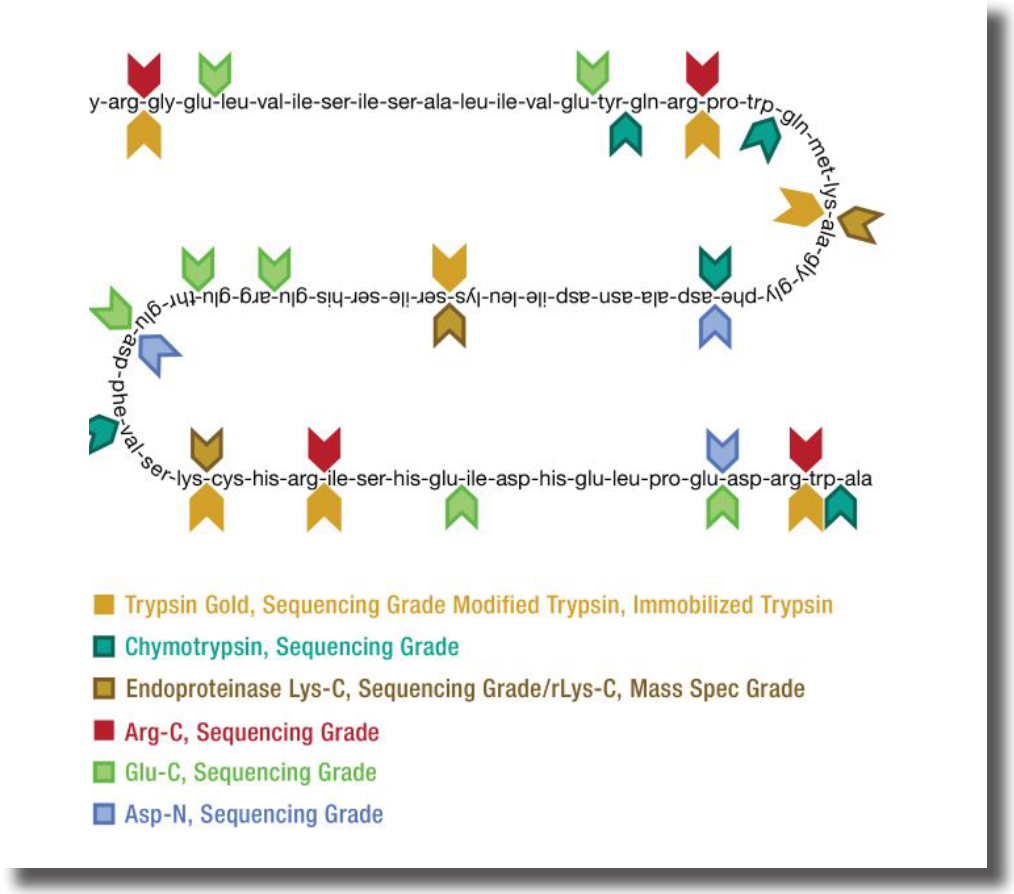
Promega

Reagents for Mass Spectrometry

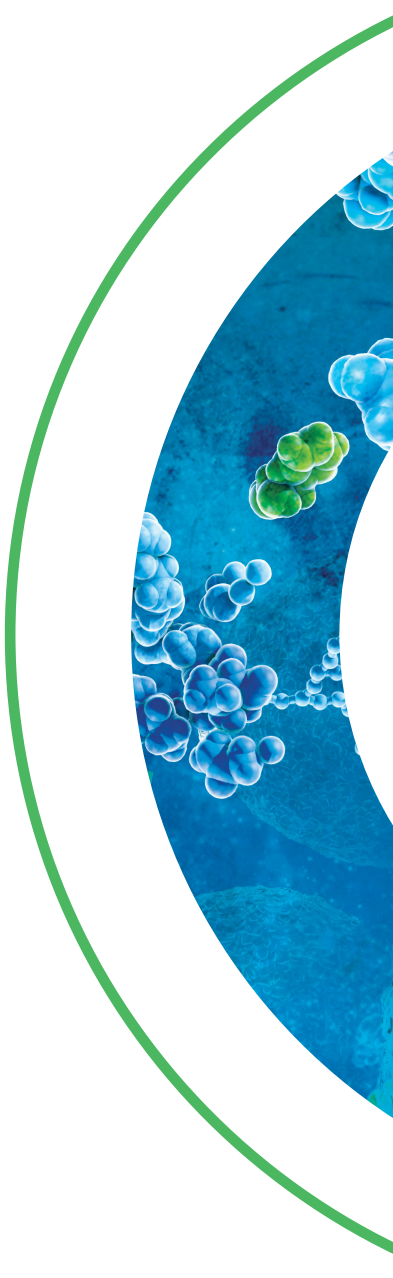
Reagents for Mass Spectrometry

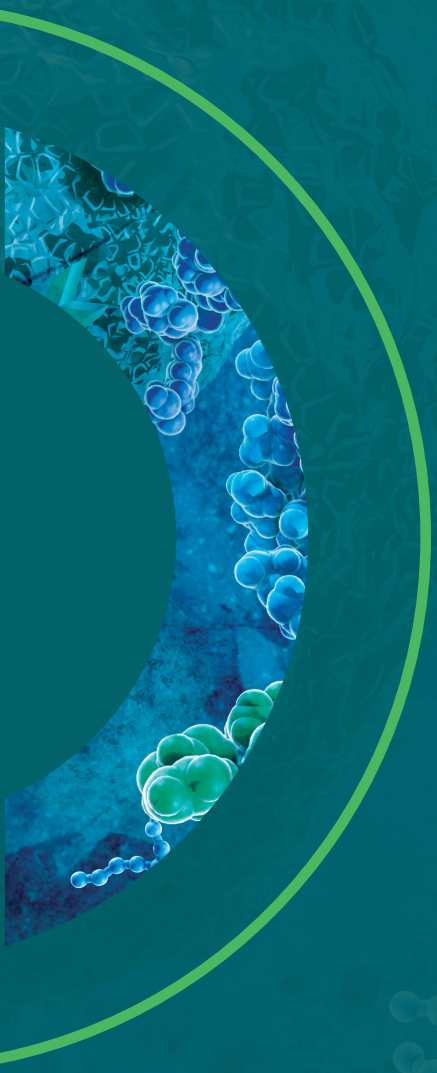
Mass spectrometry (MS) has become a powerful tool in proteomics for proteome-wide analysis and characterisation of proteins from a variety of organisms and cell types. Recent advances in mass spectrometry provide tools for protein identification, characterisation, relative and absolute quantitation, and the study of post-translational modifications and protein:protein interactions. Proteins are generally digested with proteases to generate peptides for MS analysis followed by sequencing (tandem MS or MS/MS).

Promega provides high-quality proteases and other reagents that are critical to sample preparation for mass spectrometry. Also available are protein extracts and peptide reference mixes for Liquid Chromatography-Mass Spectrometry (LC-MS) instrument performance monitoring and method optimisation.



Cut sites for Arg-C, Sequencing Grade; Glu-C, Sequencing Grade; Asp-N, Sequencing Grade; Trypsin Gold, Mass Spectrometry Grade; Sequencing Grade Modified Trypsin; Immobilized Trypsin; Chymotrypsin, Sequencing Grade; Endoproteinase Lys-C, Sequencing Grade; rLys-C, Mass Spec Grade.





Trypsin

Trypsin is the most widely used protease in mass spectrometry sample preparation. It is a highly specific serine protease that cleaves at the carboxylic side of lysine and arginine residues. Tryptic peptides have a strong C-terminal charge and therefore can be efficiently ionised. Promega's high quality trypsin is derived from porcine trypsin and modified to give the highest proteolytic activity and cleavage specificity.

Alternative proteases

There are certain proteins and protein mixtures for which trypsin digestion alone is not efficient enough. Examples include digestion of membrane proteins and analysis of histone post-translational modifications. 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. Promega provide a wide range of alternative proteases that can help improve sequence coverage and enhance digestion.

Rapid digestion kits

One significant drawback to trypsin digestion is the long sample preparation times, which typically range from four hours to overnight for most protocols. Achieving efficient digestion usually requires that protein substrates first be unfolded either with surfactants or denaturants such as urea or guanidine. In an effort to shorten the time required to prepare samples for LC-MS analysis, we have developed a specialised trypsin preparation that supports rapid and efficient digestion at temperatures as high as 70°C, with no chemical additives necessary.

Low pH digestion kits

Trypsin and other proteases commonly used in peptide mapping sample preparation favour alkaline pH in order to efficiently digest proteins. To avoid artificial non-enzymatic post-translational modifications that can be induced in these conditions, Promega has developed a low pH digestion kit, in which a modified trypsin has been supplemented with a special, low pH-resistant recombinant Lys-C (rLys-C) protease to achieve efficient tryptic digestion at low pH.

Glycosidases

Glycosylation is the most abundant post-translational modification in eukaryotic proteins. Glycosylation structures on proteins have diverse functions and can serve as disease biomarkers, so tools that facilitate glycoprotein characterisation are extremely important. Endoglycosidases such as PNGase F and Endoglycosidase H are tools for structural and functional analysis of glycoproteins.

Mass spec-compatible protein extracts

Promega offers mass spec-compatible yeast and human protein extracts, designed for monitoring mass spectrometry instrument performance. Each extract is available in a pre-digested, tryptic peptide form, used primarily for instrument performance monitoring. The yeast and human extracts are also offered in an intact, undigested form to provide a test material for optimising mass spectrometry protein sample preparation.

Peptide reference mixes

For optimal Liquid Chromatography (LC) performance, parameters like retention time, peak width and peak height are typically reported. Commonly monitored MS parameters include mass accuracy, mass resolution, signal-to-noise, sensitivity, limit of detection (LOD), limit of quantitation (LOQ) and dynamic range. Promega has developed a peptide reference mix that provides a convenient way to assess LC column performance and MS instrument parameters, including sensitivity and dynamic range, in a single run.

Proteases for Mass Spectrometry

Trypsin

Fast and convenient digestions

Trypsin is a serine protease that specifically cleaves at the carboxylic side of lysine and arginine residues. The stringent specificity of trypsin is essential for protein identification. Native trypsin is subject to autolysis, generating pseudotrypsin, which exhibits a broadened specificity including a chymotrypsin-like activity. Such autolysis products would result in additional peptide fragments that could interfere with database analysis of the mass of fragments detected by mass spectrometry. Lysine residues in the porcine trypsin of **Sequencing Grade Modified Trypsin** and **Trypsin Gold**, **Mass Spectrometry Grade** have been 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. The treated trypsin is then purified by affinity chromatography.

Improved sequence coverage

It is resistant to mild denaturing conditions such as 0.1% SDS, 1M urea or 10% acetonitrile and retains 50% of its activity in 2M guanidine HCl.

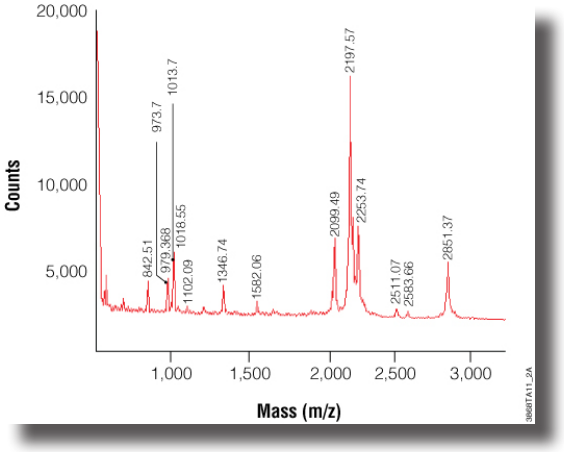
Sequencing Grade Modified Trypsin

Referenced in thousands of papers, Sequencing Grade Modified Trypsin has been manufactured to provide maximum specificity. It is available as 20µg or 100µg aliquots of lyophilised product, or as frozen liquid in 50mM acetic acid. Resuspension buffer is provided, making it easy to use. Quality control is performed by reverse-phase High-Performance Liquid Chromatography (HPLC).

Enhanced data from minimal sample material

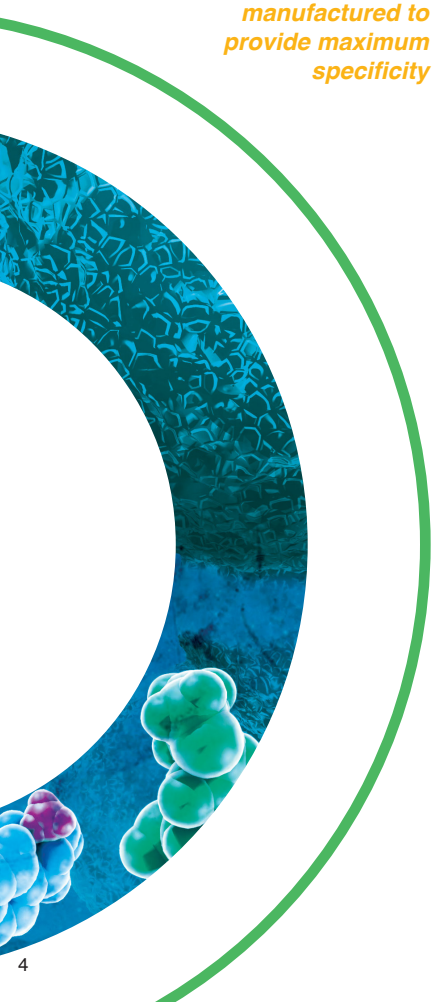
Trypsin Gold, Mass Spectrometry Grade

Trypsin Gold has maximum digest specificity and extreme resistance to autolytic digestion. It is application qualified for both in-gel digestion and in-solution digestion and is available in 100µg lyophilised aliquots. It has undergone an extra step of quality control by mass spectrometry to ensure application/instrumentation compatibility.



Spectrogram of bovine carbonic anhydrase II digested by Trypsin Gold, Mass Spectrometry Grade.

Promega's trypsin products are manufactured to provide maximum specificity



Immobilized Trypsin

Immobilized Trypsin provides a fast and convenient method for digesting a range of concentrations of purified protein or complex protein mixtures. Digestion occurs in a spin column in as little as 30 minutes, and digested peptides are easily separated from the Immobilized Trypsin as they flow through to the collection tube as the trypsin does not pass through the column, reducing enzyme interference during analysis. The adjustable protocol easily accommodates various protein concentrations and no shaking or water baths are necessary.

