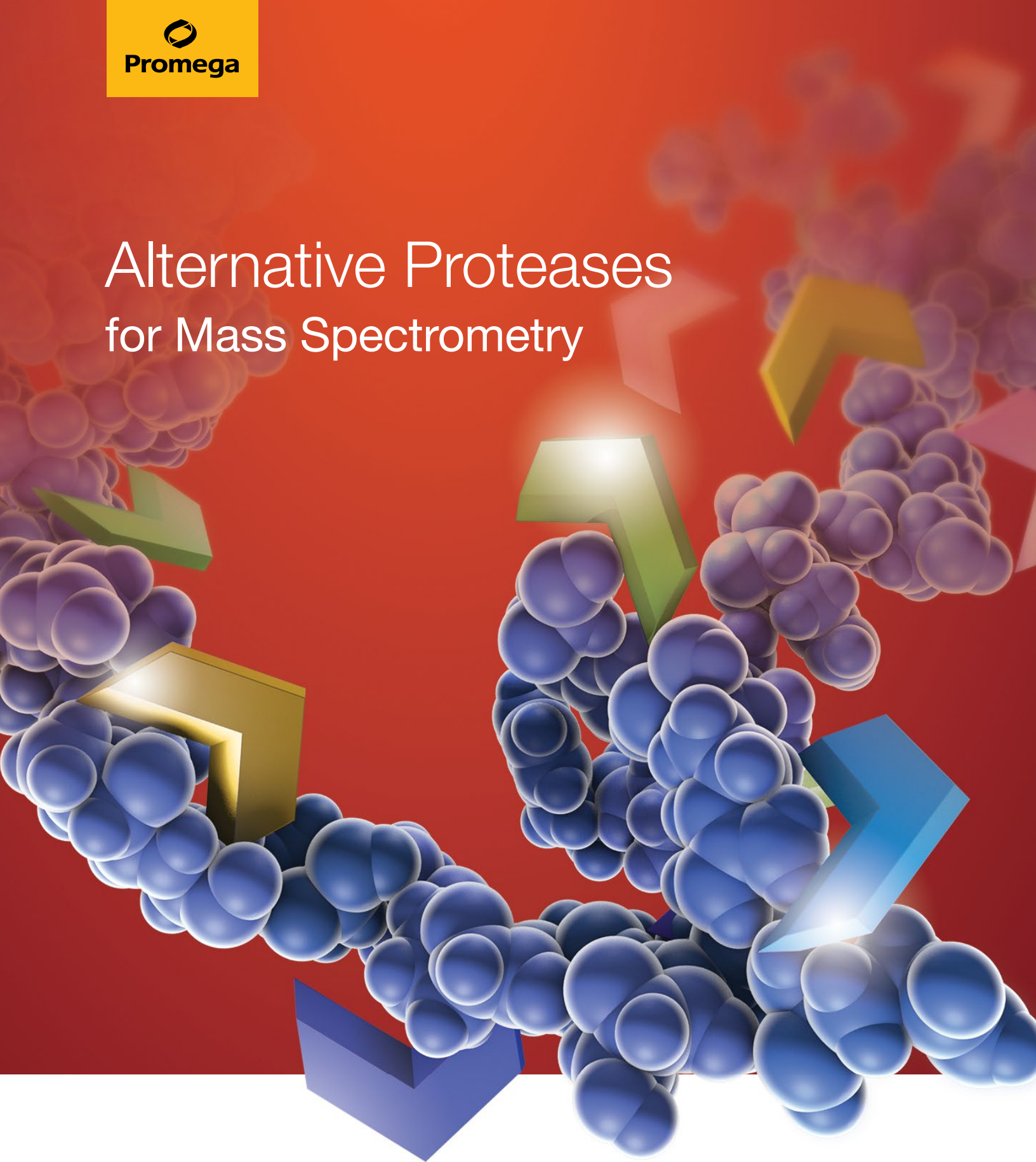


Alternative Proteases for Mass Spectrometry



**Fast and Convenient Digestion
with Improved Sequence Coverage**

Alternative Proteases for Mass Spectrometry

Trypsin, the most widely used protease in mass spectrometry analysis, utilizes 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) present a different challenge for trypsin because glycans often limit trypsin access to cleavage sites, and acetylation or di- and trimethylation of lysine and arginine residues make them resistant to trypsin digestion. Promega offers several alternative proteases that complement standard trypsin digestions and provide enhanced data.

