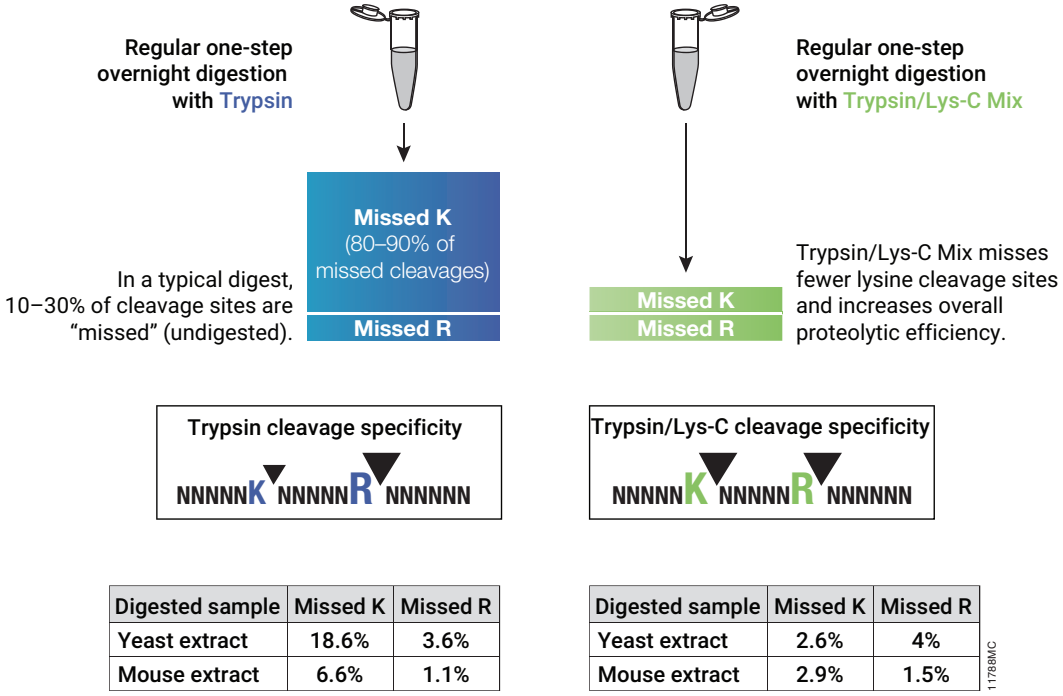
- **Simple to Use:** Use standard overnight digestion with non-denaturing conditions.
- **Enhanced Proteolysis:** Increase peptide recovery by enhancing cleavage efficiency at lysine residues and eliminating the majority of missed cleavages (**Figures 5 and 6**).
- **Tolerant to Trypsin-Inhibiting Contaminants:** Generate mass spectrometry data from low-quality sample material.

References

Saveliev, S. et al. (2013) Trypsin/Lys-C protease mix for enhanced protein mass spectrometry analysis. Nature Methods 10, i-ii

Ordering Information

Trypsin/Lys-C Mix, Mass Spec Grade
(Cat.# V5071, V5072, V5073)



Digested sample	Missed K	Missed R
Yeast extract	18.6%	3.6%
Mouse extract	6.6%	1.1%

Digested sample	Missed K	Missed R
Yeast extract	2.6%	4%
Mouse extract	2.9%	1.5%

Figure 5. *Side-by-side comparison of cleavage sites missed by trypsin or the Trypsin/Lys-C Mix using a standard digestion protocol.*

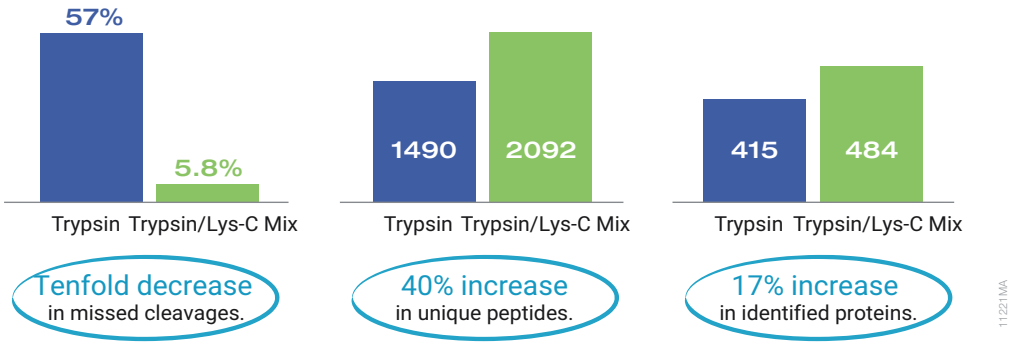


Figure 6. *Improved mass spectrometry analysis of a protein extract containing residual amounts of methanol and chloroform, which inhibit trypsin but have no effect on Trypsin/Lys-C Mix performance.*

Rapid Digestion-Trypsin and Rapid Digestion-Trypsin/Lys-C Kits

Shorten digestion time to as little as 60 minutes for more efficient workflow.

Description

The Rapid Digestion-Trypsin and Rapid Digestion-Trypsin/Lys-C Kits are designed to shorten protein digestion times to 60 minutes versus the typical 4–18 hours required for protein digestion. Both kits contain three components: i) protease (Trypsin or Trypsin/Lys-C Mix); ii) protease Resuspension Buffer; and iii) Rapid Digestion Buffer optimized for faster digestions. Protein digestion with these kits follows a simple-to-use protocol that is both fast and efficient. The protocol is flexible, and can accommodate a large range of sample volumes and protein concentrations.

Principle

In an effort to shorten the time required to prepare samples for LC-MS/MS analysis, we developed a specialized trypsin preparation that supports rapid and efficient digestion at temperatures as high as 70°C. There are several benefits to this approach. First, proteolytic reaction times are dramatically shortened. Second, because no chemical denaturants have been added, off-line sample cleanup is not necessary, leading to shorter preparation times and diminished sample losses.The Rapid Digestion Kits are highly flexible. They can accommodate a variety of additives, including reducing and alkylating agents.

We also developed a Rapid Digestion-Trypsin/Lys-C Kit. Like the Trypsin/Lys-C Mix previously developed to prepare maximally efficiently proteolytic digests, particularly for complex mixtures, Rapid Digestion-Trypsin/Lys C is ideally suited for studies that require improved reproducibility across samples. In many examples the quality of data generated by using the Rapid Digestion format exceeds that obtained with the conventional overnight procedure (**Figure 7**).

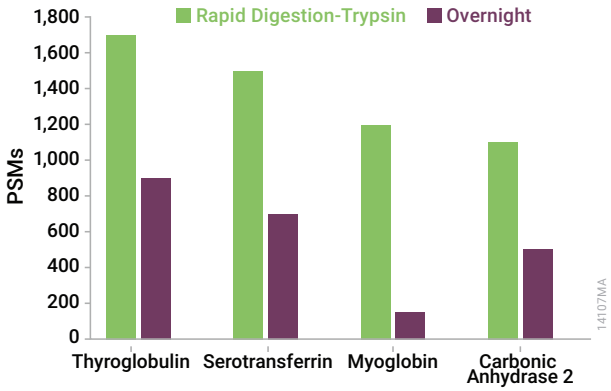


Figure 7. *Comparison of peptide spectral matches, Rapid Digestion versus overnight digestion.*

Ordering Information

Rapid Digestion-Trypsin Kit
(Cat.# VA1060)

Rapid Digestion-Trypsin/Lys-C Kit
(Cat.# VA1061)

2. Alternative Proteases for Mass Spectrometry

OVERVIEW

Trypsin is the most widely used endoprotease. It cleaves proteins in a highly specific manner and typically generates peptides 7–20 amino acids in length, with a strong C-terminal charge, making trypsin ideal for mass spectrometry analysis.

However, there are certain proteins and protein mixtures where trypsin digestion alone is not efficient enough. Examples include digestion of membrane proteins and analysis of histone post-translational modifications (PTMs). Furthermore, certain tryptic peptides are too small or too large for mass spectrometry analysis. In these situations alternative proteases provide a viable solution, either alone or in combination with trypsin (**Table 2**).

Table 2. *Alternative Proteases and Corresponding Cleavage Site*

Protease	Cleavage Site	pH Range	Example of Use
Lys-C and rLys-C Specific Protease	NNNN**K** ⌄ NNN (**K** is lysine)	7.0–9.0	Digests membrane and other proteolytically resistant proteins; generates larger peptides than trypsin, an advantage for certain mass spectrometry methods (for example, ETD).
Arg-C Specific Protease	NNNN**R** ⌄ NNN (**R** is arginine) Arg-C can, at a lesser degree, cleave at lysine also.	7.6–7.9	Facilitates analysis of histone post-translational modifications; used in proteome-wide analysis.
Glu-C Specific Protease	NNNN**E** ⌄ NNN (**E** is glutamate) Glu-C can, at a lesser degree, cleave at aspartate residue also.	4.0–9.0	Used as alternative to trypsin if trypsin produces too short or too long peptides or if tryptic cleavage sites are not accessible.
Asp-N and rAsp-N Specific Protease	NNNN ⌄ **D**NNN (**D** is aspartate)	4.0–9.0	Similiar to Glu-C.
ProAlanase Specific Protease	NNN**P** ⌄ NNN (**P** is proline) NNN**A** ⌄ NNN (**A** is alanine)	1.5–5.5	Used to reach areas of proteome not accessible with traditional proteases in a variety of applications: protein characterization, peptide mapping, disulfide bond mapping, *de novo* sequencing, HDX-MS.
Chymotrypsin Low Specific Protease	NNNN(**F**/**Y**/**W**) ⌄ NNN (**F**, **Y** and **W**) are aromatic residues phenylalanine, tyrosine and tryptophan)	7.0–9.0	Digests hydrophobic proteins (for example, membrane proteins).
Pepsin Nonspecific Protease	Nonspecific Protease (advantage: most active at low pH)	1.0–3.0	Used in structural protein studies and antibody analysis; digests proteolytically resistant, tightly folded proteins.
Thermolysin Nonspecific Protease	Nonspecific Protease (advantage: remains active at high temperature)	5.0–8.5	Digests proteolytically resistant, tightly folded proteins; used in structural protein studies.
Elastase Nonspecific Protease	Nonspecific Protease	9.0	Used to increase protein coverage.

2 Alternative Proteases

Digestion with Alternative Proteases Increases Protein Coverage

Digestion with an alternative protease, individually or in combination with trypsin, creates a unique peptide map that may include sequences not seen with trypsin digestion alone. Overlaying peptides obtained with alternative proteases and trypsin, increases protein coverage and overall confidence in protein identification (**Figure 8**).

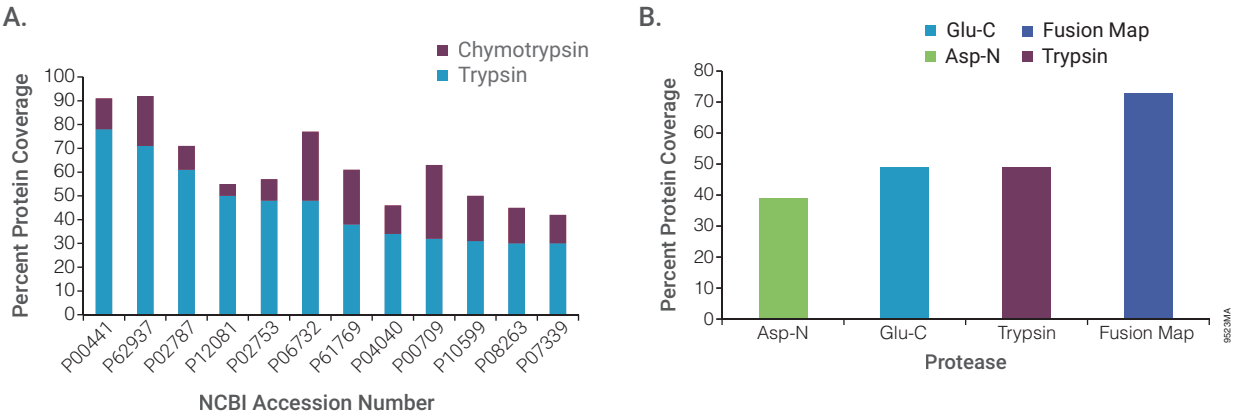


Figure 8. *Increased protein coverage using **(Panel A)** both trypsin and chymotrypin; **(Panel B)** multiple proteases in separate digestion reactions. Note that the fusion map is the combination of the results obtained by digestion with each protease alone.*

Alternative Proteases Improve Analysis of Post-Translational Modifications (PTMs)

In certain cases, trypsin is not suitable for PTM analysis. For example, histone PTMs are complex and some, such as acetylation and methylation, prevent trypsin digestion. As shown in **Figure 9**, trypsin digestion of histone H4 identified several PTMs; however, certain PTMs were missing. By digesting histone H4 with Arg-C, additional PTMs were identified, including mono- and dimethylated, and acetylated lysine and arginine residues.