Lys-C and Lys-N

Lys-C, Mass Spec Grade

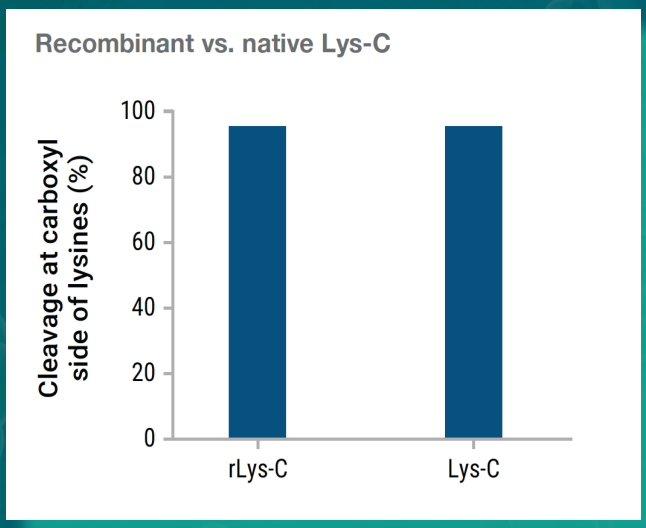
Endoproteinase Lys-C is a serine protease isolated from *Lysobacter enzymogenes* as a highly purified protease that hydrolyses specifically at the carboxyl side of lysine residues. Lys-C retains proteolytic activity under protein denaturing conditions such as 8M urea, which is used to improve digestion of proteolytically resistant proteins. Lys-C activity is optimal 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.

rLys-C, Mass Spec Grade

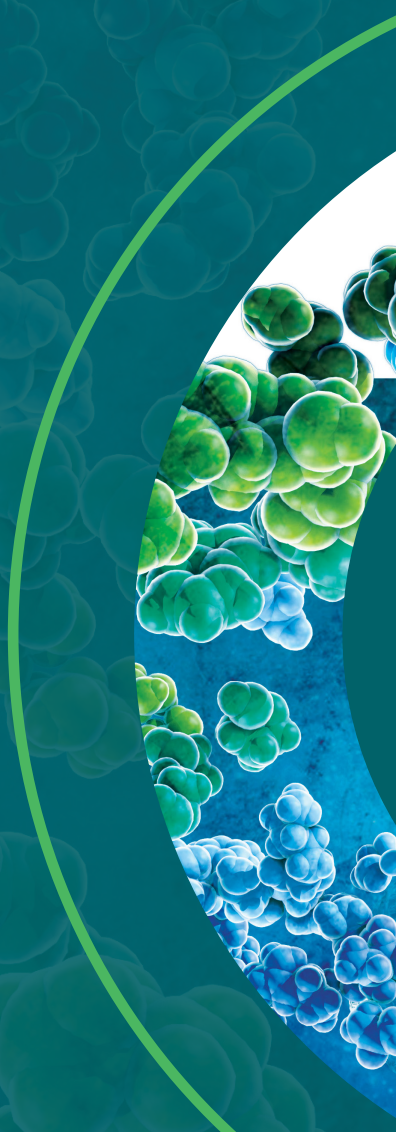
rLys-C is a recombinant Lys-C protease expressed in *E. coli*. The sequence origin of rLys-C is protease IV from *Pseudomonas aeruginosa.* Similar to native Lys-C, rLys-C cleaves at the carboxyl side of lysine residues with exceptional specificity. It retains proteolytic activity under protein denaturing conditions such as 8M urea. rLys-C activity is optimal in the pH range of 8.0-9.0. rLys-C is recommended for digestion of single proteins and complex protein mixtures in-solution and in-gel.

Lys-N, Mass Spec Grade

Endoproteinase Lys-N is a metalloprotease that cleaves at the amino side of lysine residues. Lys-N is a zinc metalloprotease derived from *Grifola frondosa* that retains proteolytic activity under protein denaturing conditions such as 8M urea, which is used to improve digestion of proteolytically resistant proteins. Charged amino-terminal peptide fragments generated by Lys-N are useful for de novo sequencing with Electron Transfer Dissociation (ETD) fragmentation techniques.

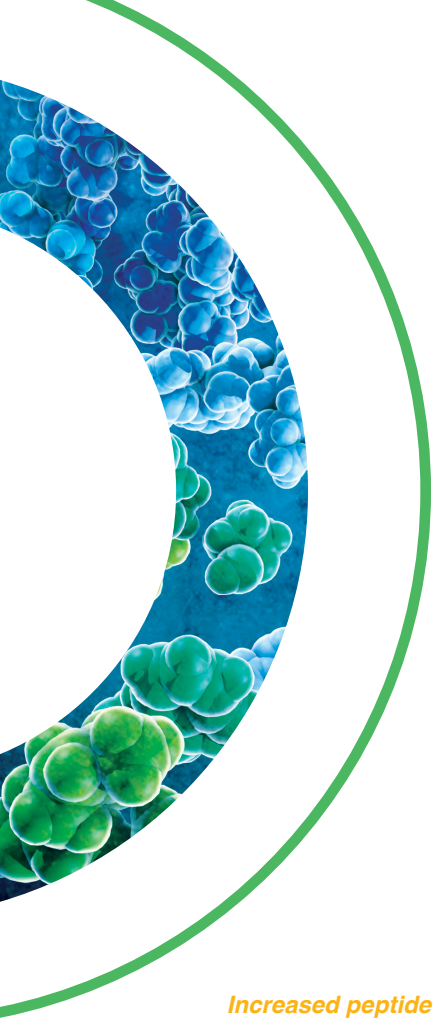


Comparison of lysine-specific cleavages in total yeast protein extract. Mass Spec-Compatible Yeast Protein Extract, Intact (Cat. No. V7341) was digested with either rLys-C (P. aeruginosa) or native Lys-C (Lysobacter sp.) at 1:100 ratio.



Ordering Information

Product	Size	Cat. No
Sequencing Grade Modified Trypsin, Lyophilized	5 x 20µg	V5111
	1 x 100µg	V5117
Sequencing Grade Modified Trypsin, Frozen	5 x 20µg	V5113
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Immobilized Trypsin	2ml	V9012
	4ml	V9013
Lys-C, Mass Spec Grade	20µg	VA1170
rLys-C, Mass Spec Grade	15µg	V1671
Lys-N, Mass Spec Grade	20µg	VA1180



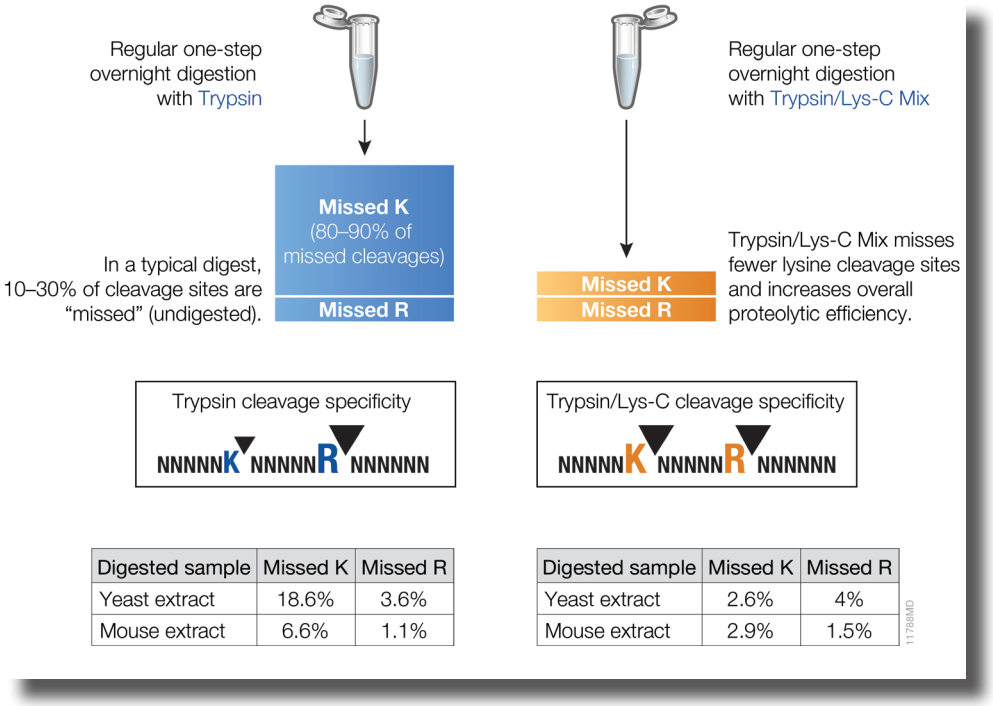
Trypsin/Lys-C Mix, Mass Spec Grade

Trypsin/Lys-C Mix is a unique mixture of Trypsin Gold, Mass Spectrometry Grade and rLys-C, Mass Spec Grade. It is designed to improve the digestion of proteins or protein mixtures in solution over trypsin alone using standard digestion conditions, increasing peptide recovery and resulting in better protein quantitation.

Many proteins are resistant to trypsin due to tight conformation, and although protein denaturation enables proteolysis, trypsin is inactive under these conditions. Trypsin/Lys-C mix overcomes this barrier as it remains active even under strong denaturing conditions and is tolerant to trypsin-inhibiting contaminants, enabling digestion of even proteolytically resistant proteins.

The enhanced proteolytic activity of Trypsin/Lys-C Mix results in a 20-40% increase in the number of peptides generated, which enables a higher number of proteins to be identified. The majority of missed lysine cleavages that can occur using trypsin alone are eliminated due to the inclusion of Lys-C. Reproducibility of digestion is also improved compared to trypsin digestion alone.

Increased peptide recovery, enhanced protein quantitation



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%

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Tolerant to trypsin-inhibiting contaminants

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

Ordering Information

Product	Size	Cat No.
Trypsin/Lys-C Mix, Mass Spec Grade	20µg	V5071
	1 x 100µg	V5072
	5 x 20µg	V5073

Asp-N

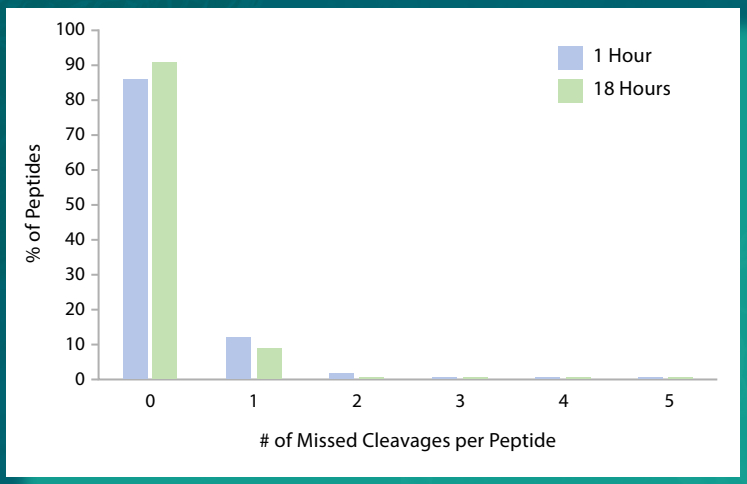
Asp-N is an endoproteinase that hydrolyses peptide bonds on the N-terminal side of aspartic and cysteic acid residues (Asp and Cys). Asp-N activity is optimal in the pH range of 4.0-9.0.

Asp-N, Sequencing Grade

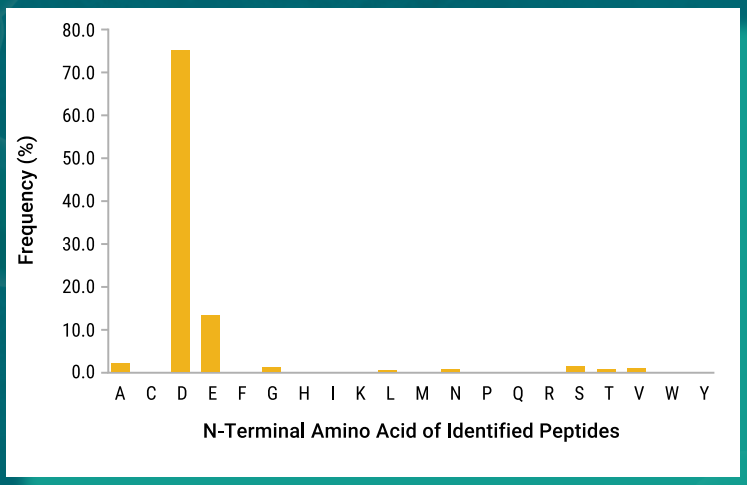
Asp-N, Sequencing Grade is a highly pure and specific endoproteinase for use alone or in combination with other proteases to produce protein digests for peptide mapping applications, or for protein identification by peptide mass fingerprinting or MS/MS spectral matching. It is suitable for both in-solution and in-gel digestion reactions. It is available in 2µg vials.

Recombinant Asp-N (rAsp-N), Mass Spec Grade

rAsp-N, Mass Spec Grade is a recombinant protease cloned from *Stenotrophomonas maltophilia* and expressed in *E. coli*. It is a highly purified 25kDa Zn-metalloprotease with a C-terminal His tag, and has a pH optimum of 8.0-9.0. rAsp-N is a highly active protease suitable for proteomic analysis of complex mixtures as well as peptide mapping of purified proteins, such as therapeutic monoclonal antibodies. The protease is provided in 10µg aliquots in a conical vial for easy and consistent resuspension. It is a less expensive alternative to native Asp-N.



Recombinant Asp-N provides efficient digestion of a yeast extract in 1 hour. Digestion efficiency was approximately 85% after 1 hour and increased only slightly (to approximately 91%) after overnight digestion.



rAsp-N, Mass Spec Grade digests yeast extract proteins primarily N-terminal to aspartic acid residues. Weak digestion at glutamic acid is observed in some cases.

Glu-C, Sequencing Grade

Glu-C, Sequencing Grade is a serine protease that specifically cleaves at the C-terminus of either aspartic or glutamic acid residues. In ammonium bicarbonate and ammonium acetate, the enzyme specificity is higher at the glutamic residues. In phosphate buffers, cleavage occurs at aspartic and glutamic residues. Glu-C activity is optimal in the pH range of 4.0-9.0. This sequencing grade enzyme can be used alone or in combination with other proteases to produce protein digests for peptide mapping applications, or for protein identification by peptide mass fingerprinting or MS/MS spectral matching. It is suitable for digestion reactions in solution but not recommended for in-gel digestions.

Arg-C, Sequencing Grade

Arg-C, Sequencing Grade is an endopeptidase (clostripain) that cleaves at the C-terminus of arginine residues, including the sites next to proline. Cleavage can also occur at lysine residues. This sequencing grade enzyme can be used alone or in combination with other proteases for protein analysis by mass spectrometry and other applications. Arg-C activity is optimal in the pH range of 7.6-7.9.