- Increased Protein Coverage
- Characterize Post-translational Modifications
- Enhanced Digestion of Difficult-to-digest Proteins

Table 1. Comparison of proteases and corresponding cleavage sites.

Protease	Cleavage site	Example of use
Trypsin Specific protease	NNNR▼NNNNNK N (R is arginine, K is lysine)	Protease of choice for standard digestions due to size and resulting charge on peptide
ProAlaase Specific protease	NNNA▼NNN (A is alanine) NNNP▼NNN (P is proline)	Used to reach areas of proteome not accessible with traditional proteases in variety of applications: protein characterization, peptide mapping, disulfide bond mapping, de novo sequencing, HDX-MS
Lys-C Specific protease	NNNNK▼NNN (K is lysine)	Digests membrane and other proteolytically resistant proteins; generates larger peptides than tryptic peptides—advantage for certain mass spec methods (for example, ETD)
Arg-C Specific protease	NNNNR▼NNN (R is arginine) Arg-C can, to a lesser degree, cleave at lysine also	Facilitates analysis of histone post-translational modifications; used in proteome-wide analysis
Glu-C Specific protease	NNNNE▼NNN (E is glutamate) Glu-C can, to a lesser degree, cleave at aspartate also	Used as an alternative to trypsin if trypsin produces too short or too long peptides or if tryptic cleavage sites are not accessible
Asp-N Specific protease	NNNN▼DNNN (D is aspartate)	Similar to Glu-C
Chymotrypsin Low specific protease	NNNN(F/Y/W)▼NNN (F , Y and W are aromatic residues phenylalanine, tyrosine and tryptophan)	Digests hydrophobic proteins (for example, membrane proteins)
Pepsin Nonspecific protease	Nonspecific protease (advantage – most active at low pH)	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)	Digests proteolytically resistant, tightly folded proteins; used in structural protein studies
Elastase Nonspecific protease	Nonspecific protease	Used to increase protein coverage

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 (Figures 1 and 2). When alternative proteases are used, larger peptides are cleaved into smaller fragments, which are more manageable for mass spectrometry instruments.

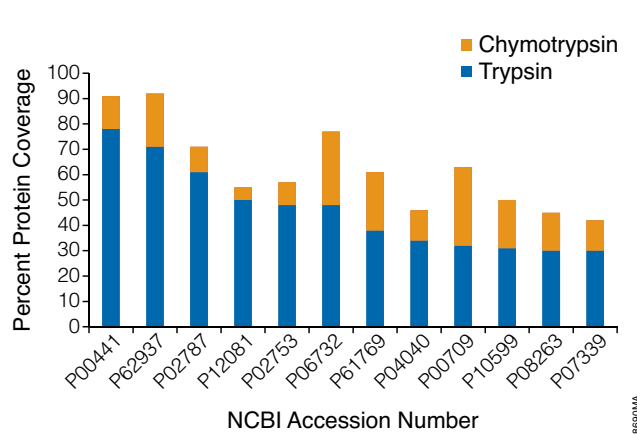


Figure 1. Increased protein coverage using both trypsin and chymotrypsin.

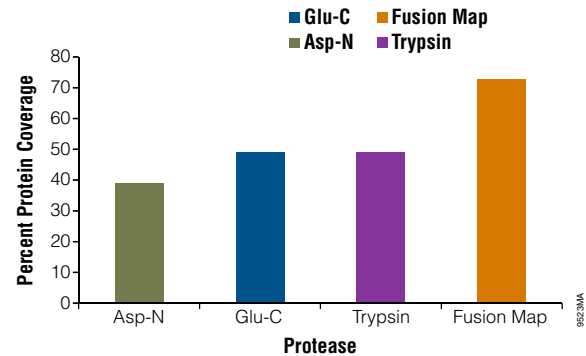


Figure 2. Increased protein coverage using multiple proteases.