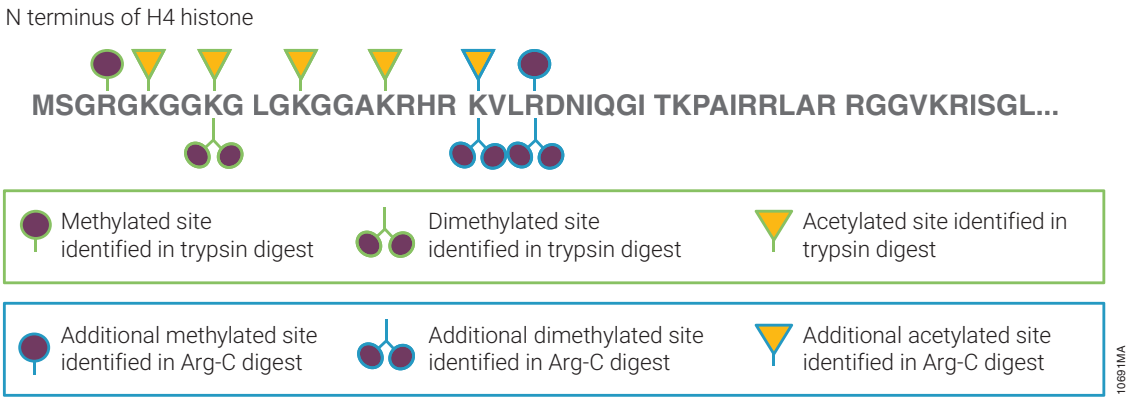


Figure 9. *Histone H4 post-translational modifications identified in trypsin and Arg-C digests.*

Alternative Proteases

Improve protein sequence coverage, enhance digestion of tightly-folded proteins, improve the identification of post-translation modifications (PTMs).

Specific Proteases

Arg-C (Clostripain), Sequencing Grade, is an endopeptidase isolated from *Clostridium histolyticum* that cleaves at the C-terminus of arginine residues, including the sites next to proline. Arg-C is active in the pH range of 7.6–7.9.

Asp-N, Sequencing Grade, is an endoproteinase that hydrolyzes peptide bonds on the N-terminal side of aspartic acid residues. Asp-N is active in the pH range of 4.0–9.0.

Glu-C, Sequencing Grade, is a serine protease that specifically cleaves at the C-terminus of glutamic acid residues. In ammonium bicarbonate and ammonium acetate the enzyme specificity is higher at the glutamic residues. In phosphate buffers cleavage occurs at the aspartic and glutamic residues. Glu-C is active in the pH range of 4.0–9.0.

Lys-C, Mass Spec Grade, is a serine protease isolated from *Lysobacter enzymogenes* as a highly purified protease that hydrolyzes specifically at the carboxyl side of lysine residues. Lys-C retains proteolytic activity under protein denaturing conditions such as 8M urea, which is used to improve digestion of proteolytically resistant proteins. Lys-C is active in the pH range of 7.0–9.0. This protease can be used alone or in combination with other proteases to produce protein digests for peptide mapping applications or protein identification by peptide mass fingerprinting or MS/MS spectral matching.

rAsp-N, Mass Spec Grade, is a recombinant protease that cleaves proteins N-terminal to aspartic acid residues. The enzyme was cloned from *Stenotrophomonas maltophilia* and purified from *E. coli*. rAsp-N is a highly active protease suitable for proteomic analysis of complex mixtures as well as peptide mapping of purified proteins, including therapeutic monoclonal antibodies. The protease is provided in 10µg quantities, in a conical tube, for simple and consistent resuspension.

rLys-C, Mass Spec Grade, is a recombinant Lys-C expressed in *E. coli*. The sequence origin of rLys-C is Protease IV from *Pseudomonas aeruginosa*. Similarly to a native Lys-C, rLys-C cleaves at the C-terminus of lysine residues with exceptional specificity. rLys-C retains proteolytic activity under protein denaturing conditions such as 8 M Urea, used to improve digestion of proteolytically resistant proteins. rLys-C is active in the pH range of 8.0–9.0.

ProAlanase, Mass Spec Grade, is an endoprotease that preferentially cleaves proteins on the C-terminal side of proline and, to a lesser extent, alanine amino acids. Isolated and purified from the fungus *Aspergillus niger*, enzyme is also known as An-PEP or EndoPro. Peptides derived from protein digestion with ProAlanase are suitable for identification and characterization by mass spectrometry. ProAlanase is active in acidic pH (1–5.5) with a pH optimum of ~1.5 and has ideal digestion time of 1–2 hours.

2 Alternative Proteases

Low-specific Protease

Chymotrypsin, Sequencing Grade is a highly purified serine endopeptidase derived from bovine pancreas that preferentially hydrolyzes at the carboxyl side of aromatic amino acids: tyrosine, phenylalanine and tryptophan. Chymotrypsin is active in the pH range of 7.0–9.0.

Nonspecific Proteases

Elastase is a serine protease that preferentially cleaves at the C-terminus of alanine, valine, serine, glycine, leucine or isoleucine. Elastase is active at pH 9.0.

Pepsin preferentially cleaves at the C-terminus of phenylalanine, leucine, tyrosine and tryptophan. Pepsin is active in the pH range of 1.0–3.0.

Thermolysin is a thermostable metalloproteinase. Thermolysin preferentially cleaves at the N-terminus of the hydrophobic residues leucine, phenylalanine, valine, isoleucine, alanine and methionine. The optimal digestion temperature range is 65–85°C. Thermolysin is active in the pH range of 5.0–8.5.

Posters

Enhancing Trypsin Digestion with Lys-C and Arg-C Proteases. Promega Scientific Poster: **www.promega.com/resources/scientific-poster/posters/enhancing-trypsin-digestion-with-lysc-and-argc-proteases-scientific-poster/**

A Recombinant Asp-Specific Protease for Bottom-up Mass Spectrometry Workflows Poster: **www.promega.com/resources/scientific-poster/posters/a-recombinant-asp-specific-protease-for-bottomup-mass-spectrometry-workflows-poster/**

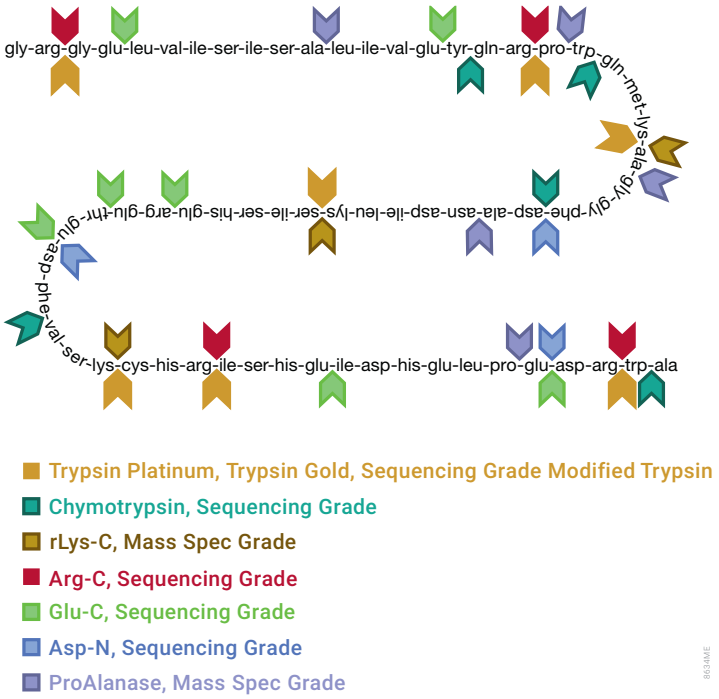


Figure 10. *Cleavage sites of proteases frequently used in mass spectrometry sample preparation.*

Ordering Information

Specific Proteases:

Lys-C, Mass Spec Grade (Cat.# VA1170)
Arg-C (Clostripain), Sequencing Grade (Cat.# V1881)
Asp-N, Sequencing Grade (Cat.# V1621)
Glu-C, Sequencing Grade (Cat.# V1651)
rLys-C, Mass Spec Grade (Cat.# V1671)
rAsp-N, Mass Spec Grade (Cat.# VA1160)
ProAlanase, Mass Spec Grade (Cat.# VA2161)
ProAlanase Plus, Mass Spec Grade (Cat.# VA2171)

Low-specific Proteases:

Chymotrypsin, Sequencing Grade (Cat.# V1061, V1062)

Nonspecific Proteases:

Elastase (Cat.# V1891)
Pepsin (Cat.# V1959)
Thermolysin (Cat.# V4001)

ProAlanase, Mass Spec Grade

New, site-specific endoprotease that targets proline and alanine.

Description and Principle

ProAlanase is an endoprotease that preferentially cleaves proteins on the C-terminal side of proline and, to a lesser extent, alanine amino acids (**Figure 11**). Isolated and purified from the fungus *Aspergillus niger*, ProAlanase is active at acidic pH (~1.0–5.5), unlike most proteases used in bottom-up proteomics, which require slightly basic pH (~7.0–9.0).

Utilizing alternative proteases helps to provide complete sequence coverage and to identify all post-translational modifications (PTMs). However, like trypsin, alternative proteases such as Lys-C, Asp-N, Glu-C & Arg-C also cleave at charged residues, introducing bias to regions within proteins that are digested. Use of ProAlanase prevents this bias because it cleaves at unique, non-charged sites in the proteome.

Applications

As shown in studies by Samadova D *et al.*, ProAlanase is useful for a wide variety of proteomics applications including:

- Protein analysis/characterization
- *de novo* sequencing
- Peptide mapping
- Disulfide bond mapping
- PTM analysis of proteins such as histones
- Paleoproteomics

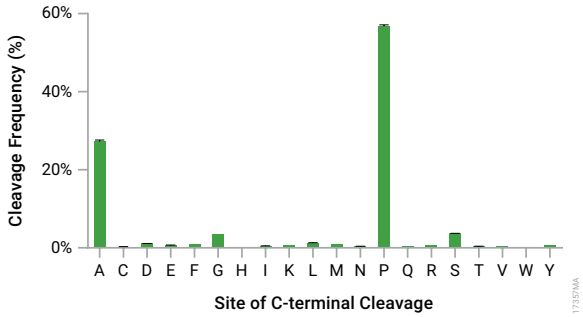


Figure 11. *The C-terminal cleavage specificity of ProAlanase. Human K562 extract was digested with ProAlanase at pH 1.5 for 2 hours at 37°C using a 1:100 enzyme:substrate ratio.*

Features and Benefits

- **Unique Cleavage Profile:** Cleavage C-terminal to proline and alanine residues can increase protein sequence coverage as well as PTM identifications by cleaving at unique sites in the proteome not targeted with conventional proteases.
- **Digestion at Low pH:** Activity at acidic pH (~1.0–5.5) minimizes disulfide bond scrambling and other artificial non-enzymatic PTMs like deamidation. Additional denaturants are typically not needed at this low pH.
- **Fast Digestion:** Digest samples in 1 to 2 hours rather than the typical 4 hours to overnight.

References

Samodova, D. *et al.* (2020) ProAlanase is an effective alternative to trypsin for proteomics applications and disulfide bond mapping. Mol Cell Proteomics **19(12)**, 2139–56
Link under: https://pubmed.ncbi.nlm.nih.gov/33020190/

Ordering Information

ProAlanase, Mass Spec Grade, 0.2 µg/µL
(5 µg, Cat.# VA2161)

ProAlanase Plus, Mass Spec Grade, 0.5 µg/µL
(15 µg, Cat.# VA2171)

3. Glycosidases

OVERVIEW

Glycosylation is the most abundant post-translational modification (PTM) in eukaryotic proteins. Glycosylation structures on proteins have diverse functions and can serve as disease biomarkers, such as for the progression of cancer. Therefore, tools that facilitate glycoprotein characterization are extremely important.