Chymotrypsin, Sequencing Grade

Chymotrypsin is a highly-purified serine endopeptidase derived from bovine pancreas that preferentially cleaves at the carboxyl side of the aromatic amino acids tyrosine, phenylalanine and tryptophan. Cleavage may also be observed, but at a lower rate, at leucine and methionine. Chymotrypsin activity is optimal at pH 7.0-9.0. This sequencing grade enzyme can be used alone or in combination with other proteases to produce protein digests for peptide mapping applications, or for protein identification by peptide mass fingerprinting or MS/MS spectral matching. It is suitable for digestion reactions in-solution or in-gel.

Elastase

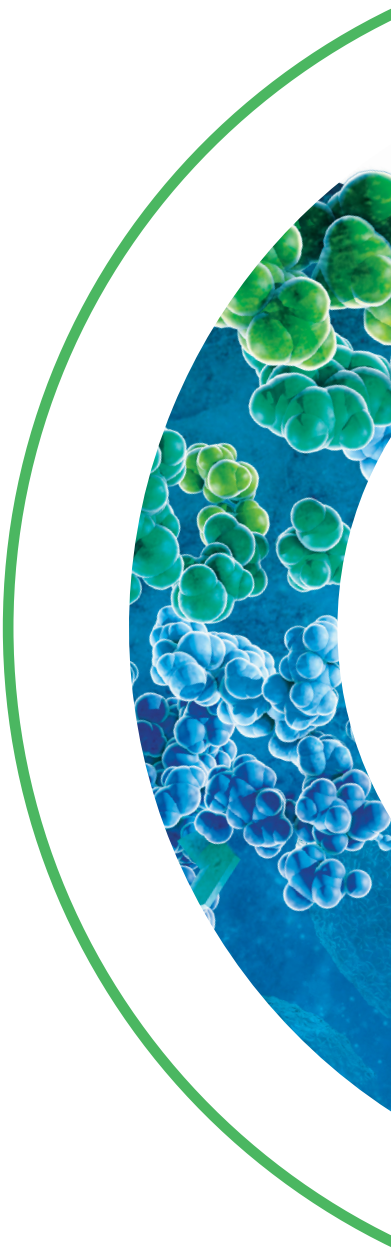
Elastase is a serine protease that preferentially cleaves at the C-terminus of alanine, valine, serine, glycine, leucine or isoleucine residues. Elastase has a unique ability to digest elastin. This enzyme can be used alone or in combination with other proteases for protein analysis by mass spectrometry and other applications. Elastase activity is optimal at pH 9.0.

Pepsin

Pepsin cleaves at the C-terminus of phenylalanine, leucine, tyrosine and tryptophan residues. This protease can be used alone or in combination with other proteases for protein analysis by mass spectrometry and other applications. Pepsin activity is optimal at pH 1.0-3.0.

Thermolysin

Thermolysin is a thermostable metalloproteinase. The high digestion temperature stability of this enzyme may be useful as an alternative to denaturants to improve digestion of proteolytically resistant proteins. 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 activity is optimal at pH 5.0-8.5.



Ordering Information

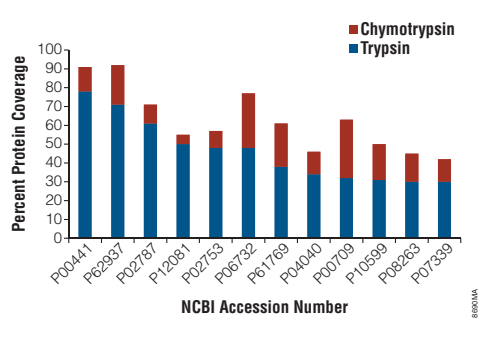
Product	Size	Cat No.
Asp-N, Sequencing Grade	2µg	V1621
rAsp-N, Mass Spec Grade	10µg	VA1160
Glu-C, Sequencing Grade	50µg	V1651
Arg-C, Sequencing Grade	10µg	V1881
Chymotrypsin, Sequencing Grade	25µg	V1061
	100µg	V1062
Elastase	5mg	V1891
Pepsin	250mg	V1959
Thermolysin	25mg	V4001

Multiple enzyme use

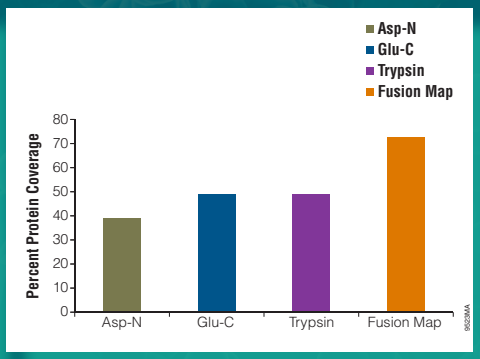
Trypsin, the most widely used protease in mass spectrometry analysis, utilises its high specificity to cleave proteins, resulting in peptides with a strong C-terminal charge. Tightly folded proteins can resist trypsin digestion, resulting in an inadequate distribution of trypsin cleavage sites and peptides that are either too long or too short for effective mass spectrometry analysis. Post-translational modifications (PTMs) can also present a challenge for trypsin. The addition of alternative proteases can complement standard trypsin digestions and provide enhanced data.

Increased protein coverage

Digestion with an alternative protease individually or in combination with other proteases creates a unique peptide map that may include sequences not seen with trypsin digestion. Overlaying peptides obtained with alternative proteases, along with those obtained with trypsin, increases protein coverage and overall confidence in protein identification.



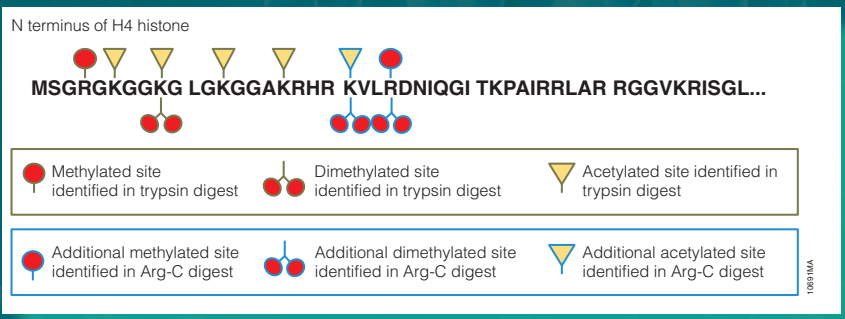
Increased protein coverage using both trypsin and chymotrypsin.



Increased protein coverage using multiple proteases.

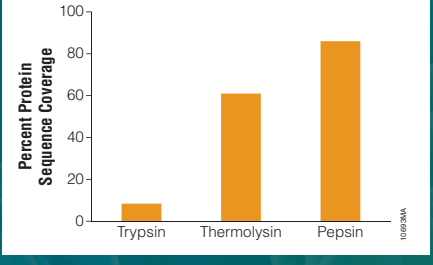
Characterise post-translational modifications

Some PTMs can impede analysis with trypsin. For example, histone PTMs are complex and some, such as acetylation and methylation, prevent trypsin digestion. As shown in the figure below, trypsin digestion of histone H4 identified several PTMs; however, certain PTMs were missing. By digesting histone H4 with Arg-C, several PTMs that were missed by trypsin were identified, including mono-, dimethylated and acetylated lysine and arginine residues.

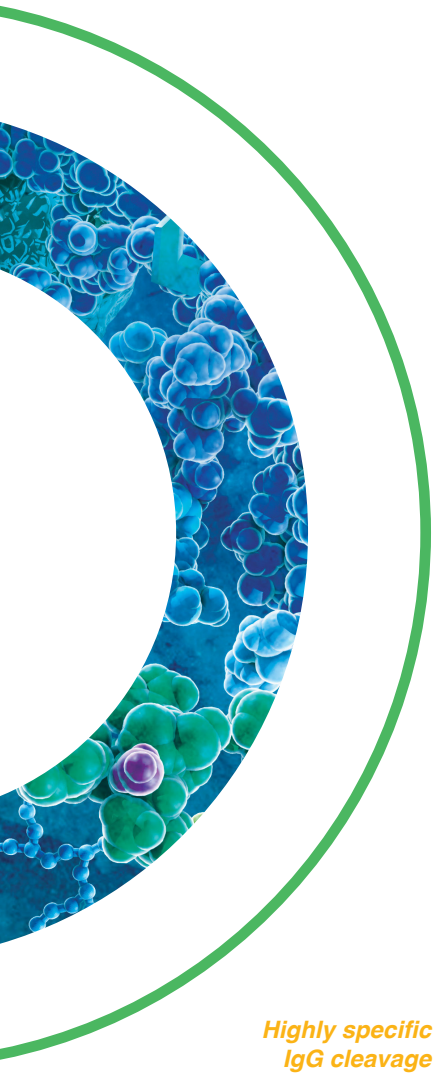


Enhanced digestion of difficult-to-digest proteins

Thermolysin and pepsin are unique because they tolerate extreme conditions-high temperatures and low pH, respectively. Because they remain active under these denaturing conditions, thermolysin and pepsin are ideal proteases for the digestion of proteolytically resistant, tightly folded proteins and can cleave previously inaccessible sites.



Bacteriorhodopsin sequence coverage obtained from trypsin, thermolysin and pepsin.



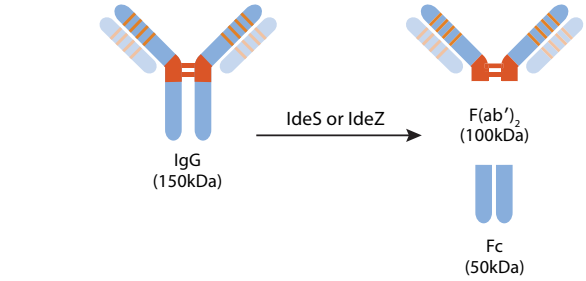
Highly specific IgG cleavage

IdeS and IdeZ Proteases

IdeS and IdeZ Proteases are immunoglobulin G (IgG)-degrading enzymes that are valuable tools for the characterisation of therapeutic antibodies, Fc fusion proteins and antibody-drug conjugates. Both proteases effectively cleave human IgG1, IgG2, IgG3 and IgG4, monkey, sheep, rabbit, humanised and chimeric IgGs as well as Fc-fusion proteins.

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.

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.



IgG1 Hinge sequence: S-C-D-K-T-H-T-C-P-P-C-P-A-P-E-L-L-G-G-P-S-V

IdeS or IdeZ cleavage site

Schematic showing cleavage specificity of IdeS and IdeZ Proteases. The orange cysteines (C) indicate where the interchain disulphide bonds are formed to the opposite heavy chain. The orange glycines (G) indicate the IdeS/ IdeZ cleavage site.

IdeS and IdeZ Proteases are fast, easy-to-use proteases; complete digestion can be achieved in 30 minutes without optimisation. Cleavage is highly reproducible and specific.

Key applications

Fast and comprehensive analysis:

- Sequence verification
- Analysis of both small and large post-translational modifications (PTMs)
- Analysis of antibody drug conjugates.

Rapid comparisons of sample types:

- Clone comparisons
- Innovator mAb to biosimilar
- Lot-to-lot variability
- Monitor process manufacturing changes.

Rapid generation of highly pure fragments

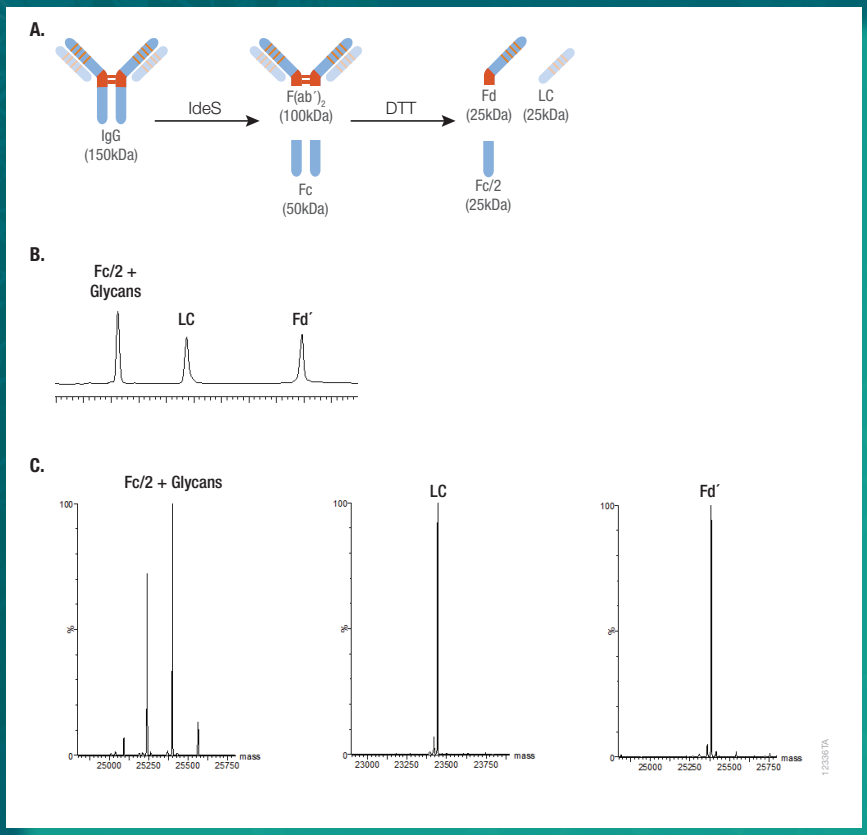
Core and lower hinge sequence of human and mouse IgG subclasses

Subclass	Hinge/CH2 Sequence	IdeS Activity	IdeZ Activity
Human			
IgG1	CPPCPAPELLGGPSVF	++++	++++
IgG2	CPPCPAPP_VAGPSVF	++++	++++
IgG3	CPRCPAPELLGGPSVF	++++	++++
IgG4	AHHAQAPEFLGGPSVF	++++	++++

Subclass	Hinge/CH2 Sequence	IdeS Activity	IdeZ Activity
Mouse			
IgG1	PCICTVPEV___SSVF	–	–
IgG2a	CPPCAAPNLLGGPSVF	+	++++
IgG2b	CHKCPAPNLEGGPSVF	–	–
IgG3	GSSCPAGNILGGPSVF	+	++++

Characterise therapeutic antibody candidates using LC-MS

IdeS digestion of IgG produces three fragments of approximately 25kDa (Fd', Fc/2 and LC) that are ideal for characterisation by LC-MS. The small fragments facilitate accurate mass measurements that enable detection o fPTMs such as glycoform profiles, C-terminal lysine variants, N-terminal pyroglutamate and oxidation.