Characterize Post-translational Modifications

Some post-translational modifications (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 Figure 3, 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.

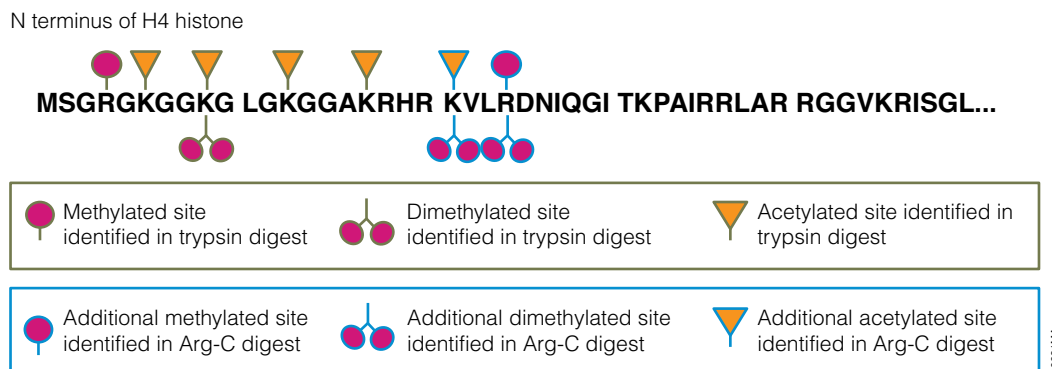


Figure 3. Histone H4 post-translational modifications identified in trypsin and Arg-C digests.

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 (Figure 4).

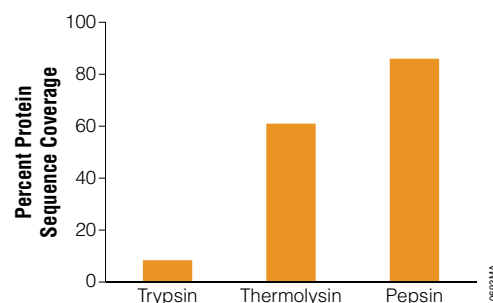


Figure 4. Bacteriorhodopsin sequence coverage obtained from trypsin, thermolysin and pepsin.

Ordering Information

Product	Size	Cat.#
AccuMAP™ Low pH Protein Digestion Kit	1 each	VA1040
Arg-C, Sequencing Grade	10µg	V1881
Asp-N, Sequencing Grade	2µg	V1621
Chymotrypsin, Sequencing Grade	25µg	V1061
	100µg (4 x 25µg)	V1062
Elastase	5mg	V1891
Glu-C, Sequencing Grade	50µg (5 x 10µg)	V1651
Pepsin	250mg	V1959
ProAlanase, Mass Spec Grade	5µg	VA2161
ProAlanase Plus, Mass Spec Grade	15µg	VA2171
Rapid Digestion Trypsin	100µg	VA1060
Rapid Digestion Trypsin/Lys-C	100µg	VA1061
Lys-C, Mass Spec Grade	20µg	VA1170
rLys-C, Mass Spec Grade	15µg	V1671
Sequencing Grade Modified Trypsin	100µg (5 x 20µg)	V5111
	100µg (1 x 100µg)	V5117
Sequencing Grade Modified Trypsin, Frozen	100µg (5 x 20µg)	V5113
Thermolysin	25mg	V4001
Trypsin Gold, Mass Spec Grade	100µg (1 x 100µg)	V5280
	20µg	V5071
Trypsin/Lys-C Mix, Mass Spec Grade	100µg (1 x 100µg)	V5072
	100µg (5 x 20µg)	V5073
Trypsin Platinum	100µg	VA9000

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Learn more about our alternative proteases, visit: www.promega.com/proteases



Promega GmbH

Gutenbergring 10
69190 Walldorf · Germany
www.promega.com

Germany/Austria

Phone +49 6227 6906-290
de_techserv@promega.com

Poland

Phone +48 22 531 06 68
pl_techserv@promega.com

Central/Eastern Europe

Phone +49 6227 6906-0
de_ceesales@promega.com