Endoglycosidases and exoglycosidases are tools for structural and functional analysis of glycoproteins. Endoglycosidases like PNGase F (**Figure 12**) and Endo H are routinely used to remove carbohydrates from N-glycosylated proteins. For the analysis of O-glycosylated proteins, a mixture of endoglycosidases and exoglycosidases is recommended.

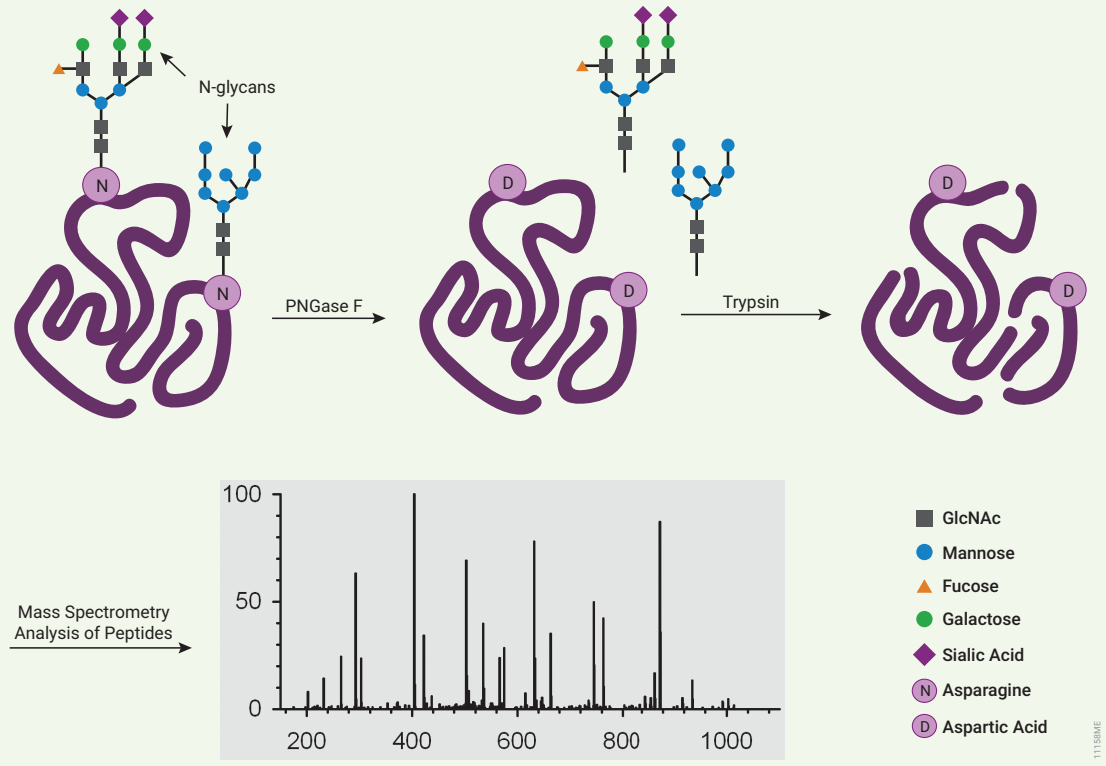


Figure 12. *Identification of N-glycosylated sites in proteins. Asn-linked glycans can be cleaved enzymatically by PNGase F, yielding intact oligosaccharides and a protein in which Asn residues at the site of N-glycosylation are converted to Asp.*

Glycosidases

- Identification of glycosylation sites by LC-MS/MS
- Characterization of glycoproteins and glycan structures
- Monitoring of protein trafficking (endoplasmic reticulum, golgi)
- Correlation studies of structural and functional data

Description

PNGase F is a recombinant glycosidase cloned from *Elizabethkingia miricola* and overexpressed in *E. coli.* PNGase F has a molecular weight of 36 kDa. PNGaseF catalyzes the cleavage of N-linked oligosaccharides between the innermost N-Acetylglucosamine (GlcNAc) and asparagine residues (Asn) of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins (**Figure 13**). Due to the enzymatic reaction, intact oligosaccharides are released and Asn residues are converted to aspartic acids (Asp) at the former sites of N-glycosylation. The conversion (deamination) of Asn to Asp leads to a monoisotopic mass shift of 0.9840 Da that is used in tandem mass spectrometry (MS/MS) for the identification of N-glycosylated sites in proteins. PNGase F will not remove oligosaccharides containing α-(1,3)-linked core fucose commonly found on plant glycoproteins.

Endo H is a recombinant glycosidase cloned from *Streptomyces plicatus* and overexpressed in *E. coli.* Endo H cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins. In contrast to PNGase F, EndoH does not cleave N-glycosylated proteins of the complex type. The enzymatic activity of EndoH leaves one GlcNac residues at the N-glycosylated protein site leading to a shift in molecular weight (M.W.). This M.W. shift is used in mass spectrometry for the identification of N-glycosylated sites.

Fetuin is a glycoprotein with O- and N-linked glycosylation sites. It is applied as deglycosylation substrate control for PNGase F and EndoH activity monitoring.

Ordering Information

PNGase F (Cat.# V4831)

Endo H (Cat.# V4871, V4875)

Fetuin (Cat.# V4961)

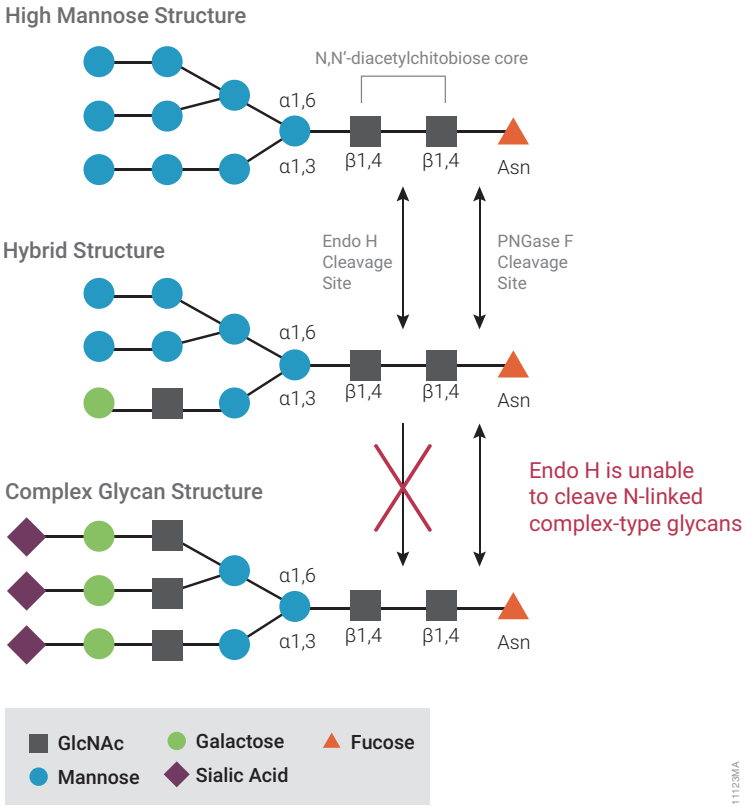


Figure 13. *Cleavage specificity of PNGase F and Endo H on N-glycans.*

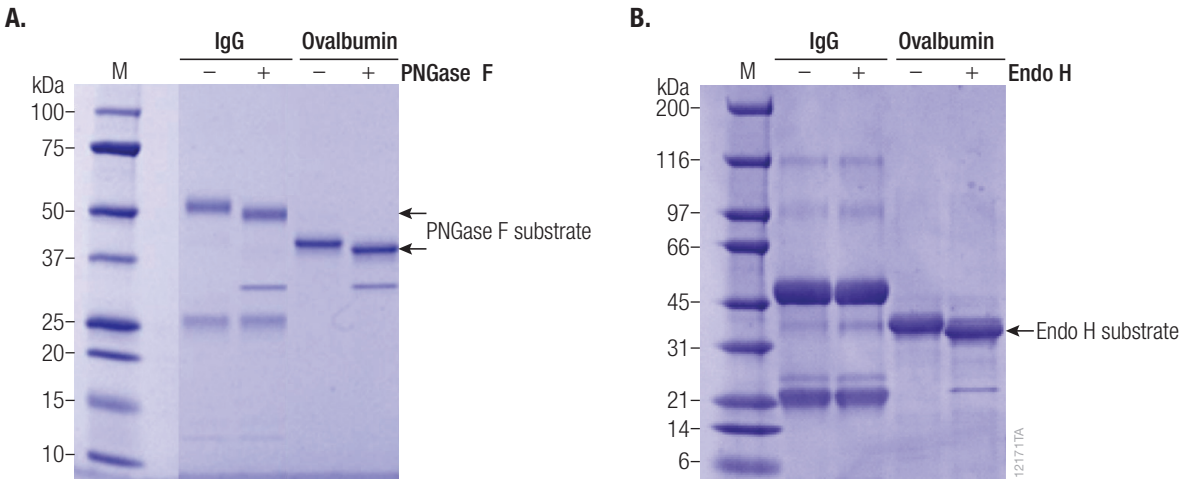


Figure 14. *Detection of N-glycosylated proteins in SDS-PAGE. After glycan cleavage, the respective proteins show a higher mobility in SDS-PAGE. The application of both PNGase F* ***(Panel A)*** *and Endo H* ***(Panel B)*** *is informative for the presence of complex glycan structures, as shown here for immunoglobulin G (IgG).*

4. Surfactants

OVERVIEW

Incomplete solubilization and digestion and poor peptide recovery are frequent limitations in protein sample preparation for mass spectrometry (MS) analysis. Detergents such as SDS or urea can improve protein solubilization and denaturation; however, they are not compatible with MS instruments and can inhibit the activity of proteases like trypsin if present in concentrations greater than 1% in the final digest. These components need to be diluted or removed prior to mass spectrometry analysis. Alternatively, mass spectrometry compatible surfactants can be used.

Here we present two novel surfactants: an acid- and thermo-labile for in-gel digestion surfactant, ProteaseMax™, and SoluMAX™ for in-solution digestion. Both highly improve solubilization, digestion and peptide recovery while avoiding negative effects observed with common solubilizing agents.

Figure 15. *Multiple applications of ProteaseMAX™ Surfactant for improved protein mass spectrometry sample preparation.*

ProteaseMAX™ Surfactant, Trypsin Enhancer

Improved in-gel and in-solution protein digestions by trypsin performance enhancement.

Description

ProteaseMAX™ Surfactant, Trypsin Enhancer is designed to improve in-gel and in-solution protein digestions. It ensures fast and efficient protein digestion with proteases such as Trypsin, Chymotrypsin and Lys-C. ProteaseMAX™ Surfactant degrades over the course of a digestion reaction (**Figure 16**), resulting in products that are compatible with mass spectrometry (MS) and liquid chromatography (LC). No long-term negative effect of the residual surfactant on the ion optics and capillary of mass spectrometry instruments has been observed. ProteaseMAX™ Surfactant can be used with existing in-gel or in-solution digestion protocols.

Principle

In-gel protein digestion is the major application for ProteaseMAX™ Surfactant. Benefits for in-gel digestion include increased peptide recovery from gels, minimized peptide absorption to plasticware and a streamlined digestion procedure. Trypsin and ProteaseMAX™ Surfactant (final concentration 0.01 %) are added to a gel slice containing the protein band of interest. The digestion is incubated at 50°C for 1 hour. ProteaseMAX™ Surfactant also improves recovery of longer peptides that are retained in the gel under a standard extraction protocol.
ProteaseMAX™ Surfactant solubilizes proteins, including difficult proteins (i.e., membrane proteins) and enhances in-solution protein digestion by providing a denaturing environment. For cytoplasmic proteins, add ProteaseMAX™ Surfactant to a final concentration of 0.03 %; for membrane proteins add to a final concentration 0.05 %.