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

	MWavg (Theoretical) (Da)	MWavg (Observed) (Da)	Error (Da)
Fd'	25383.6	25383.3	-0.3
Fc/2 + G0	25090.2	25091.0	0.8
Fc/2 + G0F	25236.3	25236.9	0.6
Fc/2 + G1F	25398.5	25398.5	0.0
Fc/2 + G2F	25560.6	25561.8	1.2
LC	23443.1	23443.1	0.0

Observed, deconvoluted fragment masses. Separation of antibody fragments was achieved using HPLC performed on a Waters BEH300 C4 column. MS analysis was performed on a Waters Xevo® G2 QTof Instrument.

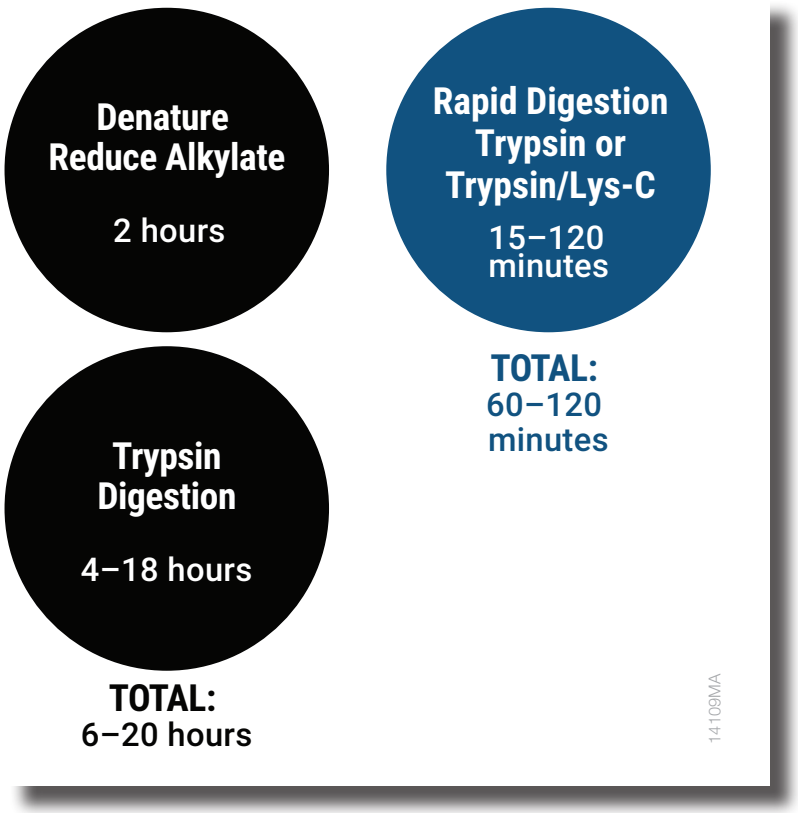
Ordering Information

Product	Size	Cat.No
IdeS Protease	5,000 units	V7511
IdeS Protease	25,000 units	V7515
IdeS Protease, Frozen	2,000 units	V7512
IdeZ Protease	5,000 units	V8341
IdeZ Protease	25,000 units	V8345
IdeZ Protease, Frozen	2,000 units	V8342

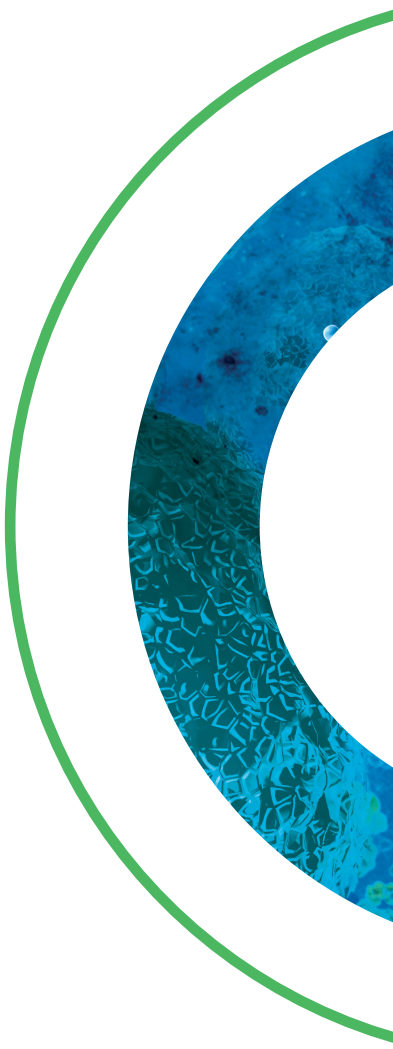
Rapid Digestion – Trypsin and Rapid Digestion – Trypsin/Lys-C Kits

One significant drawback to trypsin digestion is long sample preparation times, ranging from four hours to overnight. Achieving efficient digestion requires that protein substrates first be unfolded using surfactants or denaturants, like urea or guanidine. However, such chemicals can have negative side effects and usually must be removed prior to analysis.

To shorten the time required to prepare samples for LC-MS analysis, Promega has developed an optimised protocol in which rapid and efficient digestion is easily achieved with minimal protocol steps.



Shorten digestion time to as little as 60 minutes



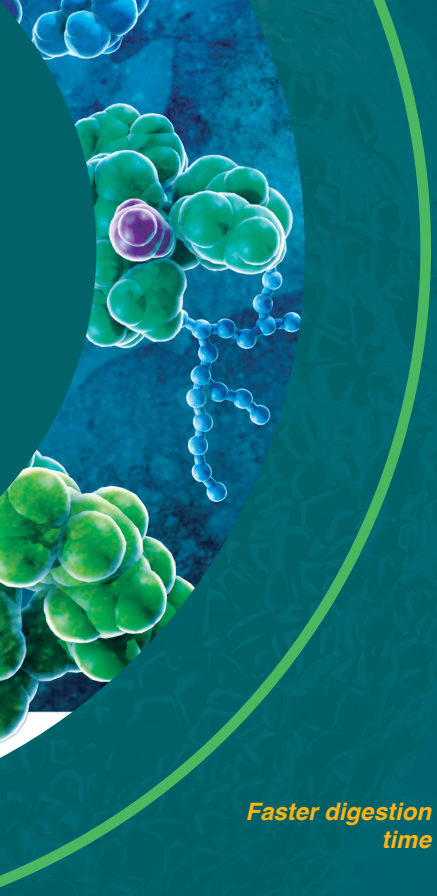
Rapid Digestion - Trypsin and Rapid Digestion - Trypsin/Lys-C Kits enable dramatically shortened proteolytic reaction times through protein digestion at 70°C.

Both kits contain three components: i) a protease (Trypsin or Trypsin/Lys-C Mix); ii) a protease resuspension Buffer; and iii) a Rapid Digestion Buffer optimised for faster digestions. The simple-to-use protocol is fast, efficient and flexible, accommodating a large range of sample volumes and protein concentrations.

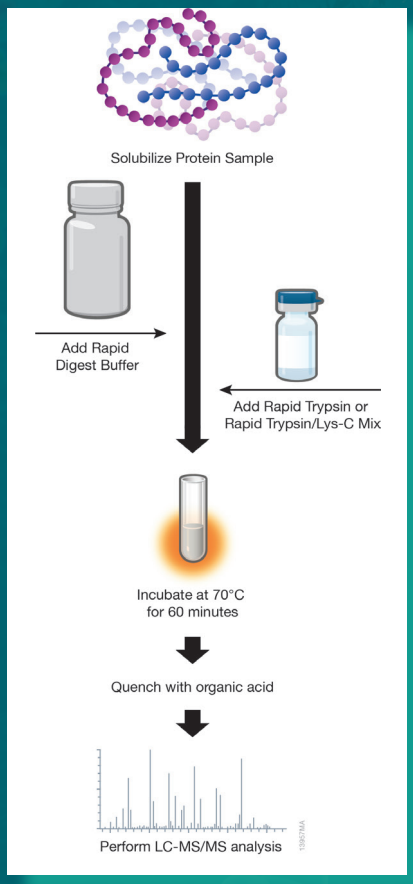
Digestion is achieved completely using an in-solution approach; as the enzyme is not immobilised on beads, the protocol does not have strict requirements for rapid shaking and off-line filtering to remove beads.

No chemical denaturants, special laboratory equipment or off-line desalting is required, so sample cleanup for downstream LC-MS is unnecessary, leading to shorter preparation times and diminished samples losses.

Scale up or adjust protease/substrate ratio to optimise reaction conditions as required. Optimise complete digestion of target proteins and improve reproducibility across samples.



Faster digestion time



The Rapid Digestion Kit workflow. After solubilising the protein substrate, add Rapid Digest Buffer (3x the protein substrate sample volume) along with enzyme (Rapid Trypsin or Rapid Trypsin/Lys-C). Heat at 70°C (typically ~60 minutes) to rapidly digest the protein substrate. Quench the digest with organic acid (i.e., neat formic acid) then perform direct LC-MS/MS analysis.

Streamlined workflow

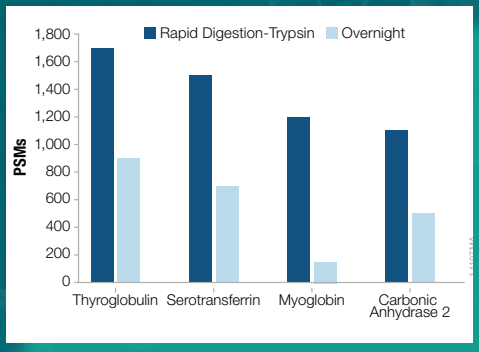
Quality data in less time

In many examples the quality of data generated using the Rapid Digestion format exceeds that obtained with the conventional overnight protocol.

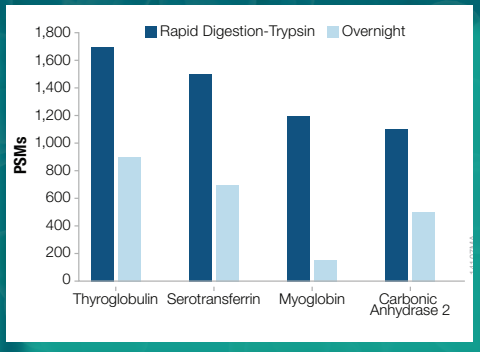
Flexibility for protocol optimisation

Reduction and alkylation are not required for most samples when using the Rapid Digestion Kits, minimising protocol steps. However, unlike other commercial kits, the Rapid Digestion Kit formats can be used with reduction/alkylation, leading to improved sequence coverage in certain cases.

Tighter coefficients of variation



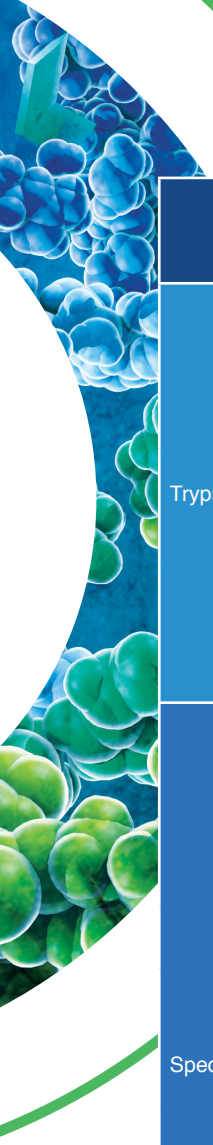
Comparison of peptide spectral matches: Rapid Digestion - Trypsin and standard digestion. The increase in spectra noted for Rapid Digestion - Trypsin sample preparation indicates more complete digestion compared to standard overnight digestion.



Both Rapid Digestion - Trypsin and Rapid Digestion - Trypsin/Lys-C Kits are compatible with reduction and alkylation steps, resulting in increased sequence coverage.

Ordering Information

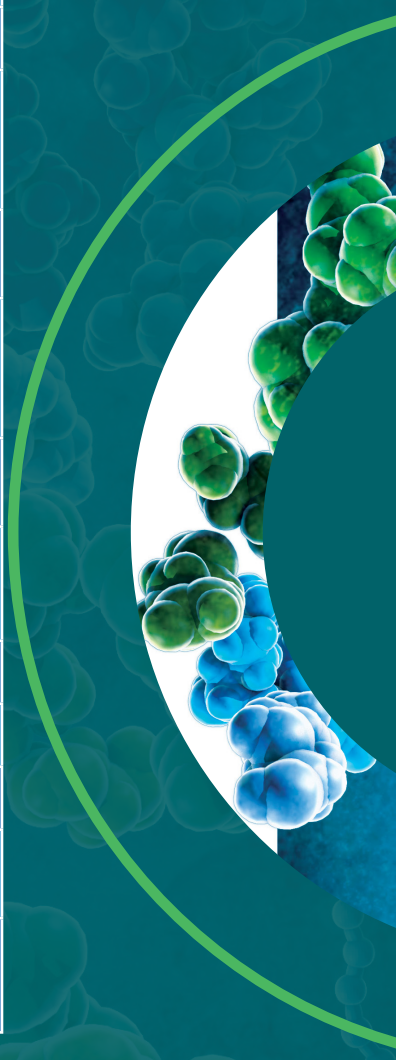
Product	Size	Cat.No
Rapid Digestion - Trypsin	100µg	VA1060
Rapid Digestion - Trypsin/Lys-C	100µg	VA1061



Characteristics and reaction conditions for proteases

	Protease	Source and size	Cleavage sites
Trypsin	Trypsin Gold, Sequencing Grade Modified Trypsin, Sequencing Grade Modified Trypsin, Frozen	Porcine Pancreas	C-term 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.
	Immobilized Trypsin	Porcine Pancreas	C-term 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.
	Trypsin/Lys-C Mix	Mix of Tryspin Gold & rLys-C	C-term 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 and Arg.
Specific alternative proteases	rLys-C	Biological origin - *Pseudomonas* aeruginosa. Expressed in *E.coli*	C-term of Lys. Does not cleave if Lys is followed by Pro. Asp and Glu at C-terminal side of Lys inhibit cleavage.
	Lys-C	*Lysobacter enzymogenes*	C-term of Lys. Does not cleave if Lys is followed by Pro.
	Arg-C	*Clostridium histolyticum* (Subunits: 45kDa and 12kDa)	C-term of Arg. Also cleaves at Lys albeit at lower efficiency.
	Asp-N	*Pseudomonas fragi*	N-term of Asp.
	rAsp-N	*Stenotrophomonas maltophilia*	N-term of Asp. Weak digestion at Glu in some cases.
	Glu-C	*Staphylococcus aureus V8*	C-term of Glu. Low level cleavages might occur at Asp residues too albeit at 100-300 fold lower efficiency.
	Lys-N	*Grifola frondosa*	N-term of Lys.
Low specific alternative protease	Chymotrypsin	Bovine pancreas	Preferentially C-term of Trp, Tyr and Phe but it also cleaves at other residues albeit at a lower efficiency.
Non-specific proteases	Elastase	Porcine pancreas	Preferentially C-term of Ala, Val, Ser, Gly, Leu and Ile.
	Pepsin	Porcine stomach	Preferentially C-term of Phe, Leu, Tyr and Trp.
	Thermolysin	*Bacillus thermoproteolyticus rokko*	Preferentially N-term of Leu, Phe, Val, Ile and Met.
IgG-cleaving proteases	IdeS Protease	*Streptococcus pyogenes*	IgG molecules at a single site below the hinge region, yielding F(ab')2 and Fc fragments.
	IdeZ Protease	*Streptococcus equi* subspecies *zooepidemicus*	IgG molecules at a single site below the hinge region, yielding F(ab')2 and Fc fragments.

	Protease: protein ratio (w/w)	pH range digestion	Additional Information
s u	1: 20 to 1: 100	pH 7.0-9.0	50-100mM Tris-HCl (pH 8) or 50-100mM NH4HCO3(pH 7.8). Digest overnight hours at 37°C.
s u	See Technical Manual	pH 5.0-9.0	Use if rapid digestion is required. Urea ≤ 4M; guanidine HCl ≤ 3M; methanol < 60%; DTT ≤ 50mM; TCEP ≤ 5mM; pH 5–9 pH; Iodoacetamide ≤ 300mM.
s	1: 25 to 1: 50	pH 8.0	Trypsin/Lys-C mix is the most robust trypsin preparation. It is designed to improve general protein digestion. Trypsin/Lys-C mix uses the same regular overnight digestion at 37°C under non- or mildly denaturing conditions as trypsin alone. It digests proteins and tolerates inhibitors and denaturing agents such as urea guanidine HCL more efficiently than trypsin.
	1: 20 to 1: 50	pH 8.0-9.0	Inexpensive alternative to a native Lys-C protease. Similarly to a native protease, rLys-C tolerates high denaturing conditions such as 8M urea. Used to digest tightly folded proteolytically resistant proteins. Also used as a trypsin alternative if larger peptides are preferable for the analysis.
	1: 20 to 1: 100	pH 7.0-9.0	Tolerates high denaturing conditions such as 8M urea. Used to digest tightly folded proteolytically resistant proteins. Also used as a trypsin alternative if larger peptides are preferable for analysis.
t	1: 20 to 1: 350	pH 7.6-7.9	Used in analysis of histone modifications. Requires DTT, cysteine or other reducing agent and CaCl2 for activity.
	1: 20 to 1: 200	pH 4.0-9.0	Can be used as a trypsin alternative to achieve better distribution of cleavage sites. 100% activity retained in the presence of urea (up to 3.5M), guanidine HCL (1M), SDS (up to 0.028%), ProteaseMAX Surfactant (up to 0.026%), acetonitrile (up to 60%), EDTA (up to 2 mM); DTT or ß-mercaptoethanol.
	1: 10 to 1: 100	pH 6.0-9.0	Inexpensive alternative to native Asp-N protease. Provided in a larger aliquot in a conical vial for easier, more convenient resuspension. Can be used as a trypsin alternative to achieve better distribution of cleavage sites.
t	1: 20 to 1: 200	pH 4.0-9.0	Can be used as a trypsin alternative to achieve bettter 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 Glu and Asp in phosphate buffer.
	1: 20 to 1: 100	pH 7.0-9.0	Retains proteolytic activity under protein denaturing conditions such as 8M urea. Charged amino-terminal peptide fragments generated by Lys-N are useful for de novo sequencing with ETD fragmentation techniques.
e it	1: 20 to 1: 200	pH 7.0-9.0	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%).
',	1: 20 to 1: 100	pH 9.0	Used as a trypsin alternative to increase protein coverage.
	1: 20 to 1: 100	pH 1.0-3.0	Used in structural protein studies (HDX exchange based) and antibody analysis; used to digest proteolytically resistant, tightly folded proteins.
e	1: 20 to1: 50	pH 5.0-8.5	Used to digest proteolytically resistant, tightly folded proteins and in structural studies.
e	I unit of protease per 1µg of IgG	pH 6.6-7.4	Cleaves human IgG1, IgG2, IgG3 and IgG4, monkey, sheep, rabbit, humanised and chimeric IgGs as well as Fc-fusion proteins. Digestion can be achieved in 30 minutes with no optimisation and high specificity.
e	I unit of protease per 1µg of IgG	pH 6.6-7.4	Cleaves human IgG1, IgG2, IgG3 and IgG4, monkey, sheep, rabbit, humanised and chimeric IgGs as well as Fc-fusion proteins, as well as mouse IgG2a and IgG3 subclasses. Digestion can be achieved in 30 min-utes with no optimisation and high specificity.



AccuMAP™ Low pH Protein Digestion Kits

Suppress artificial post-translational modifications; maximise peptide mapping results

Non-enzymatic post-translational modifications (PTMs) such as deamidation, disulphide bond scrambling and oxidation spontaneously occur in biotherapeutic proteins during manufacturing and storage. These modifications can affect the efficacy and stability of these biotherapeutic proteins and are the subject of careful monitoring.

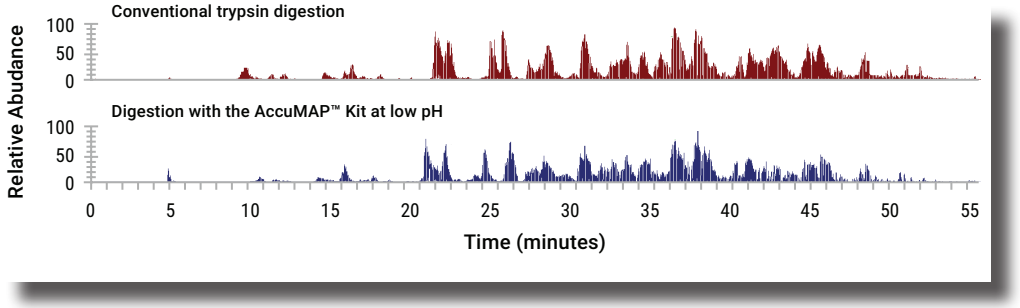
Non-enzymatic PTMs can also be induced during protein sample preparation for peptide mapping and can compromise analysis. Major causes of non-enzymatic PTMs induced during sample preparation include alkaline pH as well as excipients and impurities that have protein oxidising activity.

Protein digestion at low pH

Highly reproducible digestion results

Trypsin and other proteases commonly used in peptide mapping sample preparation favour alkaline pH in order to efficiently digest proteins. To avoid artificial non-enzymatic PTMs induced at these conditions, we developed a kit which allows trypsin digestion at low pH.

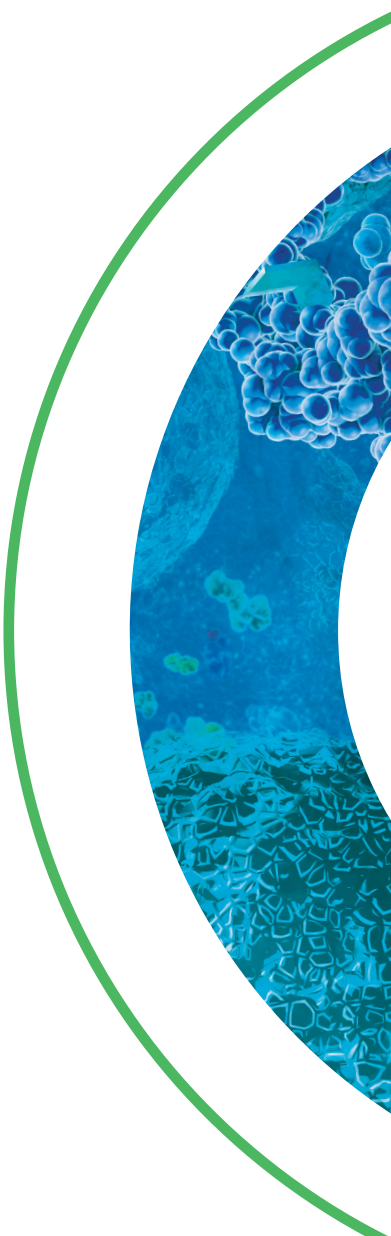
To restore trypsin cleavage efficiency at lysine sites trypsin is supplemented with a special, low pH-resistant recombinant Lys-C (rLys-C) protease, resulting in efficient tryptic digestion at low pH.



Efficient digestion at low pH. Rituximab antibody was digested according to a conventional protocol (at pH 8) or with the AccuMAP™ Low pH Protein Digestion Kit at low pH and analysed by LC-MS. Digestion at low pH was comparable to digestion at conventional conditions.

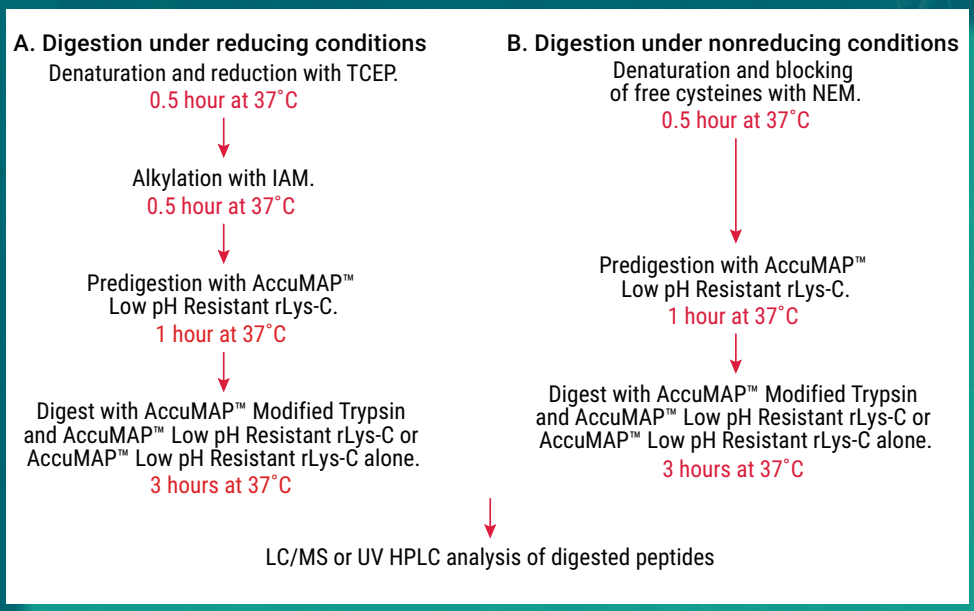
AccuMAP provides conditions for the most accurate characterisation of biotherapeutic proteins:

- Reduction, alkylation and digestion is conducted at low pH to suppress sample preparation-induced artificial PTMs
- Contains low pH-resistant rLys-C which is active under denaturing conditions and ensures maximal cleavage efficiency at lysine sites
- High digestion efficiency results in sequence coverage comparable to standard digestion conditions
- Flexible protocol gives the option to use in reducing or non-reducing conditions with alternative reducing and alkylating agents, and to perform a desalting step
- Sample preparation can be completed in 4.5-5 hours
- Kit is amenable to both UV-HPLC and mass spec applications.



Use for reduced and non-reduced proteins

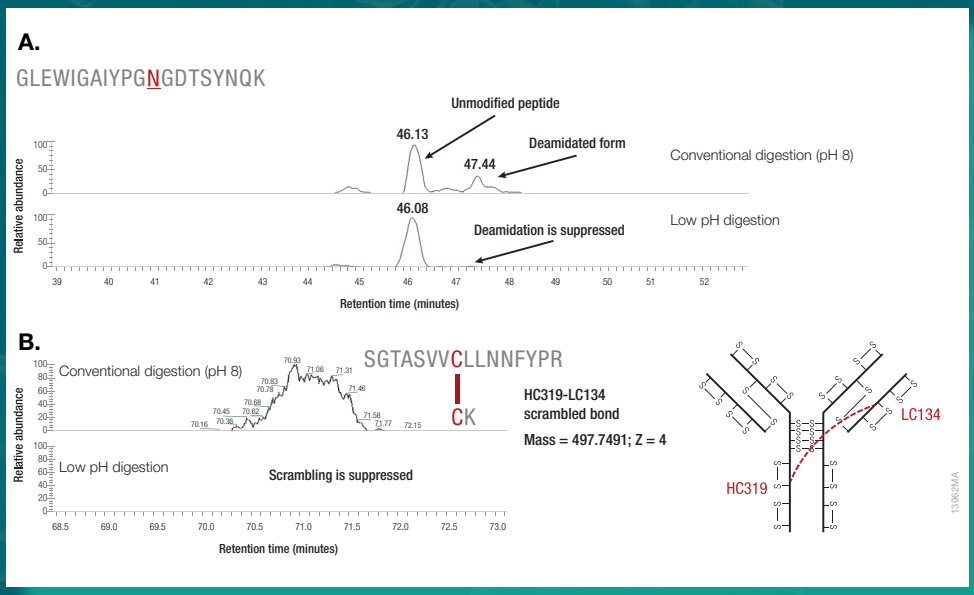
The AccuMAP™ Low pH Protein Digestion Kits suppress artificial non-enzymatic PTMs, as sample preparation is performed at low pH under either reducing or non-reducing conditions.



Schematic diagram of sample preparation performed with the AccuMAP™ Low pH Protein Digestion Kit.

Suppression of deamidation and disulphide bond scrambling at low pH

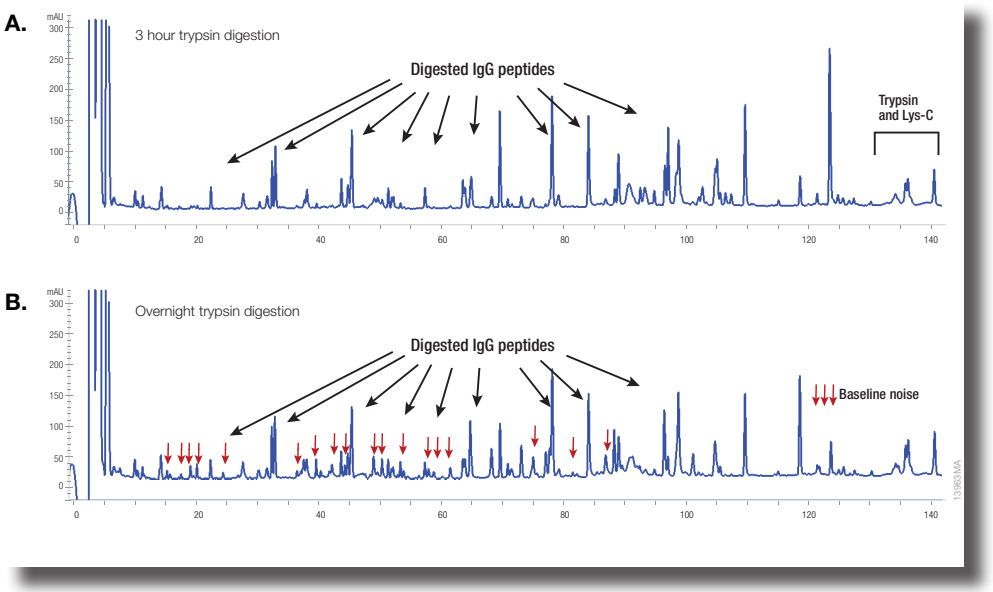
Deamidation and disulphide bond scrambling modifications are artificially introduced at alkaline pH during sample preparation. By digesting at a lower pH, AccuMAP effectively reduces occurrence of both types of PTMs, while pre-existing scrambled bonds remain intact.