Table 3. *Comparison of Cell-lysis/Protein Extraction Methods*

Cell-lysis/ Extraction Buffer	Number of Peptides	Number of Proteins
Urea	17,024 ± 148	3,326 ± 20
SDC*	22,171 ± 403	3,698 ± 18
ProteaseMAX™ Surfactant	29,884 ± 228	4,465 ± 100
ProteaseMAX™ Surfactant and Cell Debris**	33,098 ± 283	4,655 ± 51

** SDC: Sodium desoxycholate.*
*** Cell debris was included in the trypsin digestion step.*

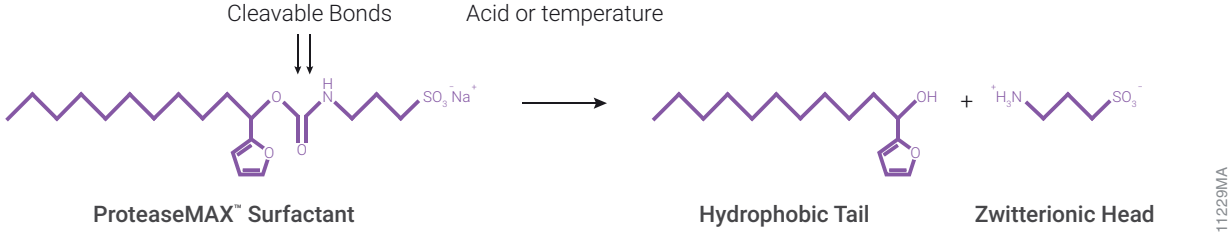


Figure 16. *Chemical Structure of ProteaseMAX™ Surfactant and its decomposition pathway.*

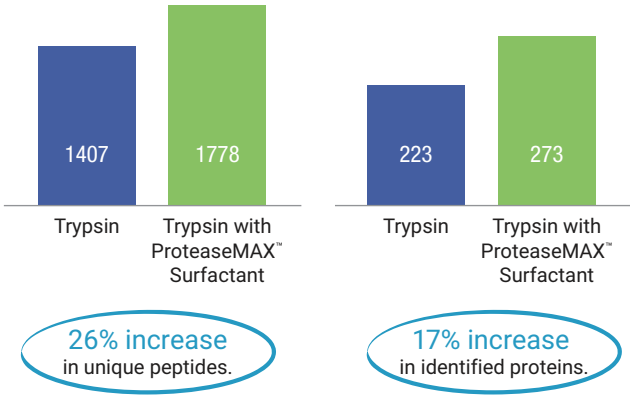


Figure 17. *Example of improved peptide/protein identifications when using ProteaseMAX™ Surfactant for in-gel digestion of a complex protein sample.*

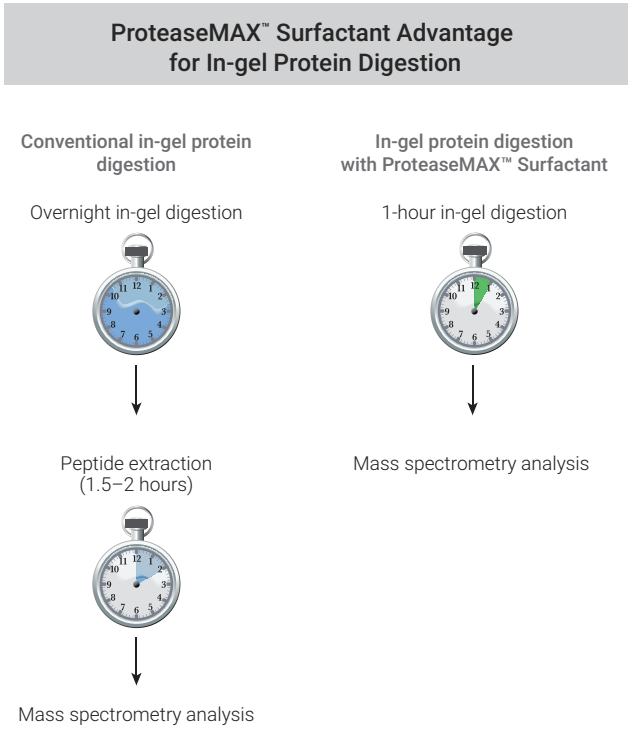


Figure 18. *Comparison of ProteaseMAX ™ Surfactant-assisted versus standard in-gel digestion procedure ProteaseMAX™ Surfactant offers time and labor savings.*

Features and Benefits

- **No Interference with Mass Spec Analysis:** Avoid the negative effects of common solubilizing agents (such as SDS).
- **Improved Peptide Recovery from Gels:** Protein sequence coverage and identification increased.
- **Enhanced Protein Solubilization at Room Temperature:** Preventing precipitation by avoiding high temperature.
- **Enhanced Proteolysis Rate:** Better access to cleavage sites.
- **Degrades Over Course of Digestion Reaction:** No need for post-digestion inactivation.

References

Saveliev, S. (2013) Mass spectrometry compatible surfactant for optimized in-gel protein digestion. Anal. Chem. **85(2)** 907-14.

Pirmoradian, M. *et al.* (2013) Rapid and deep human proteome analysis by single-dimension shotgun proteomics. Mol. Cell Prot. **12(11)**, 3330-38.

Kalashnikova, A. *et al.* (2013) Linker histone H1.0 interacts with an extensive network of proteins found in the nucleolus. Nucl. Acids. Res. **41(7)**, 4026-35.

Silva, C. *et al.* (2013) Interaction of Mycobacterium leprae with human airway epithelial cells: adherence, entry, survival, and identification of potential adhesins by surface proteome analysis. Infect. Immun. **81(7)**, 2645-59.

Pearson, R. *et al.* (2013) Regulation of H2O2 stress-responsive genes through a novel transcription factor in the protozoan pathogen Entamoeba histolytica. J. Biol. Chem. **288(6)**, 4462-74.

Ordering Information

ProteaseMAX™ Surfactant, Trypsin Enhancer (Cat.# V2071, V2072)

SoluMAX™, Mass Spec Compatible Surfactant

Novel surfactant for high temperature protein sample preparation to enhance in-solution protein extraction, solubilization and digestion.

Description

SoluMAX™ is designed to improve protein mass spectrometry sample preparation. It maximizes protein extraction and solubilization and increases proteolytic efficiency with trypsin and alternative proteases. The performance of this surfactant matches or exceeds sodium dodecylsulfonate (SDS) in terms of protein solubilization and extraction efficiency (**Figure 19**). Additionally, it minimizes peptide loss commonly observed in acid degradation step with currently available mass spectrometry compatible surfactants.

Principle

All steps of the protein sample preparation with SoluMAX™ are performed at high temperature (50°C). SoluMAX™ has been specifically selected for compatibility with trypsin activity at these conditions. The combination of surfactant and high temperature ensure maximal protein and peptide solubilization, as well as denaturation, along with significantly enhanced protein digestion. This leads to improvement in protein and peptide identification and protein sequence coverage.

Features and Benefits

- **Improved Protein Solubilization and Extraction**, particularly for large, hydrophobic proteins.
- **Enhanced Protein Digestion** with trypsin at high temperatures.
- **Increased Peptide Recovery**
- **Compatible with MS Instruments** – no removal necessary prior analysis.

Ordering Information

SoluMAX™ Surfactant (Elite Access Material)

Please contact your local Promega representative for a quote.

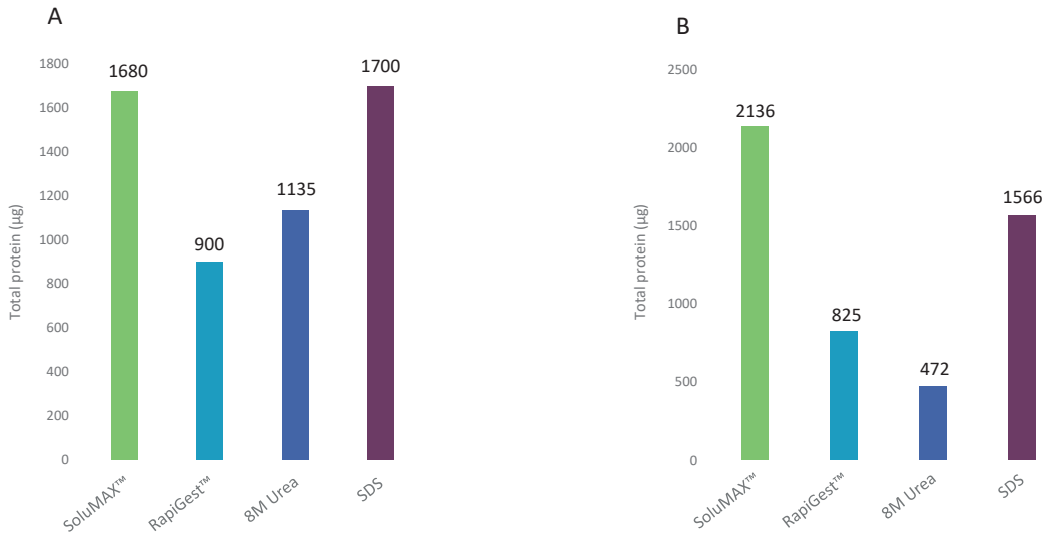


Figure 19. *SoluMAX™ matches SDS in protein solubilizing efficiency* ***(Panel A)****. SoluMAX™ maximized protein extraction from the tested tissue comparing to commonly used extraction agents* ***(Panel B)****.*

5. Tools for LC/MS Instrument Performance Monitoring

OVERVIEW

Adequate monitoring of instrument performance for proteomic studies requires complex protein reference materials. Whole-cell protein extracts provide the needed complexity. We offer mass spectrometry-compatible whole-cell reference protein extracts from yeast and human cells, in a convenient, pre-digested lyophilized form. Consistency is monitored by various protein and peptide qualitative and quantitative methods including amino acid analysis and LC/MS analysis (**Table 4**).

For routine reporting on MS instrument sensitivity and dynamic range as well various LC parameters, we recommend use of the 6 x 5 LC-MS/MS Peptide Reference Mix. The product is a mixture of 30 peptides: 6 sets of 5 isotopologues of the same peptide sequence. The isotopologues differ only by the number of stable, heavy-labeled amino acids incorporated into the sequence. The labels consist of uniform 13C and 15N atoms. Chromatographically, each of the isotopologues is indistinguishable; however, since they differ in mass, they are clearly resolved by mass spectrometry. The isotopologues of each peptide are present in a series of tenfold dilutions. This format allows assessment of instrument dynamic range and sensitivity from a single run.

Table 4. *Promega Human Extract has Enhanced Quality Control Specifications and Higher Performance than Other Commercial Extracts.*

	Promega (MS Compatible Human Protein Extract, Digest, Cat.# V6951)	Vendor X (HeLa Protein Digest Standard)
Nonbiological post-translational modifications	Deamidation spectra: < 12 %	Not tested; no specification
	Oxidation spectra: < 5 %	Oxidation spectra: < 10 %
	Carbamylation spectra: < 5 %	Carbamylation spectra: < 10 %
Missed clevages	< 10 %	< 10 %
Peptide quality	Measured by Amino Acid Analysis (AAA) to quantitate the amount of each amino acid in the sample	A_{280}
	Protein fragmentation: 1 % or less	Not tested; no specification
	Matching spectra: > 65 %	Not tested; no specification
Lot-to-lot consistency	≥ 12,462 unique peptides	LC-MS chromatogram conforms to reference
	Total proteins: ≥ 1805	4.8 %
	Protein identification > 85 % of 1,194 human core peptides	Ratio of peptide area to reference = 0.75–1.125
	Protein quantitation reproducibility is monitored by relative abundance of ten reference proteins	Not tested

MS Compatible Human Protein Extract, Digest

Ready-to-use predigested human extracts for LC/MS instrument performance monitoring and method development.

Description

MS Compatible Human Protein Extract, Digest is a lyophilized tryptic peptide mixture from K562 human cells (myelogenous leukemia cell line) designed for monitoring of LC/MS instrument performance. The digest is also suitable for MS method development such as isobaric mass tag labeling. Stringent quality control over the manufacturing process assures reproducibility of this reference material. MS Compatible Human Protein Extract, Digest is provided in ready-to-use format eliminating the need for sample preparation prior to MS analysis.

Preparation

MS Compatible Human Protein Extract, Digest, is prepared by digestion of K562 whole-cell protein extract with the Trypsin/Lys-C Mix. The digestion procedure is optimized to assure the most efficient proteolysis with less than 8 % tryptic missed cleavages. The digest is SPE (C18) cleaned-up to remove nonpeptide material and lyophilized. The digest can be reconstituted in formic acid or TFA and used for instrument performance monitoring. In general, 1µg of digest per injection is recommended.