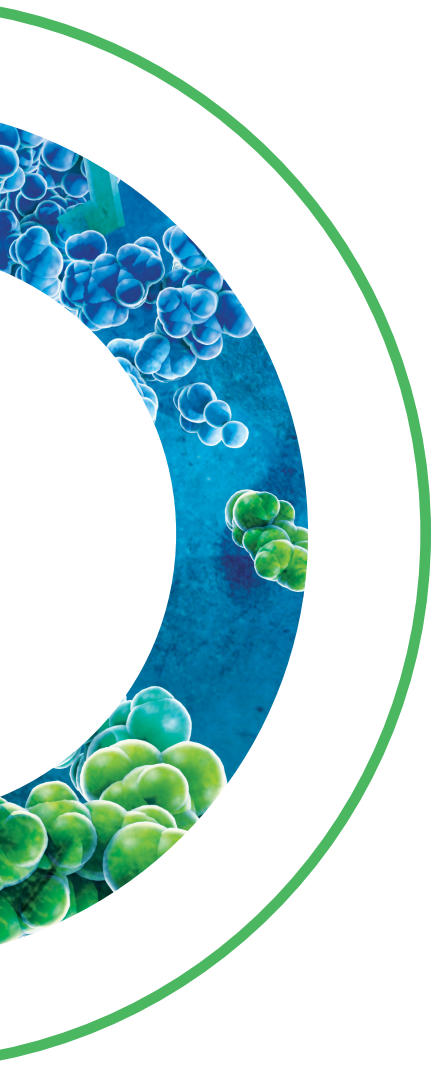
Complete suppression of deamidation and disulphide bond scrambling in IgG digested with AccuMAP™ Low pH Protein Digestion Kit. (A) An extracted ion chromatogram of a peptide from Rituximab antibody digested in conventional conditions (pH 8.0) and at low pH. The data show that asparagine (highlighted in red) was deamidated in this peptide at pH 8.0. In contrast, deamidation was fully suppressed at low pH. (B) An extracted ion chromatogram of a peptide with a scrambled disulphide bond from Panitumumab antibody digested under conventional conditions (pH 8.0) and scrambling suppression at low pH. Disulphide bond scrambling is evident at pH 8.0 while it is fully suppressed at low pH.

Minimal baseline noise in tryptic digestion

The major causes of baseline noise in tryptic digests are autoproteolysis and trypsin over-digestion, which leads to the formation of semi-tryptic peptides. Proteases in the AccuMAP Low pH Digestion kit are stable over the course of digestion reactions and baseline noise is minimal.



UV-HPLC chromatograms of Panitumumab digests. Panitumumab was predigested with AccuMAP™ Low pH Resistant rLys-C, the reaction was diluted and digestion was completed by incubation with AccMAP™ Modified Trypsin for (A) 3 hours or (B) overnight. In this experiment, a 1:5 trypsin:protein ratio was used. Note: the accumulation of baseline noise for the overnight digest shown in B.



Ordering Information

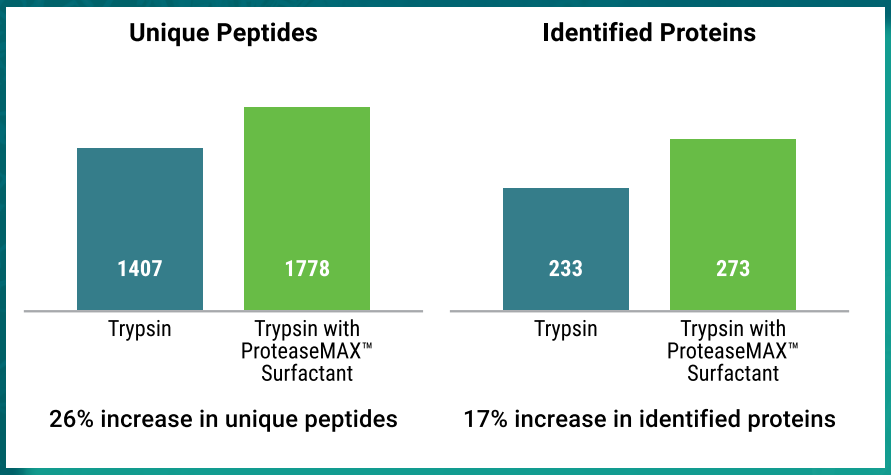
Product	Size	Cat No.
AccuMAP™ Low pH Protein Digestion Kit, Mini Contains sufficient reagents to prepare and digest 500µg of protein.	10 reactions	VA1040
AccuMAP™ Low pH Protein Digestion Kit, Maxi Contains sufficient reagents to prepare and digest 5mg of protein.	100 reactions	VA1050

Available Separately

Product	Size	Cat No.
AccuMAP™ Denaturing Solution	1ml	VA1000
AccuMAP™ 10X Low pH Reaction Buffer	1ml	VA1010
AccuMAP™ 100X Oxidation Suppressant	50µl	VA1020
AccuMAP™ Modified Trypsin Solution	120µl	V5285
AccuMAP™ Low pH Resistant rLys-C solution	120µl	VA1030
TCEP	15mg	VB1000
Iodoacetamide	15mg	VB1010

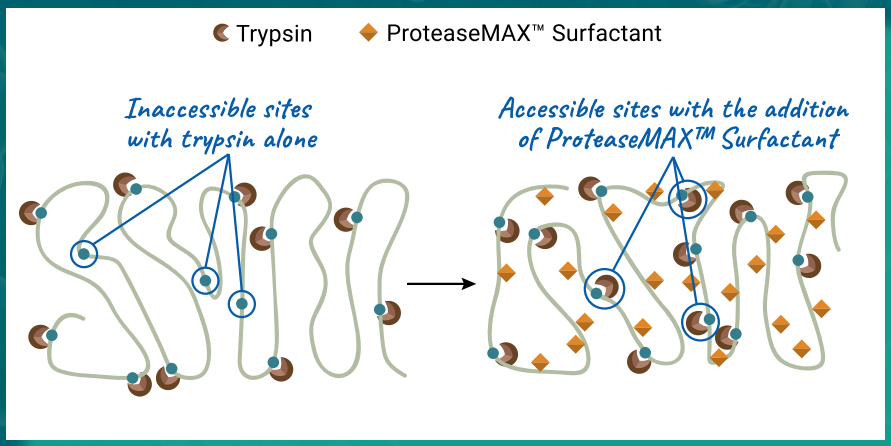
ProteaseMAX™ Surfactant, Trypsin Enhancer

ProteaseMAX Surfactant ensures fast and efficient protein digestion with proteases such as trypsin, chymotrypsin and lys-C. For in-gel protein digestion, ProteaseMAX Surfactant offers time and labour savings. Digestion is complete in one hour, and the surfactant provides concurrent extraction of peptides from gels, eliminating the need for post-digestion peptide extraction. It also improves peptide recovery, including longer peptides that are retained in the gel under a standard extraction protocol. This results in increased sequence coverage, increasing confidence of protein identification.



For in-solution digestions, ProteaseMAX Surfactant enhances protein solubility, including difficult proteins (i.e. membrane proteins). It enhances protein digestion by providing a denaturing environment prior to protease addition.

ProteaseMAX Surfactant degrades over the course of a digestion reaction, yielding products that are compatible with downstream methods such as mass spectrometry and liquid chromatography. Inactivation steps such as heating or acid treatment are not required. No long-term negative effect of the residual surfactant on the ion optics and capillary of mass spectrometers has been observed. It can be used with existing in-gel or in-solution digestion protocols.

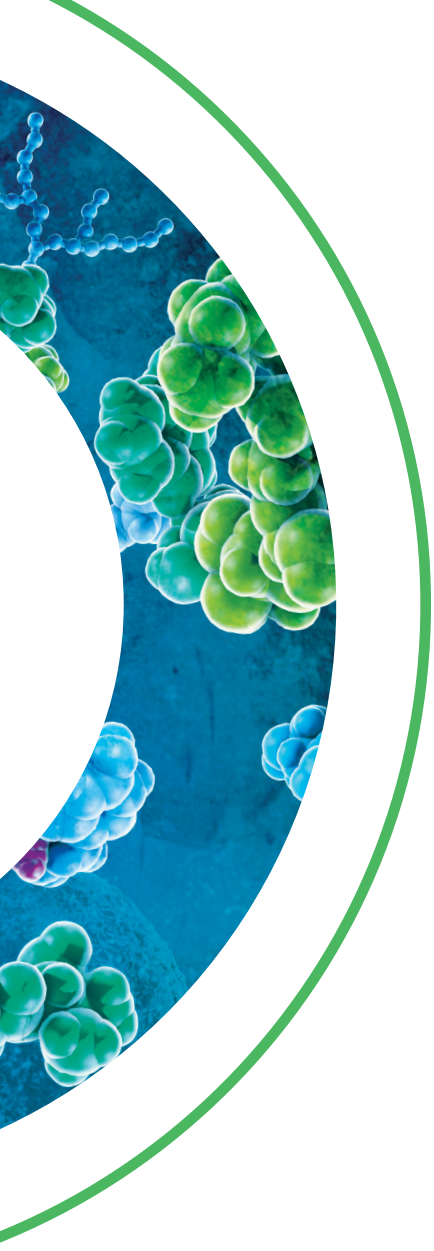


Schematic illustrating the enhanced digestion of proteins with ProteaseMAX™ Surfactant

Enhances the performance of trypsin, chymotrypsin and lys-C

Ordering Information

Product	Size	Cat.No
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg	V2071
	5 x 1mg	V2072

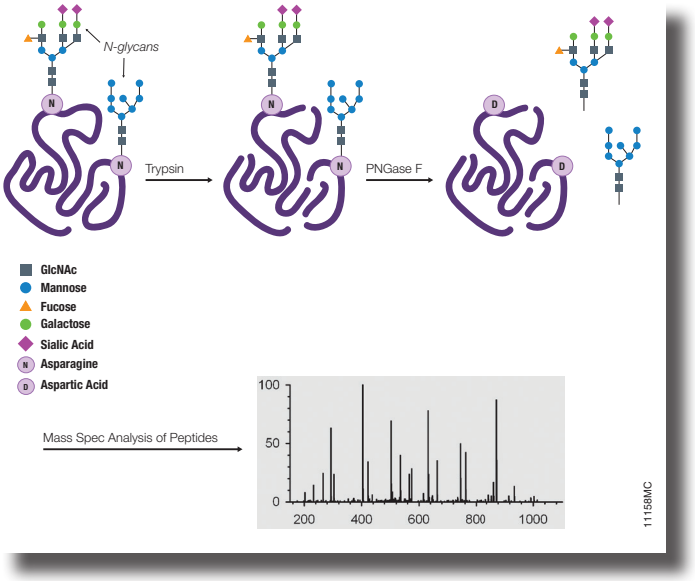


Glycosidases

Promega's glycosidases are used to monitor protein trafficking and to determine the location and structure of glycans via mass spectrometry.

PNGase F

PNGase F is a 36kDa recombinant glycosidase cloned from *Elizabethkingia miricola* and overexpressed in *E. coli*. PNGase F catalyses the cleavage of N-linked oligosaccharides between the innermost N-acetylglucosamine (GlcNAc) and asparagine residues of high mannose, hybrid and complex oligosaccharides from N-linked glycoproteins. PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins. It can be used to determine protein glycosylation status and location and to characterise the glycan structure by mass spectrometry. It can also be used to monitor protein trafficking.



Schematic illustrating the use of PNGase F and mass spec analysis of N-glycosylation.

Endoglycosidase H

Endoglycosidase H (Endo H) is a 29kDa recombinant glycosidase cloned from *Streptomyces plicatus* and overexpressed in *E. coli*. Endo H cleaves the chitobiose core of high mannose and a limited number of hybrid oligosaccharides from N-linked glycoproteins. It does not cleave complex glycans. Enzymatic cleavage is between the two GlcNAc residues in the diacetylchitobiose core of the oligosaccharide, leaving one GlcNAc residue on the asparagine. This is in contrast to PNGase F, which cleaves all asparagine-linked oligosaccharides. It can be used to determine the state of protein glycosylation and to characterise glycan structure.

Fetuin

Fetuin is a glycoprotein with O- and N-linked glycosylation sites. It is an ideal glycosylation substrate control for PNGase F or Endo H activity monitoring.

Ordering Information

Product	Size	Cat No.
PNGase F	500 units	V4831
Endoglycosidase H	10,000 units	V4871
	50,000 units	V4875
Fetuin	500µg	V4961

Additional Reagents

Factor Xa Protease

Factor Xa Protease preferentially cleaves after the arginine residue in the amino acid sequence Ile-Glu-Gly-Arg. It is purified from bovine plasma and activated by treatment with an enzyme from Russell's Viper Venom. Its main application is for cleavage of proteins and fusion proteins containing Factor Xa Protease sites, including fusion proteins generated with the PinPoint™ Vectors.

ProTEV Plus

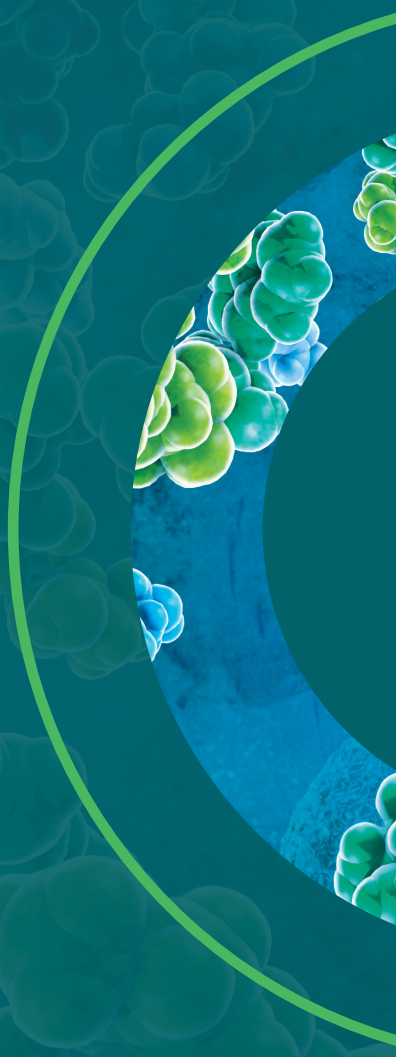
ProTEV Plus is an improved 48kDa version of the Nla protease from Tobacco Etch Virus (TEV) that has been engineered to be more stable than native TEV protease for prolonged enzymatic activity. It is a highly specific proteolytic enzyme that cleaves within a seven amino acid sequence (ENLYFQ(G/S)) with minimal off-target effects. ProTEV Plus is active over a wide range of pH values (5.5-8.5) and temperatures (4-30°C). It can be used to cleave individual protein fusions that have been engineered with the above amino acid sequence at the desired cleavage site. The enzyme is easy to use in multiple experimental formats, being compatible for both in-solution and on-column cleavage reactions. ProTEV Plus also contains a HQ tag (analogous to His tag) located at the N-terminus of the protein, which allows it to be immobilised on Ni-based affinity resins and removed from the cleavage reactions.

Proteinase K

Proteinase K, produced by the fungus *Tritirachium album* Limber, is a serine protease that exhibits broad cleavage activity. It cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids and is useful for general digestion of protein in biological samples. It has been purified to remove RNase and DNase activities. The stability of Proteinase K in urea and SDS and its ability to digest native proteins make it useful for a variety of applications including preparation of chromosomal DNA for pulsed-field gel electrophoresis, protein fingerprinting and removal of nucleases from DNA and RNA preparations. It is available in both lyophilised form and in solution.

Ordering Information

Product	Size	Cat.No
Factor Xa Protease	50µg	V5581
ProTEV Plus	1,000 units	V6101
	8,000 units	V6102
Proteinase K (Lyophilized)	100mg	V3021
Proteinase K (PK) Solution	4ml	MC5005
	16ml	MC5008



Protein Extracts

Mass spec-compatible protein extracts

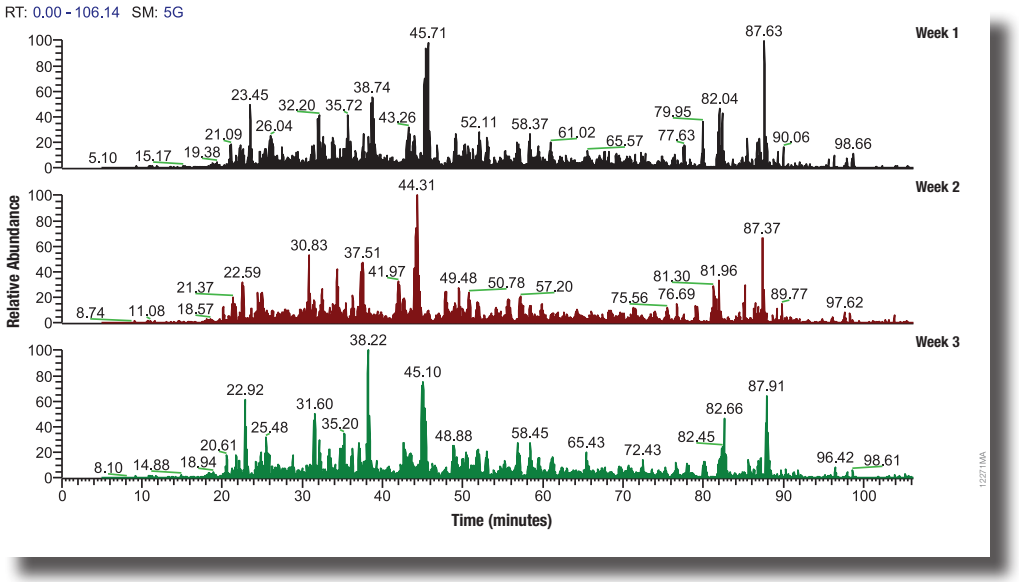
Human and yeast protein extracts for easier instrument monitoring and method development

Monitoring of Liquid Chromatography (LC) and Mass Spectrometry (MS) instrument performance is required to ensure efficient proteomic analysis. Promega's protein extracts provide complex reference material that offers consistent peptide retention time, signal intensity and other critical performance parameters.

Both human and yeast extracts are available in a pre-digested, tryptic peptide form, used primarily for instrument performance monitoring. They have been cleaned up by solid-phase extraction for immediate use in LC-MS analysis. The extracts are also offered in an intact undigested form to provide a test material for optimising mass spectrometry protein sample preparation.

Yeast extract benefits users who prefer working with a relatively compact and well-studied proteome, whereas human extract provides an opportunity for working with a complex proteome having a large dynamic range.

LC-MS performance: consistent mass spec profiles

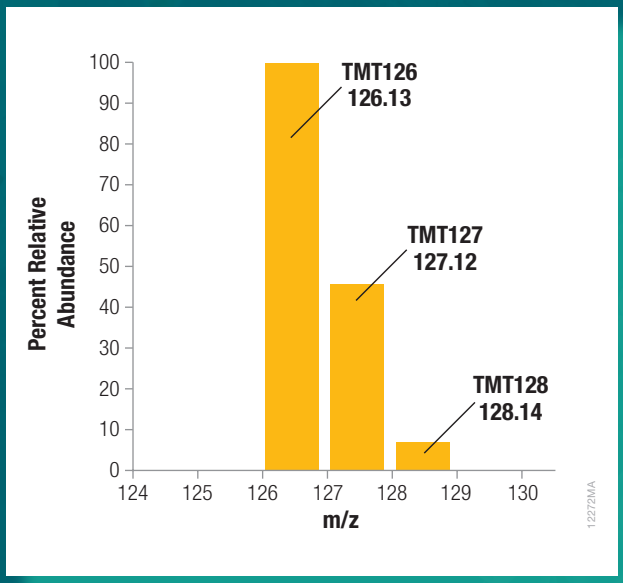


Consistent mass spec profile of complex protein extracts. Each run used 1µg of human predigested protein extract injected into the instrument (Waters NanoAquity HPLC system interfaced to a ThermoFisher Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer). Peptides were resolved with two hour gradient. Weekly monitoring with the human extract ensured consistent analytical performance of the instrument.

Relevant complex proteomes

These protein extracts provide ideal model systems for developing and optimising various sample preparation methods, including isobaric tagging, enrichment and other applications, preserving your valuable sample material for experimental analysis.

Use for method development and optimisation



Example of method development, isobaric tagging. Three aliquots of the human K562 predigested extract were labelled with three different Tandem Mass Tag (TMT) isobaric mass tag reagents (TMT126, TMT127 and TMT128) and mixed at a 10:5:1 ratio. Relative quantitative ratio between the mass tags was determined with an LTQ Orbitrap Velos Pro™ mass spectrometer. The data shows good correlation between the expected and observed protein quantitation levels.

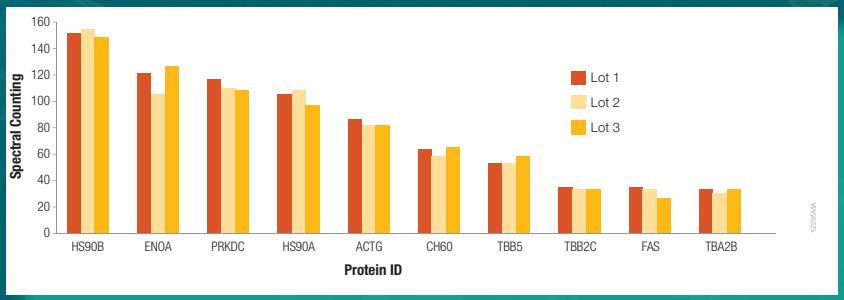
High material integrity

Promega protein extracts are manufactured to meet stringent specifications for peptide integrity and composition, in order to provide reliable and reproducible data. Examples of specifications data include:

- Marginal nonspecific fragmentation
- Acceptable levels of non-biological post-translational modifications, including deamidation, oxidation and carbamylation
- Minimal level of missed tryptic cleavages
- Reproducibility of protein composition
- Consistency of protein quantity monitored by spectral counting.

LC-MS performance: consistent mass spec profiles

Proteins	2099
Total spectra	23,876
Unique peptides	15,211
Missed tryptic cleavages	5.4%
Non-specific cleavages	0.4%
Deaminated spectra	3.3%
Oxidation spectra	0.3%
Carbamylated spectra	0.3%



Protein quantity reproducibility for the human protein extract. Each extract was analysed by LC-MS/MS. Relative amounts of protein were determined by spectral counting. The data shows high protein quantity reproducibility between the tested human extracts.

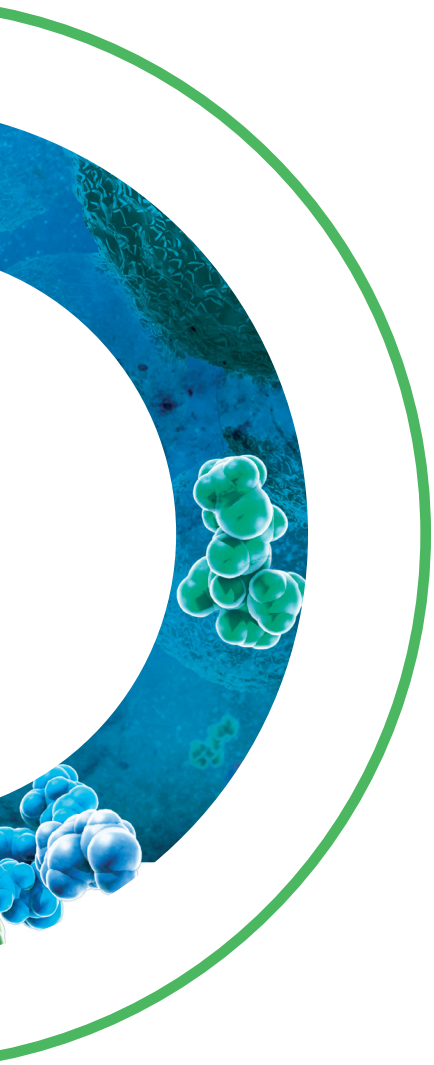
Enhanced quality control ensures reliable, reproducible performance

Monitor LC and MS instrument performance

Lot-to-lot consistency of extracts is monitored by various protein and peptide qualitative and quantitative methods, including LC-MS and amino acid analysis. Our manufacturing process assures compatibility with LC-MS by monitoring non-specific protein fragmentation, non-biological post-translational modifications and, for digested extracts, minimal undigested peptides.

QC specifications for MS-Compatible Human Protein Extract, Digest

Non-biological post-translational modifications	
Deamidation spectra	≤12%
Oxidation spectra	≤5%
Carbamylation spectra	≤5%
Missed cleavages	
Missed cleavages	≤10%
Peptide quality	
Quantification of amino acids in sample	
Protein fragmentation	1% or less
Matching spectra	>65%
Lot-to-lot consistency	
Total proteins	>1805
Unique peptides	>12,463
Quantitation reproducibility monitored by relative abundance of 10 reference proteins	



Yeast extracts are whole-cell protein extract prepared from *Saccharomyces cerevisiae* cells. Human extracts are whole-cell protein extract prepared from human K562 cells.

Digests are lyophilised tryptic peptides. Intact extracts are solubilised in 6.5M urea/50mM Tris-HCl.

Ordering Information

Product	Size	Cat No.
MS Compatible Yeast Protein Extract, Digest	100µg	V7461
MS Compatible Human Protein Extract, Digest	100µg	V6951
MS Compatible Yeast Protein Extract, Intact	1mg	V7341
MS Compatible Human Protein Extract, Intact	1mg	V6941

Reference Standards

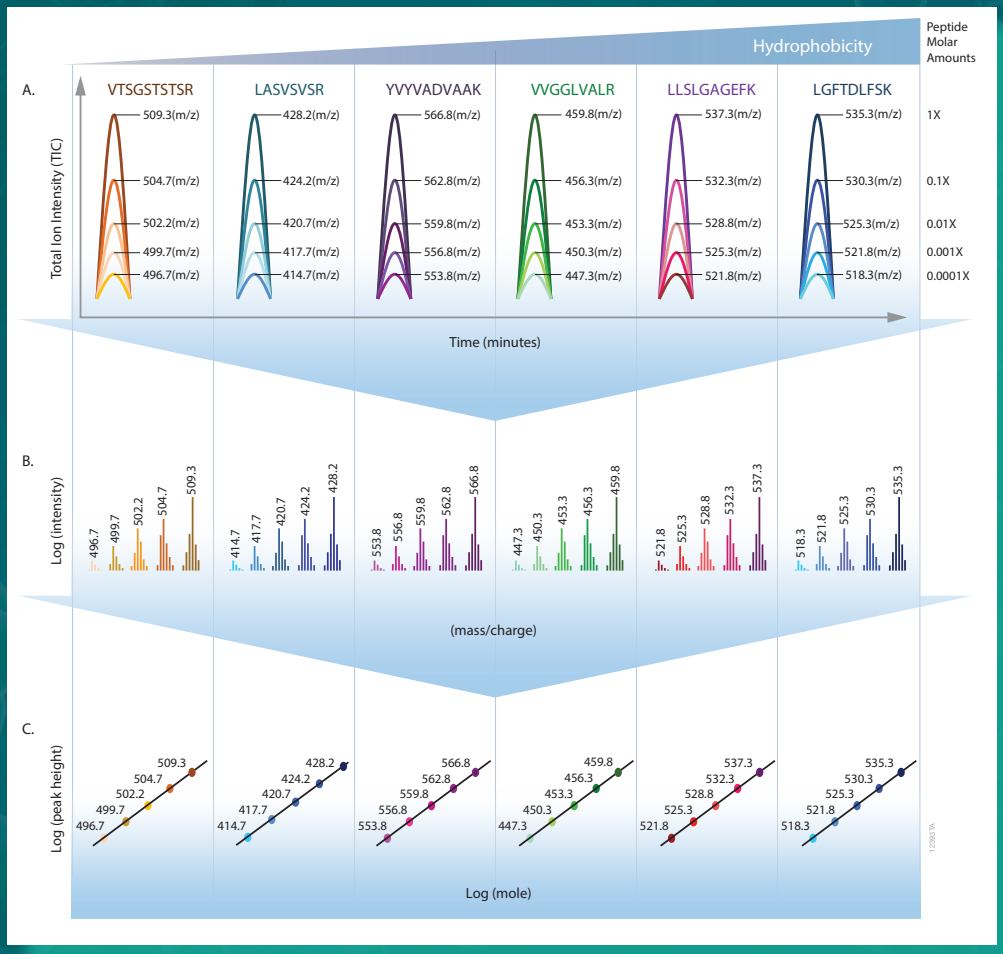
6 x 5 LC-MS/MS Peptide Reference Mix

6 × 5 LC-MS/MS Peptide Reference Mix is a unique reagent that provides a convenient way to assess liquid chromatography (LC) column performance and mass spectrometry (MS) instrument parameters, including sensitivity and dynamic range, and to assist in method development and optimisation.