Features and Benefits

- **Ready-to-Use:** No clean-up or digestion required.
- **High Material Complexity:** Complex proteome with a large dynamic range.
- **Lot-to-Lot Consistency:** Stringent QC over the production process.
- **Validated Reference Material:** Tested with all the major LC/MS platforms.

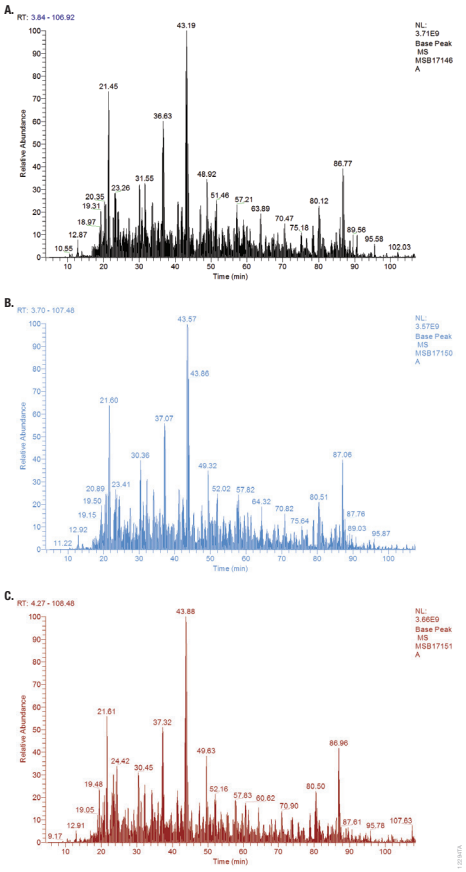


Figure 20. *Lot-to-lot reproducibility. Base peak chromatograms of three different human protein extract batches analyzed by LC/MS. Each run used 1 µg of human pre-digested protein extract injected into the instrument (Waters NanoAquity HPLC system interfaced to a ThermoFisher Q Exactive). Peptides were resolved with 2 h gradient.*

Ordering Information

MS Compatible Human Protein Extract, Digest (Cat.# V6951)

MS Compatible Human Protein Extract, Intact (Cat.# V6941)

MS Compatible Yeast Protein Extract, Digest

Ready-to-use predigested yeast extracts for LC/MS instrument performance monitoring and method development.

Description

MS Compatible Yeast Protein Extract, Digest from *Saccharomyces cerevisiae* is a lyophilized tryptic peptide mixture designed for monitoring LC/MS instrument performance. The digest is also suitable for MS method development such as isobaric mass tag labeling. Stringent quality control over the manufacturing process assures reproducibility of this reference material.

Preparation

MS Compatible Yeast Protein Extract, Digest, is prepared by digestion of yeast whole-cell protein extract with the Trypsin/Lys-C Mix. The digestion procedure is optimized to assure the most efficient proteolysis with less than 8 % tryptic missed cleavages. The digest is SPE (C18) cleaned-up to remove nonpeptide material and lyophilized. The digest can be reconstituted in formic acid or TFA and used for instrument performance monitoring. In general, 1 µg of digest per injection is recommended.

Features and Benefits

- **Ready-to-Use:** No clean-up or digestion required.
- **Well-Annotated Proteome:** The small yeast proteome is a convenient reference material.
- **Lot-to-Lot Consistency:** Stringent QC throughout the production process.
- **Validated Reference Material:** Tested with all the major LC/MS platforms.

Ordering Information

MS Compatible Yeast Protein Extract, Digest (Cat.# V7461)

MS Compatible Yeast Protein Extract, Intact (Cat.# V7341)

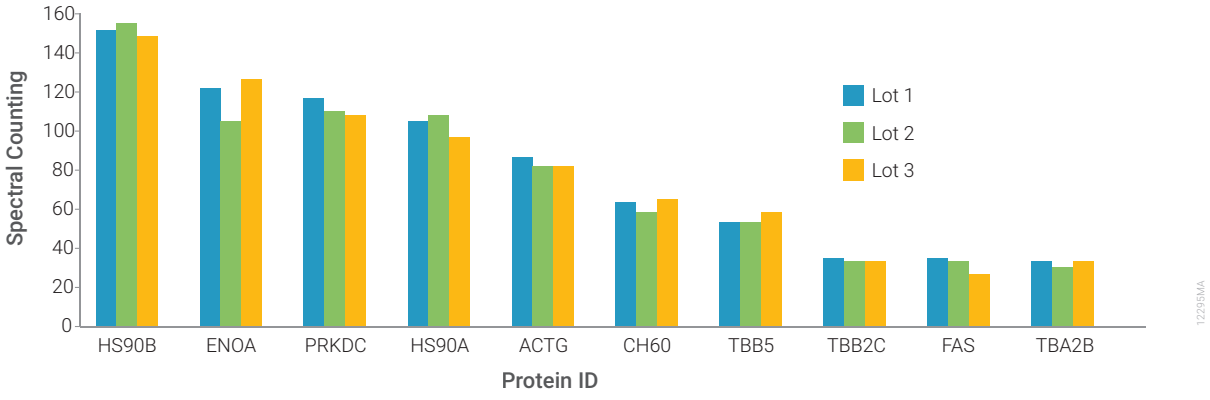


Figure 21. *Lot-to-lot quantitative reproducibility of yeast extract. Relative protein quantitative levels were determined by spectral counting. The data showed high protein quantitative reproducibility between the different lots.*

6 × 5 LC-MS/MS Peptide Reference Mix

Unique peptide mix to optimize both LC and MS methods to monitor instrument performance.

Description

The 6 × 5 LC-MS/MS Peptide Reference Mix is a unique reagent designed to monitor liquid chromatography (LC) and mass spectrometry (MS) instrument performance and to assist in method development/optimization. The product is a mixture of 30 peptides: 6 sets of 5 isotopologues of the same peptide sequence. The isotopologues differ only by the number of stable, heavy-labeled amino acids incorporated into the sequence. The labels consist of uniform 13C and 15N atoms. Chromatographically, each of the isotopologues is indistinguishable; however, since they differ in mass, they are clearly resolved by mass spectrometry. The isotopologues of each peptide are present in a series of tenfold dilutions. This format allows assessment of instrument dynamic range and sensitivity from a single run (**Figure 22**). Peptides with a wide range of hydrophobicities were chosen to enable reporting of LC column performance. In addition there is a mass separation of at least 4 daltons between the isotopologues, so that even low resolution instruments can distinguish the masses.

PReMiS™ Software Tool

The 6 × 5 LC-MS/MS Peptide Reference Mix is designed for use with the complementary, free PReMiS™ Software tool (available by download) that reports on key liquid chromatography and mass spectrometry parameters. The parameter reports can be exported to CSV or saved as ".pdf" files. In addition to the general reporting feature, performance parameters can be tracked over time, allowing a clear assessment of trends to pinpoint poor performance and maintenance needs. For those laboratories that have multiple instruments, the ability to compare parameters across instruments will also be available. Thermo (.raw) and ABSCIEX (.wiff) are available for direct importing. Other vendor formats can be imported after conversion to ".mzml."

Ordering Information

6 x 5 LC-MS/MS Peptide Reference Mix
(Cat.# V7491, V7495)

Features and Benefits

Unique Peptide Formulation

- Save time by assessing LC and MS parameters (e.g., dynamic range and sensitivity) in one run with a single reagent.
- Optimize and streamline method development.

Complementary Software Provides Routine Analysis and Historical Monitoring

- Eliminate time-consuming manual calculations.
- Ensure consistent instrument performance over time.
- Compare performance of multiple instruments over time.

Peptides are AAA Qualified

- Accurate reporting of instrument sensitivity and dynamic range.

Multiple Applications

- Compatible with neat or complex mixture analysis.

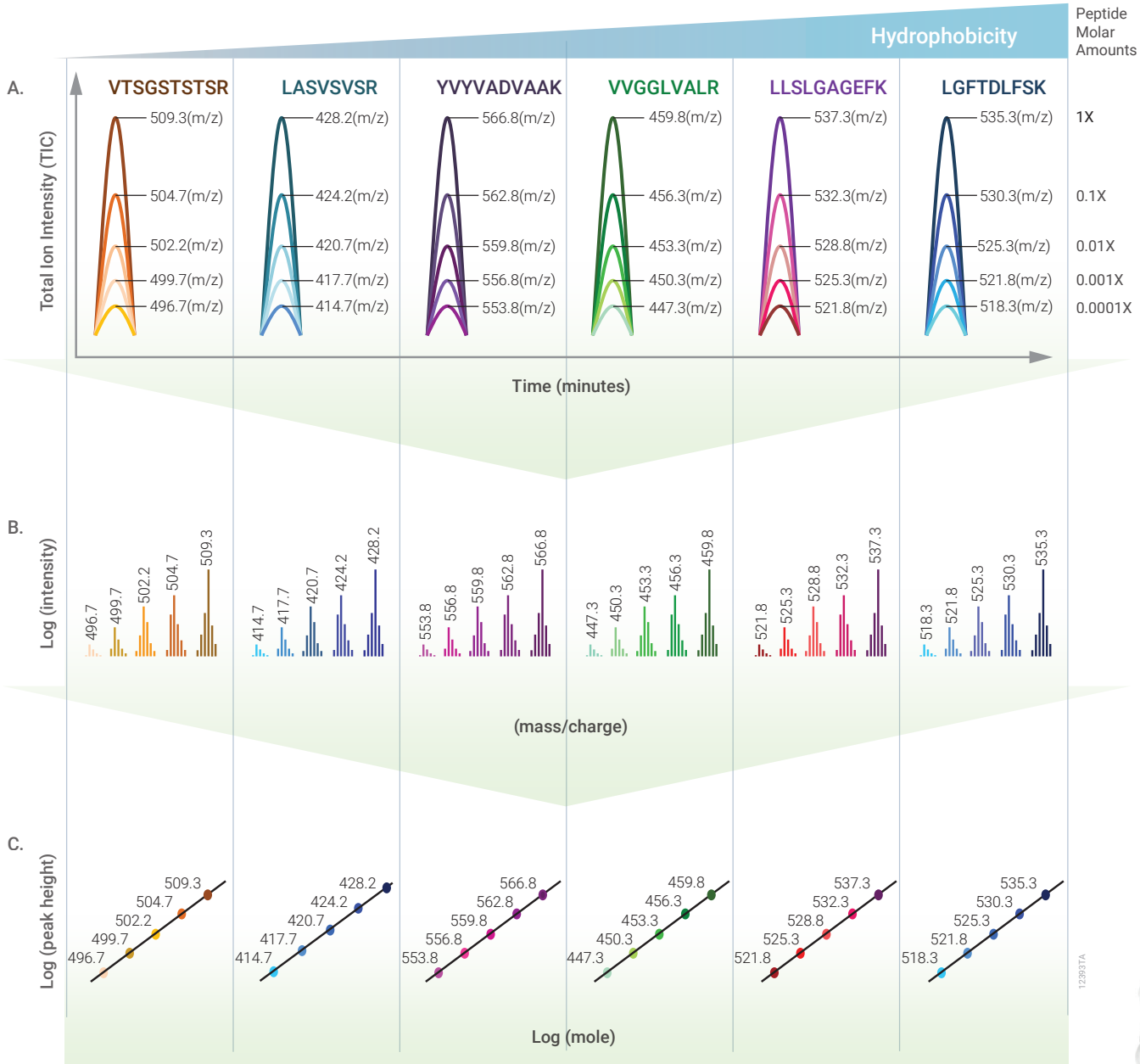


Figure 22. *Schematic illustrating standard workflow and correlating features of the 6 × 5 LC-MS/MS Peptide Reference Mix. A mixture of 6 peptide sets is actually 30 peptides, in total. Each of the peptides sets is a mixture of 5 isotopologues that are distinguished only by mass through the incorporation of stable, heavy-labeled amino acids.*

Panel A. *The isotopologues are chemically and chromatographically identical and therefore appear as 6 peaks.*

Panel B. *Each of the isotopologues is mixed with a 10-fold molar increase (lightest to heaviest).*

Panel C. *A plot of the log of the peak height versus the log (molar amount) on column has a slope of 1. This analysis enables confirmation of detection limits and can also be used to confirm linearity of up to 5 logs of dynamic range.*

6. Antibody Characterization/Fragmentation

OVERVIEW

Therapeutic monoclonal antibodies are large, complex molecules that undergo numerous post-translational modifications (PTMs). Numerous varieties of PTMs are known to exist on antibodies. Because these PTMs can affect the efficacy and safety profile of the drug, the structural variants must be assessed and quantified. In-depth characterization of antibody PTMs remains a significant hurdle because their large size (~150 kDa) makes mass spectrometry analysis extremely challenging.

The AccuMAP™ Low pH Protein Digestion Kits suppress artificial nonenzymatic PTMs, as sample preparation is performed at low pH under either reducing or nonreducing conditions.

IdeS and IdeZ Proteases are highly specific and cleaves human (and some other species) IgG specifically at one site in the lower hinge region. Because of the exquisite specificity of these enzymes, they produce highly homogeneous Fc and Fab fragments which are then readily analyzed using techniques such as mass spectrometry or X-ray crystallography.

The ISOQUANT® Isoaspartate Detection Kit is intended for quantitative detection of isoaspartic acid residues in proteins and peptides. It allows monitoring of protein deamidation caused during storage or handling.

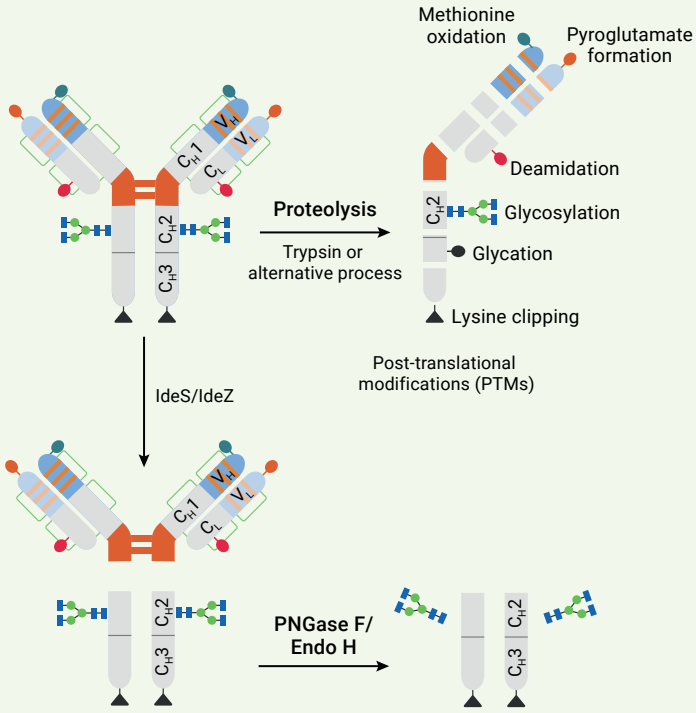


Figure 23. *Common antibody characterization methods.*

AccuMAP™ Low pH Protein Digestion Kit

Suppress nonenzymatic PTMs during protein sample preparation.

Description

The AccuMAP™ Low pH Protein Digestion Kit is designed for accurate, reproducible characterization of biotherapeutic proteins by peptide mapping using LC/MS and/or UV HPLC. The entire sample preparation procedure is performed at low (mildly acidic) pH to suppress artificial deamidation and disulfide bond scrambling. The kit also contains an optional agent for suppression of protein oxidation during sample preparation.

Principle

Nonenzymatic posttranslational modifications (PTMs) spontaneously occur in biotherapeutic proteins during manufacturing and storage. The major nonenzymatic PTMs are deamidation, disulfide bond scrambling and oxidation. These modifications can affect efficacy and stability of biotherapeutic proteins and are the subject of careful monitoring. Nonenzymatic PTMs can also be induced during protein sample preparation for peptide mapping and can compromise analysis. Major causes of nonenzymatic PTMs induced during sample preparation include alkaline pH as well as excipients and impurities that have protein oxidizing activity. The AccuMAP™ Low pH Protein Digestion Kits suppress artificial nonenzymatic PTMs, as sample preparation is performed at low pH under either reducing or nonreducing conditions (**Figure 24**).

Ordering Information

AccuMAP™ Low pH Protein Digestion Kits (Cat.# VA1040, VA1050)

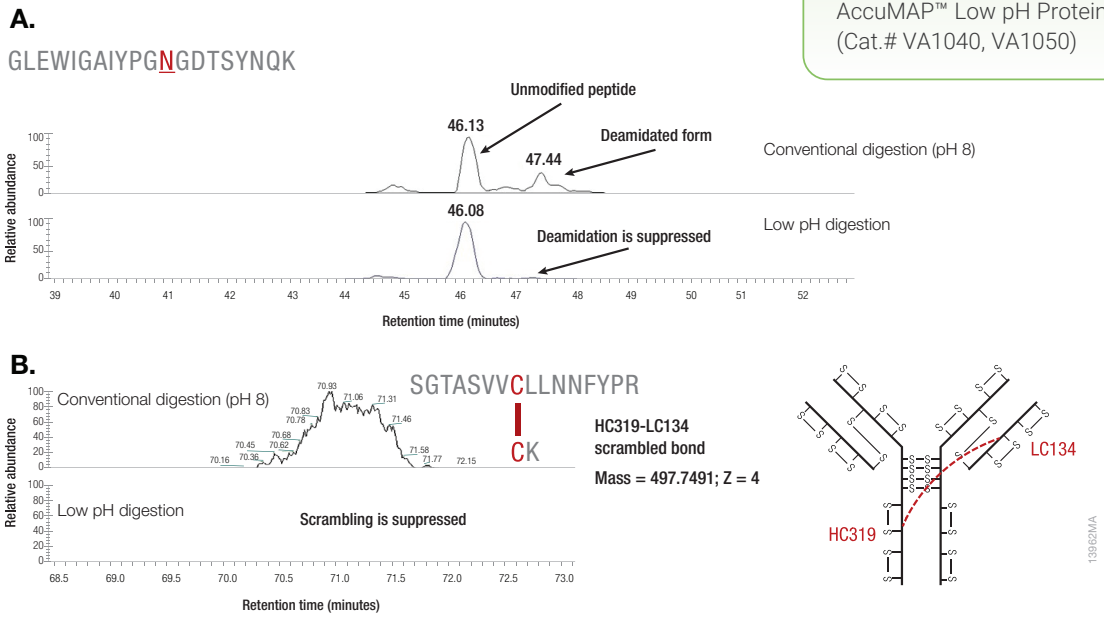


Figure 24. *Suppression of deamidation and disulfide bond scrambling in IgG digested with AccuMAP™ Low pH Protein Digestion Kit.* **Panel A** *shows extracted ion chromatogram of a peptide from Rituximab antibody digested at conventional conditions (pH 8) and at low pH. The data show that asparagine (highlighted in red) was deamidated in this peptide at pH 8. In contrast, deamidation was fully suppressed at low pH.* **Panel B** *shows an extracted ion chromatogram of a peptide with a scrambled disulfide bond from Panitumumab antibody digested under conventional conditions (pH 8) and at low pH.*

IdeS and IdeZ Proteases

Recombinant proteases engineered to simplify antibody fragmentation and characterization.

Description

IdeS and IdeZ Proteases are immunoglobulin G (IgG)-degrading proteases that cleave with high specificity. IdeS Protease is derived from *Streptococcus pyogenes*. It is an engineered, recombinant protease overexpressed in *E. coli*. IdeS Protease cleaves IgG at a single site below the hinge region, yielding $F(ab')_2$ and Fc fragments as shown here. IdeZ Protease is derived from *Streptococcus equi* subspecies *zooepidemicus*. It is also an engineered, recombinant protease overexpressed in *E. coli*. Like IdeS, IdeZ Protease specifically cleaves IgG molecules below the hinge region, yielding $F(ab')_2$ and Fc fragments. However, IdeZ Protease has significantly improved activity against mouse IgG2a and IgG3 subclasses compared to IdeS Protease.

Features and Benefits

- **Fast and Easy:** Digestion in 30 minutes with no optimization.
- **Highly Specific and Reproducible:** Cleaves exclusively at a single site below the hinge to produce $F(ab)_2$ and Fc fragments.
- **High Performance:** Essentially 100 % complete digestion.
- **Versatile:** IdeS and IdeZ both effectively cleave human IgG1, IgG2, IgG3 and IgG4, monkey, sheep, rabbit, humanized and chimeric IgGs as well as Fc-fusion proteins. However, IdeZ Protease cleaves mouse IgG2a and IgG3 significantly better than does IdeS Protease.

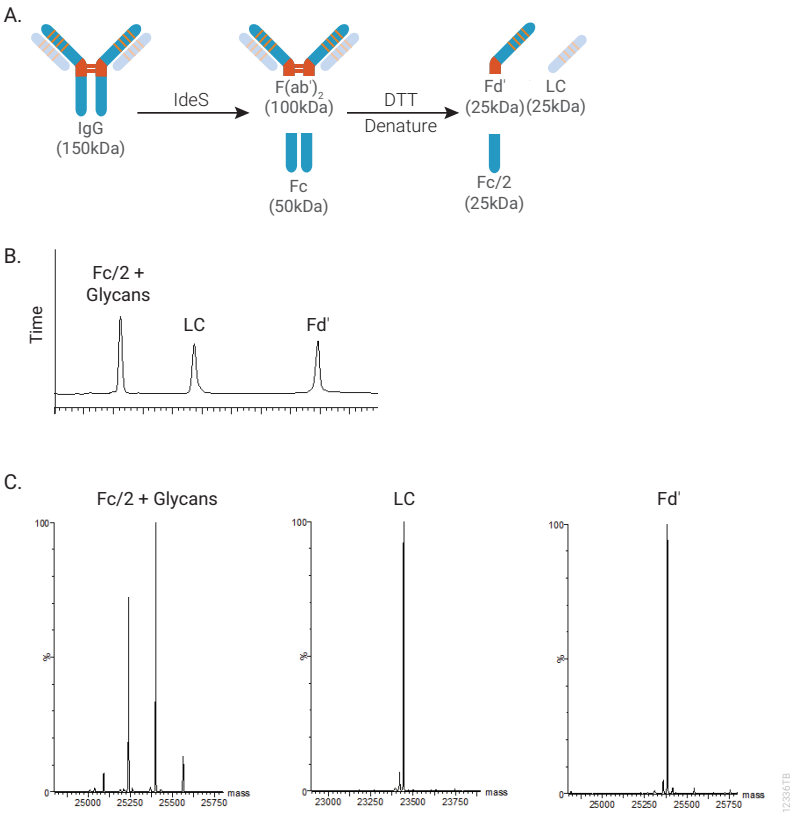


Figure 25. *Digestion with IdeS followed by reduction and denaturing produces fragments that are better separated by HPLC and are ideal for mass spectrometry analysis.* **Panel A:** *Digestion of IgG with IdeS, followed by reduction, produces three 25 kDa fragments.* **Panel B:** *Example of IdeS digestion products separated on HPLC.* **Panel C:** *Mass spectrometry results of the three IdeS digestion products.*

References

Wang, J. *et al.* (2017) Evaluation of automated Wes system as an analytical and characterization tool to support monoclonal antibody drug product development. J. Pharm. Biomed. Anal. **139**, 263–8.

Zheng, J.Y. *et al.* (2017) Excess reactive oxygen species production mediates monoclonal antibody-induced human embryonic stem cell death via oncosis. Cell Death Differ. **24**, 546-58.

Zhang, B. *et al.* (2016) Development of a rapid RP-UHPLC-MS method for analysis of modification in therapeutic monoclonal antibodies. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **1032**, 172-81.

Ordering Information

IdeS Protease (Cat.# V7511, V7515)

IdeZ Protease (Cat.# V8341, V8345)

ISOQUANT® Isoaspartate Detection Kit

Sensitive and non-radioactive isoaspartate quantitation for protein deamidation monitoring during storage and handling.