Maximise instrumentation time for sample analysis instead of for quality control

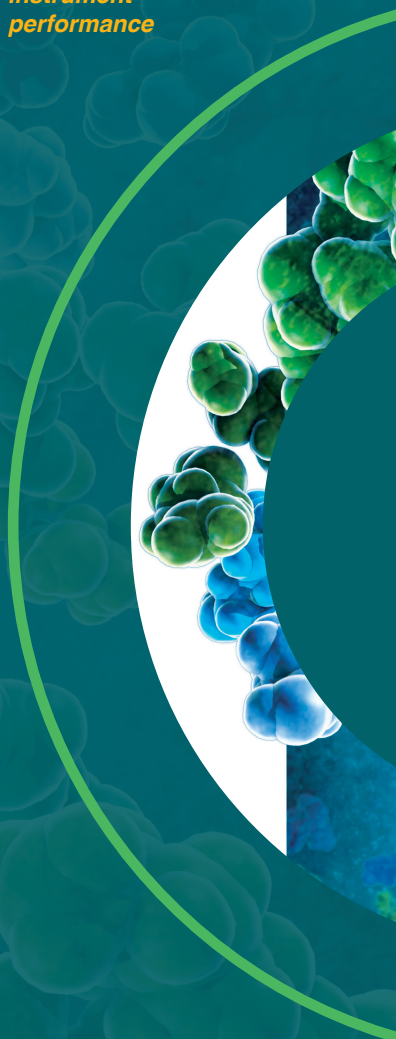
The product is a mixture of 30 peptides: 6 sets of 5 isotopologues of the same peptide sequence. The isotopologues differ only by the number of stable, heavy-labelled amino acids incorporated into the sequence. The labels consist of uniform 13C and 15N atoms. Chromatographically, each of the isotopologues 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 differences in concentration or molar abundance. If 1pmol of mixture is loaded onto an LC column, the next lighter isotopologue would be 100fmol, the next 10fmol, the second lightest 1fmol, and the lightest 100amol. This format allows assessment of instrument dynamic range and sensitivity from a single run.



Schematic illustrating the features of the 6 × 5 LC-MS/MS Peptide Reference Mix. A mixture of 6 peptide sets is 30 peptides in total (6 × 5). Each of the peptide sets is a mixture of five isotopologues, distinguished only by mass through the incorporation of stable, heavy-labelled amino acids. (A) The isotopologues are chemically and chromatographically identical and therefore appear as 6 peaks. (B) Each of the isotopologues is mixed with a tenfold molar increase (lightest to heaviest). (C) A plot of the log of peak height versus the log (molar amount) on column has a slope = 1. This analysis enables confirmation of detection limits (LOD and LOQ), and can also be used to confirm linearity up to 5 logs of dynamic range.

A complete solution for optimising and monitoring instrument performance



Monitor both LC and MS parameters with one peptide mix

Peptides with a wide range of hydrophobicities were chosen to enable reporting of LC column performance. The most hydrophilic peptide gives users a tool to optimise the capture of hydrophilic peptides that might be difficult to capture otherwise, but too precious to use for method development. In addition, the peptides were chosen for maximal stability. Amino acids prone to artificial post-translational modifications (i.e. methionine, asparagine, etc.) were excluded from the sequences. None of the peptides have internal lysines or arginines, and will therefore not be affected by trypsin or lys-C. There is also a mass separation of at least 4 Daltons between the isotopologues, so that even low resolution instruments can distinguish the masses.

No need to purchase multiple reagents

The 6 × 5 LC-MS/MS Peptides are AAA-qualified for accurate monitoring, and are compatible with multiple applications and neat or complex mixtures. The neat format can be used for standard instrument monitoring, or the peptide mix can be spiked into complex samples (i.e. human cell extract) to monitor instrument performance with relevant experimental samples.

The Peptide Reference Mix is also intended for use in method development and optimisation. To assist in data processing, we provide a complementary software tool, the 6 × 5 LC-MS/MS Peptide Reference Mix Analysis Software (PReMiS™ Software).

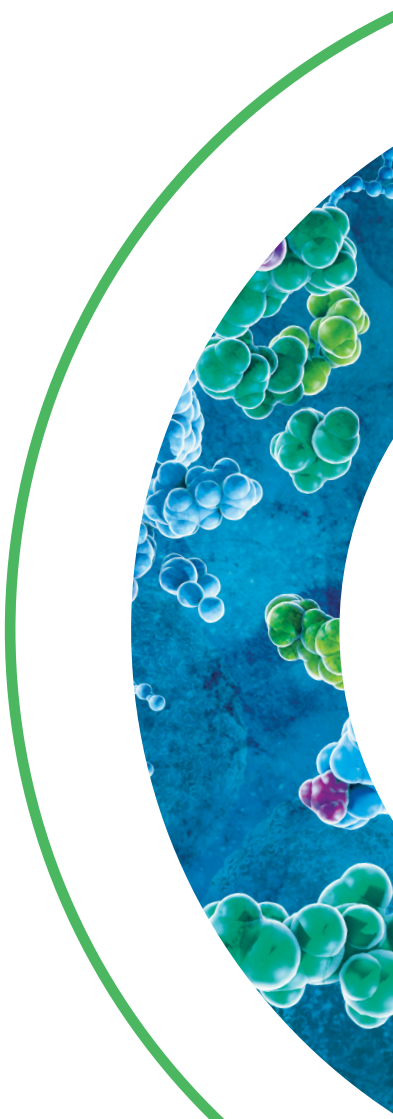
Determine instrument sensitivity and dynamic range in a single run

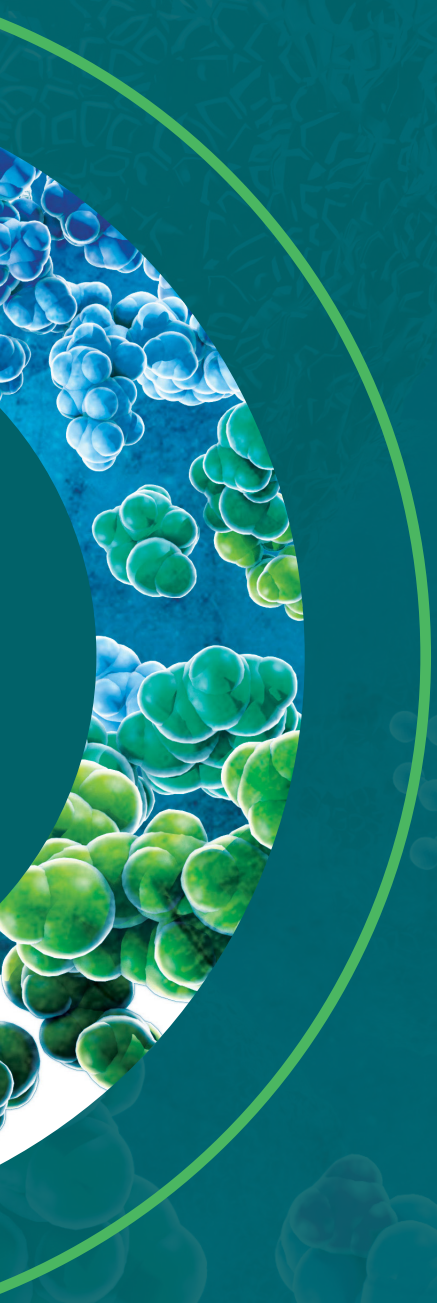
Using the included software, this product is a complete solution for optimising and monitoring instrument performance and routine QC.

Ordering Information

Product	Size	Cat No.
6 x 5 LC-MS/MS Peptide Reference Mix*	50µl	V7491
	200pmol	V7495

**Lyophilised. Includes six additional Axygen maximal recovery tubes for aliquots. For laboratory use.*





PReMIS™ Software Tool

The complimentary PReMiS™ Software streamlines routine analysis by:

- Eliminating time-consuming manual calculations
- Ensuring consistent instrument performance over time
- Allowing you to compare instrument-to-instrument performance over time.

The PReMiS Software analyses mass spectrometric data collected from the 6×5 LC-MS/MS Peptide Reference Mix, run either neat or spiked into complex backgrounds. It calculates values for parameters to report on LC-MS/MS instrument performance. Data collected from Thermo (.raw files) or Sciex (.wiff and associated files) instruments can be analysed directly. Other data formats must be converted to .mzML prior to analysis.

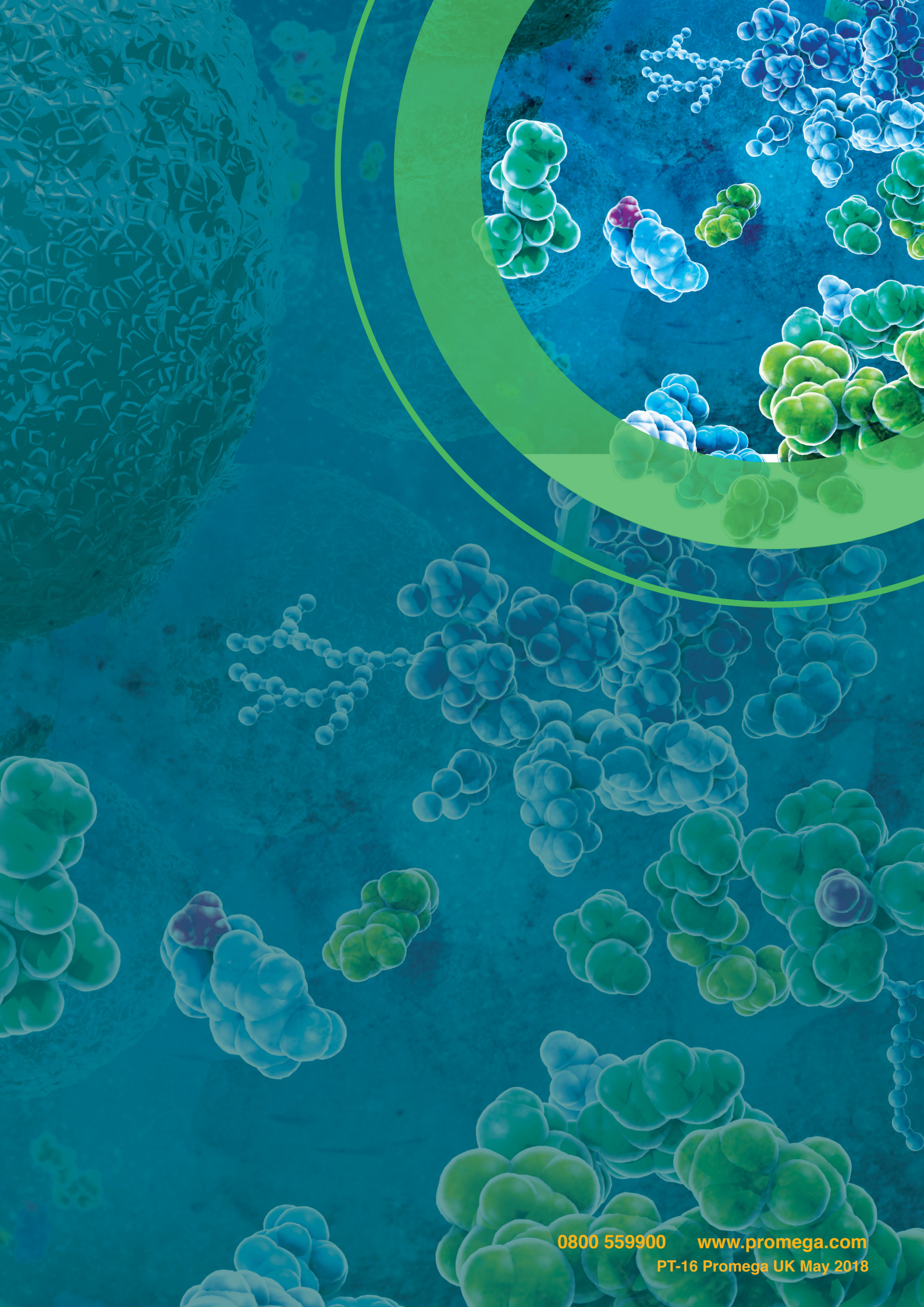
In addition to the general reporting features, the software allows you to track performance over time, enabling a clear assessment of trends to pinpoint poor performance and maintenance needs. For laboratories that have multiple instruments, the ability to compare parameters across instruments is also available.

Thermo (.raw) and Sciex (.wiff) data can be imported directly. Other data formats can be imported after conversion to .mzML.

Data reports are rapidly generated (usually less than two minutes) with clear presentation of the XIC of all 30 masses available for immediate viewing.

Parameter	Category	Calculated by software	Detail
Retention time	LC	Yes	Time required for peptide being separated to elute from the chromatography column.
Peak width	LC	Yes	Peak width is an indication of column efficiency.
Base peak height	LC	Yes	This measure of the signal response can be correlated to the ionisation efficiency of the peptide.
Graphical XIC analysis	LC	Yes	A graphical XIC (eXtracted Ion Chromatogram) will be displayed for all 30 ions for visual confirmation of the detectability of all masses. Especially important for analysis of the peptide mixture spiked into a complex background.
Lowest detectable quantity	MS	Yes	For each peptide sequence the lowest abundance detected will be reported.
Linear Fit Analysis (linear dynamic range)*	MS	Yes*	*The software does not directly calculate the full dynamic range of the instrument. However, up to five logs of dynamic range can be determined directly from the Linear Fit Analysis.
LOD and LOQ**	MS	No	Since the peptides are AAA quantified, the mixture can be used to confirm the instrument LOD and LOQ. This will serve as the basis for the amount loaded, which can then be used to routinely confirm consistency of detection limits.
Slope and r2 of sensitivity curve - graphical analysis	MS	Yes	A plot of the log of peptide amount in moles versus log of peak height is reported to provide information on LOD and LOQ.
Mass accuracy	MS	Yes	
Performance over time	Both	Yes	Parameters like LC retention time, peak height and mass accuracy are graphically displayed based on run number.
Multi instrument comparisons	Both	Yes	

* A dynamic range of up to five is confirmed. For instruments with ranges above five, multiple runs should be considered.
** The LOD and LOQ will need to be determined via titration of the peptide mixture. Once the limits are established, the reagent can be used to confirm linearity and detection of the lowest abundance isomer in a single injection.



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