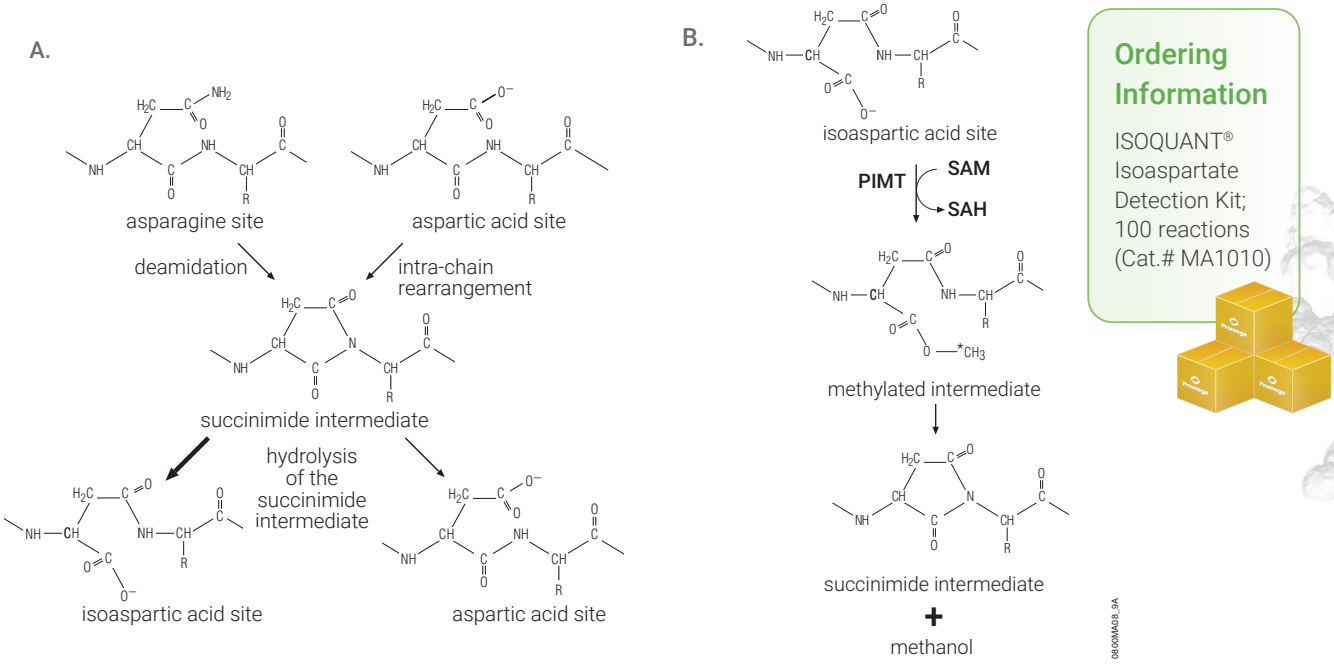
Description

The ISOQUANT® Isoaspartate Detection Kit is intended for quantitative detection of isoaspartic acid residues in peptides or proteins such as monoclonal antibodies. These isoaspartic residues can result from the gradual, non-enzymatic deamidation of asparagine or rearrangement of aspartic acid residues.

The deamidation of asparagine residues is characterized by the formation of a succinimide intermediate that resolves to form a mixture of isoaspartic acid (typically 70–85%) and aspartic acid (**Figure 26**). The ISOQUANT® Isoaspartate Detection Kit uses the enzyme Protein Isoaspartyl Methyltransferase (PIMT) to specifically detect the presence of isoaspartic acid residues in a target protein. PIMT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to isoaspartic acid. Spontaneous decomposition of this methylated intermediate results in the release of methanol and reformation of the succinimide.

Features and Benefits

- **Economical:** Detection by HPLC eliminates cost and inconvenience of handling radioactive materials.
- **Versatile:** Assay samples individually or in batches. Small sample size makes the assay suitable for research, analytical methods, formulations and process development work.
- **Robust:** Achieve detection levels as low as 10 pmol. Not affected by common buffer components.
- **HPLC Detection Method:** Fits with existing equipment and expertise.



Ordering Information

ISOQUANT® Isoaspartate Detection Kit; 100 reactions (Cat.# MA1010)

Figure 26. ***Panel A.*** *Protein deamidation at asparagine and intrachain bond rearrangement at aspartic acid. The side chains of asparagine or aspartic acid residues can spontaneously form a succinimide ring intermediate with the peptide backbone, which rearranges to form isoaspartic acid (70–85 %) and aspartic acid (15–30 %).* ***Panel B.*** *Detection of isoaspartic acid via PIMT-catalyzed generation of SAH and methanol.*

7. Detailed information on Proteases for MS Sample Preparation

Table 5. *Characteristics and Reaction Conditions for Trypsin Proteases*

	Trypsin Platinum	Trypsin Gold, Sequencing Grade Modified Trypsin, Sequencing Grade Modified Trypsin, Frozen	Immobilized Trypsin	Trypsin/Lys-C Mix
Cat.#	VA9000	V5280; V5111; V5117; V5113	V9012; V9013	V5071; V5072; V5073
Source and Size	recombinant	Porcine Pancreas (23.8kDa)	Porcine Pancreas (23.8kDa)	Mix of Trypsin Gold & rLys-C
Cleavage Sites	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. Asp and Glu at C-terminal side of Lys and Arg inhibit cleavage.	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. Asp and Glu at C-terminal side of Lys and Arg inhibit cleavage.	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. Asp and Glu at C-terminal side of Lys and Arg inhibit cleavage.	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. In contrast to trypsin, Trypsin/Lys-C efficiently tolerates presence of Glu and Asp at C-terminal side of Lys.
Protease Protein Ratio (w/w)	1:10	1:20 to 1:100	variable	1:25 to 1:50
pH Range for Digestion	pH 7–9	pH 7–9	pH 5–9	pH 8
Reaction Conditions	300mM Tris-HCl (pH 8)/ 12mM $CaCl_2$	50–100mM Tris-HCl (pH 8) or 50–100mM NH_4HCO_3 (pH7.8). Digest overnight hours at 37°C.	50mM NH_4HCO_3 (pH 7.8). Digest for 30 minutes at room temperature.	50–100mM Tris-HCl (pH 8) or 50mM NH_4HCO_3 (pH7.8). Digest overnight at 37°C.
Buffer Compatibility	Tris-HCl	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3
In-Gel Digestion Compatibility	Yes	Yes	No	Yes
ProteaseMAX™ Compatibility	Yes	Yes	Yes	Not tested
Notes	Most accurate and efficient trypsin. Designed for superior autoproteolytic resistance, outstanding cleavage specificity and improved proteolytic efficiency. An ideal tool for the biotherapeutic protein characterization.	Treated with TPCK to inactivate chymotrypsin contamination and methylated to minimize autoproteolysis. Resistant to mild denaturing conditions (1–2 M urea and 0.1 % SDS). Retain 48 % activity in 2 M Guanidine Chloride.	Used if rapid digestion is required. Urea ≤ 4 M; guanidine HCl ≤ 3 M; methanol < 60 %; DTT ≤ 50 mM; TCEP ≤ 5 mM; pH 5–9; Iodoacetamide ≤ 300 mM.	Trypsin/Lys-C mix is the most robust trypsin preparation designed to improve general protein digestion. Trypsin/Lys-C mix uses regular overnight digestion at 37°C under non- or mildly denaturing conditions.

Table 6. *Characteristics and Reaction Conditions for Low Specific Alternative Protease*

	Chymotrypsin
Cat.#	V1061; V1062
Source and Size	Bovine pancreas (25kDa)
Cleavage Sites	Preferentially C-terminal of Trp, Tyr and Phe but it also cleaves at other residues albeit at a lower efficiency
Protease Protein Ratio (w/w)	1:20 to 1:200
pH Range for Digestion	pH 7–9
Reaction Conditions	100mM Tris HCl (pH 8), 10mM CaCl2. Digestion 2–18 hours at 25°C.
Buffer Compatibility	Tris-HCl, NH4HCO3
In-Gel Digestion Compatibility	Yes
ProteaseMax™ Compatibility	Yes
Notes	Often used to digest hydrophobic proteins including membrane proteins. 80% activity retained in the presence of urea (up to 1M) or 1M guanidine HCl (up to 1M). No reduction in activity in the presence of ProteaseMAX™ Surfactant (up to 0,025%).

Table 7. *Characteristics and Reaction Conditions for Nonspecific Proteases*

	Elastase	Pepsin	Thermolysin
Cat.#	V1891	V1959	V4001
Source and Size	Porcine pancreas (25.9 kDa)	Porcine stomach (34.6 kDa)	*Bacillus thermoproteolyticus rokko* (36.2 kDa)
Cleavage Sites	Preferentially C-terminal of Ala, Val, Ser, Gly, Leu and Ile.	Preferentially C-terminal of Phe, Leu, Tyr and Trp	Preferentially N-terminal of Leu, Phe, Val, Ile and Met.
Protease Protein Ratio (w/w)	1:20 to 1:100	1:20 to 1:100	1:20 to 1:50
pH Range for Digestion	pH 9	pH 1–3	pH 5.0–8.5
Reaction Conditions	50–100 mM Tris-HCl (pH 8.5–9.5), digestion 2–18 hours at 37°C.	Adjust protein solution to pH 1–3 with 1 N HCl prior to digestion. Digestion 1–18 hours at 37°C.	50 mM Tris-HCl (pH 8), 0.5 mM $CaCl_2$. Digestion 0.5–6 hours at 70–95°C.
Buffer Compatibility	Tris-HCl, NH_4HCO_3	(—)	Tris-HCl
In-Gel Digestion Compatibility	Yes	Yes	Not tested
ProteaseMAX™ Compatibility	Yes	Yes	Yes
Notes	Used as a trypsin alternative to increase protein coverage.	Used in structural protein studies (HDX exchange based) and antibody analysis; used to digest proteolytically resistant, tightly folded proteins.	Used to digest proteolytically resistant, tightly folded proteins and in structural studies.

Table 8. *Characteristics and Reaction Conditions for Specific Alternative Proteases*

	rLys-C, Mass Spec Grade	Lys-C, Mass Spec Grade	rAsp-N, Mass Spec Grade
Cat.#	V1671	VA1170	VA1160
Source and Size	*Pseudomonas aeruginosa*. Expressed in *E.coli* (27.7 kDa)	*Lysobacter enzymogenes* (30 kDa)	*Stenotrophomonas maltophilia* (25 kDa)
Cleavage Sites	C-terminal of Lys. Does not cleave if Lys is followed by Pro. Asp and Glu at C -terminal side of Lys inhibit cleavage.	C-terminal of Lys. Does not cleave if Lys is followed by Pro. Asp and Glu at C -terminal side of Lys inhibit cleavage.	Primarily on the N-terminal side of aspartic acid residues. Cleavage on the N-terminal side of glutamic acid residues can occur at a slower rate.
Protease Protein Ratio (w/w)	1:20 to 1:50	1:20 to 1:100	1:10 to 1:100
pH Range for Digestion	pH 8–9	pH 7–9	rAsp-N has maximal activity at pH 8, but pH buffers ranging from 6–9 can be used.
Reaction Conditions	50–100 mM Tris-HCl (pH 8) or 50 mM NH_4HCO_3 (pH 7.8). Digestion 2–18 hours at 37°C.	Incubate at 37°C for 2–18 hours	Incubate at 37°C for 60 minutes
Buffer Compatibility	Tris-HCl, NH_4HCO_3	50 mM Tris (pH 8)	Ammonium acetate pH 5-6, HEPES pH 7, Tris pH 8–9, Ammonium Bicarbonate pH 8
In-Gel Digestion Compatibility	Yes	Not tested	Not tested
ProteaseMAX™ Compatibility	Yes	Not tested	Not tested
Notes	Inexpensive alternative to a native Lys-C protease. Similarly to a native protease, rLys-C tolerates high denaturing conditions such as 8 M urea. Used to digest tightly folded proteolytically resistant proteins. Also used as a trypsin alternative if larger peptides are preferable for the analysis. If urea is used in protein sample preparation, avoid high temperature. High temperature induces protein carbamylation in the presence of urea.	Tolerates high denaturing conditions such as 8 M urea. Used to digest tightly folded proteolytically resistant proteins. Also used as a trypsin alternative if larger peptides are preferable for analysis. If urea is used in protein sample preparation, avoid high temperature. High temperature induces protein carbamylation in the presence of urea.	rAsp-N, Mass Spec Grade, is lyophilized in Tris (pH 8.0) with NaCl and stabilizing sugars. Reconstitute in 50 µl of ultrapure water and mix gently. Reconstituted rAsp-N can be stored at 4°C for at least 8 weeks. For longer storage, single-use aliquots can be stored at −65°C or below. Avoid freeze-thaw cycles. rAsp-N has a histidine tag that can be used to remove it from solution.

Table 9. *Characteristics and Reaction Conditions for Specific Alternative Proteases (continued)*

	Asp-N	Arg-C	Glu-C	ProAlanase Mass Spec Grade
Cat.#	V1621	V1881	V1651	VA2161; VA2171
Source and Size	*Pseudomonas fragi* (24.5 kDa)	*Clostridium histolyticum* (Subunits: 45 kDa and 12 kDa)	*Staphylococcus aureus V8* (27 kDa)	*Aspergillus niger* (63 kD)
Cleavage Sites	N-terminal of Asp	C-terminal of Arg. Also cleaves at Lys albeit at lower efficiency	C-terminal of Glu. Low level cleavages might occur at Asp residues too albeit at 100–300 fold lower efficiency	Primarily on the C-terminal side of proline and, to a lesser extent, alanine amino acids
Protease Protein Ratio (w/w)	1:20 to 1:200	1:20 to 1:350	1:20 to 1:200	1:10 to 1:500
pH Range for Digestion	pH 4–9	pH 7.6–7.9	pH 4–9	pH 1-5.5 (optimum at 1.5)
Reaction Conditions	50 mM Tris-HCl (pH 8). Digestion 2–18 hours at 37°C	50 mM Tris-HCl (pH 7.6-7.9), 5mM $CaCl_2$, 2 mM EDTA, > 2 mM DTT. Digestion 2–18 hours at 37°C	100 mM NH_4HCO_3 (pH 7.8), 50–100 mM HCL (pH8). Digestion 2–18 hours at 37°C	Incubate at 37°C for 2 hours
Buffer Compatibility	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	NH_4HCO_3, Ammonium acetate	HCl (pH 1–2.5), glycine HCl (pH 1–2.5), sodium citrate (pH 3–4), sodium acetate (pH 4.5–5.5)
In-Gel Digestion Compatibility	Yes	Yes	Yes	Yes
ProteaseMAX™ Compatibility	Yes	Yes	Yes	Not tested
Notes	Can be used as a trypsin alternative to achieve better distribution of cleavage sites.100 % activity retained in the presence of urea (up to 3.5 M), guanidine HCL (1 M), SDS (up to 0,028 %), ProteaseMax™ Surfactant (up to 0,026 %), acetonitrile (up to 60 %), EDTA (up to 2 mM); DTT or ß-mercaptoethanol.	Used in analysis of histone modifications. Requires DTT, cysteine or other reducing agent and CaCl2 for activity.	Can be used as a trypsin alternative to achieve better distribution of cleavage sites. Glu-C activity and cleavage specificity is affected by buffer conditions. In ammonium biocarbonate and other non-phosphate buffers, Glu-C cleaves at C-term of Glu. Glu-C cleaves at C-term of Glu and Asp in phosphate buffer.	Optimization may still be necessary with the focus on: digestion time, enzyme:substrate ratio and pH. ProAlanase can be stored for 2 months at +2°C to +10°C. For longer term storage, freeze and keep at –10°C or below. Do not exceed five freeze-thaw cycles.

8. Practical Information

AMINO ACIDS, GENETIC CODE, PROTEIN CONVERSION

Table 10. *Amino Acid Abbreviations and Molecular Weights*

Amino Acid	Three-Letter Abbreviation	One-Letter Symbol	Molecular Weight
Alanine	Ala	A	89 Da
Arginine	Arg	R	174 Da
Asparagine	Asn	N	132 Da
Aspartic acid	Asp	D	133 Da
Asparagine or Aspartic acid	Asx	B	–
Cysteine	Cys	C	121 Da
Glutamine	Gln	Q	146 Da
Glutamic Acid	Glu	E	147 Da
Glutamine or Glutamic acid	Glx	Z	–
Glycine	Gly	G	75 Da
Histidine	His	H	155 Da
Isoleucine	Ile	I	131 Da
Leucine	Leu	L	131 Da
Lysine	Lys	K	146 Da
Methionine	Met	M	149 Da
Phenylalanine	Phe	F	165 Da
Proline	Pro	P	115 Da
Serine	Ser	S	105 Da
Threonine	Thr	T	119 Da
Tryptophan	Trp	W	204 Da
Tyrosine	Tyr	Y	181 Da
Valine	Val	V	117 Da

The average molecular weight of an amino acid is 110 Da.

Table 11. *Genetic Code*

1st Position	2nd Position U	2nd Position C	2nd Position A	2nd Position G	3rd Position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	**UAA** Stop	**UGA** Stop	A
	UUG Leu	UCG Ser	**UAG** Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Termination codons are in bold. AUG start codon is in bold italics.

Table 12. *Protein Conversions*

Protein Molar Conversion	
100 pmol of 100 kDa protein	10 µg
100 pmol of 50 kDa protein	5 µg
100 pmol of 10 kDa protein	1 µg
100 pmol of 1 kDa protein	100 ng

Protein/DNA Conversions	
1 kb of DNA	333 Amino Acids of Coding Capacity 37 kDa protein
270 bp DNA	10 kDa protein
810 bp DNA	30 kDa protein
1.35 kb DNA	50 kDa protein
2.7 kb DNA	100 kDa protein
Average MW of an Amino Acid	110 daltons

9. Ordering Information

PROTEIN CHARACTERIZATION BY MASS SPECTROMETRY

Product	Size	Cat.#
Trypsin		
Trypsin Platinum	100 µg	VA9000
Trypsin Gold, Mass Spectrometry Grade	100 µg	V5280
Sequencing Grade Modified Trypsin	100 µg (5 x 20 µg)	V5111
	100 µg	V5117
Sequencing Grade Modified Trypsin, Frozen	100 µg (5 x 20 µg)	V5113
Immobilized Trypsin	2 ml	V9012
	2 x 2 ml	V9013
Trypsin/Lys-C Mix, Mass Spec Grade	20 µg	V5071
	100 µg	V5072
	100 µg (5x 20 µg)	V5073
Rapid Digestion – Trypsin	100 µg	VA1060
Rapid Digestion – Trypsin/Lys-C	100 µg	VA1061
Alternative Proteases: Specific Proteases		
Arg-C, Sequencing Grade	10 µg	V1881
Asp-N, Sequencing Grade	2 µg	V1621
Glu-C, Sequencing Grade	50 µg (5 x 10 µg)	V1651
Lys-C, Mass Spec Grade	20 µg	VA1170
rAsp-N, Mass Spec Grade	10 µg	VA1160
rLys-C, Mass Spec Grade	15 µg	V1671
ProAlanase, Mass Spec Grade	5 µg	VA2161
ProAlanase Plus, Mass Spec Grade	15 µg	VA2171
Alternative Proteases: Low-Specific Protease		
Chymotrypsin, Sequencing Grade	25 µg	V1061
	100 µg (4 x 25 µg)	V1062
Alternative Proteases: Nonspecific Proteases		
Elastase	5 mg	V1891
Pepsin	250 mg	V1959
Thermolysin	25 mg	V4001

Product	Size	Cat.#
Glycosidases		
PNGase F	500 units (10 u/µl)	V4831
Endo H	10,000 units (500 u/µl)	V4871
	50,000 units (500 u/µl)	V4875
Fetuin (control protein)	500 µg (10 mg/ml)	V4961
Surfactants		
ProteaseMAX™ Surfactant, Trypsin Enhancer	1 mg	V2071
	5 x 1 mg	V2072
SoluMAX™ Surfactant	5 mg	on request
Protein Extracts for LC/MS Monitoring		
MS Compatible Human Protein Extract, Digest	100 µg	V6951
MS Compatible Human Protein Extract, Intact	1 mg	V6941
MS Compatible Yeast Protein Extract, Digest	100 µg	V7461
MS Compatible Yeast Protein Extract, Intact	1 mg	V7341
Peptide Mix For LC/MS Monitoring		
6 × 5 LC-MS/MS Peptide Reference Mix	25 pmoles	V7491*
	200 pmoles	V7495**
Antibody Characterization/Fragmentation		
AccuMAP™ Low pH Protein Digestion Kit	10 reactions	VA1040
	100 reactions	VA1050
IdeS Protease	5,000 u	V7511
	25,000 u (5 × 5,000 u)	V7515
IdeZ Protease	5,000 u	V8341
	25,000 u	V8345
ISOQUANT® Isoaspartate Detection Kit	100 reactions	MA1010

* *Supplied as frozen liquid. Includes 6 additional maximal recovery tubes for aliquots.*

** *Supplied lyophilized. Includes 6 additional maximal recovery tubes for aliquots.*

10. Custom Services

CUSTOMIZABLE REAGENTS FOR PROTEIN ANALYSIS

- **High-Quality Proteases**
  Specifically developed for mass spectrometry applications
- **Flexible Manufacturing**
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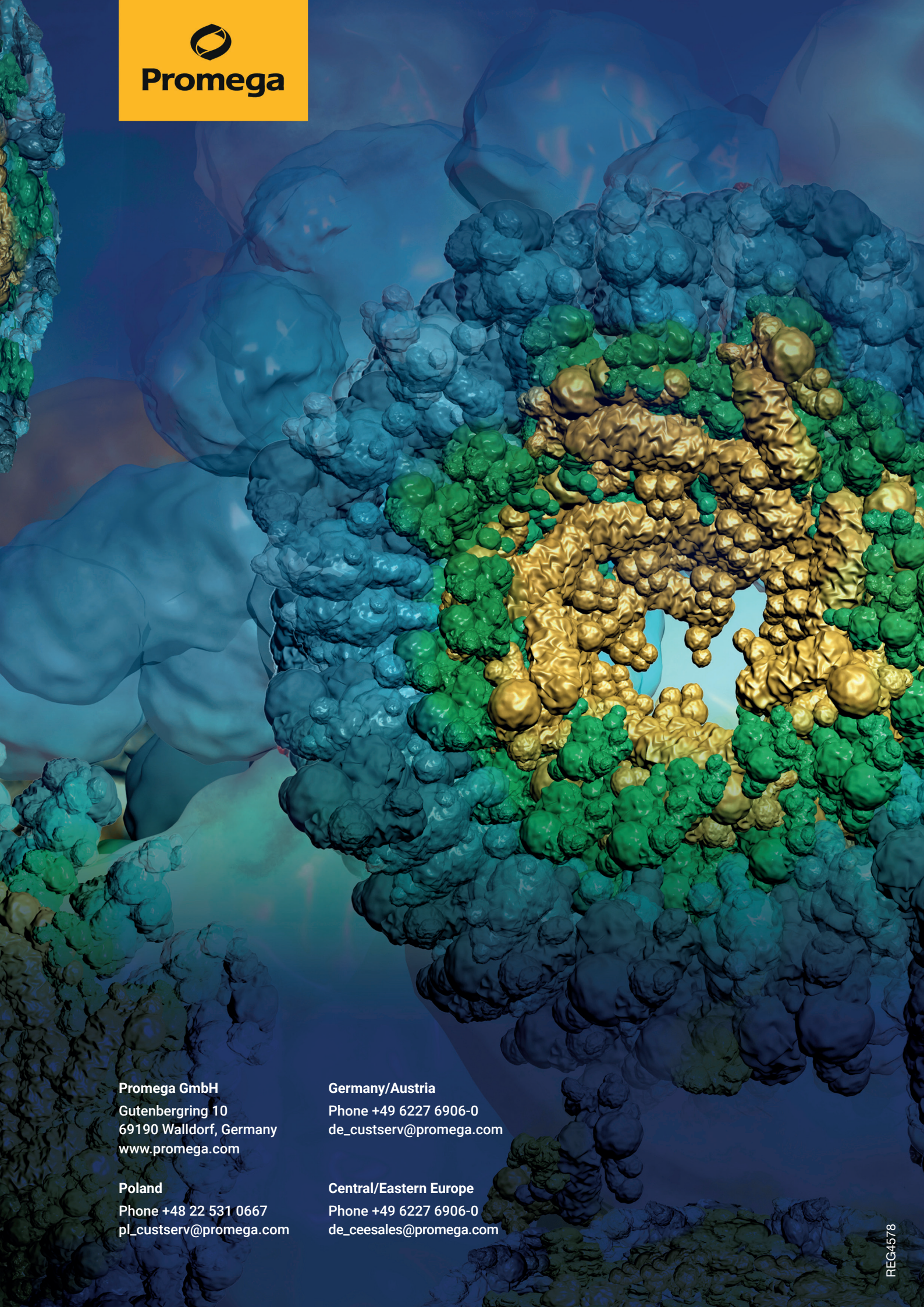

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