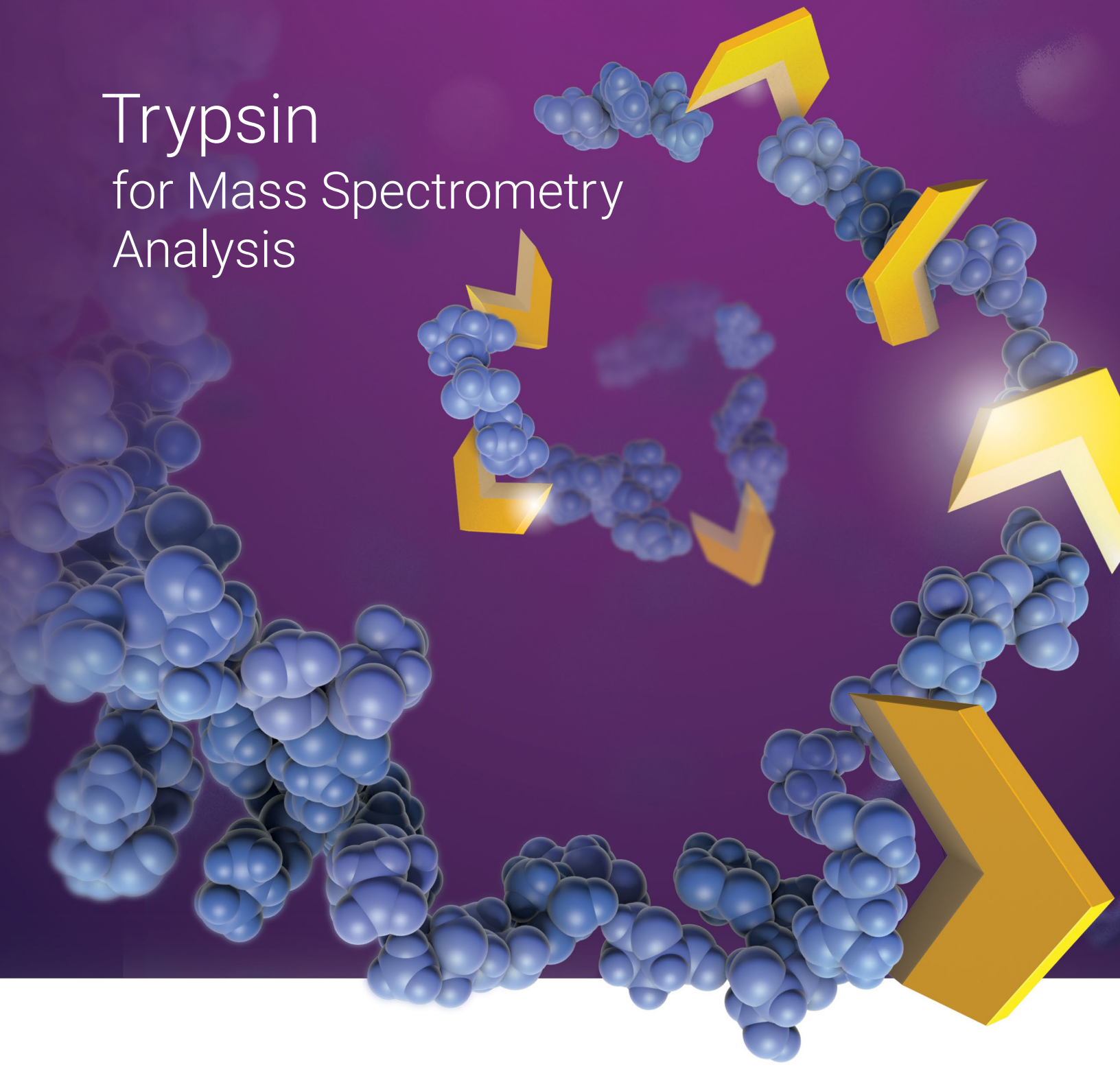
Promega

Trypsin
for Mass Spectrometry Analysis



Reliable and Customer-Proven Results

Protein Digestion with Trypsin

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 characterizing proteins using mass spectrometry. Native trypsin is subject to autolysis, generating pseudotrypsin, which exhibits a broadened specificity including a chymotrypsin-like activity. These autolysis products, when present in a trypsin preparation, result in additional peptide fragments that can interfere with database analysis of the fragments detected by mass spectrometry.

Trypsin Platinum, Mass Spectrometry Grade

Trypsin Platinum, Mass Spectrometry Grade, is a recombinant protease designed for users looking for accurate protein characterization with mass spectrometry and RP-HPLC-UV. It is free of any detectable nonspecific proteolytic activity. A novel chemical modification method assures maximal autoproteolytic resistance. Trypsin Platinum has high proteolytic efficiency and is free from contaminating proteins of animal origin.

Commercially available proteomic and mass spec grade trypsins contain nonspecific protease activity at a low but detectable level (1). Close analysis of this activity suggests it is chymotryptic in nature. The nonspecific, chymotryptic-like cleavage activity becomes evident if large amounts of trypsin are used in a digestion reaction (Figure 1, Panel A). These nonspecific cleavages compromise the quality of protein analysis. Our production procedure assures that Trypsin Platinum is free of any detectable traces of nonspecific cleavage activity (Figure 1, Panel B).

- **Optimal Tryptic Activity:** Maximum resistance to autoproteolysis.
- **Consistent Performance:** Each lot qualified by mass spectrometry to ensure compatibility with your applications/instrumentation.
- **Streamlined Data Analysis:** Recombinant protease free of nonspecific cleavage activity.

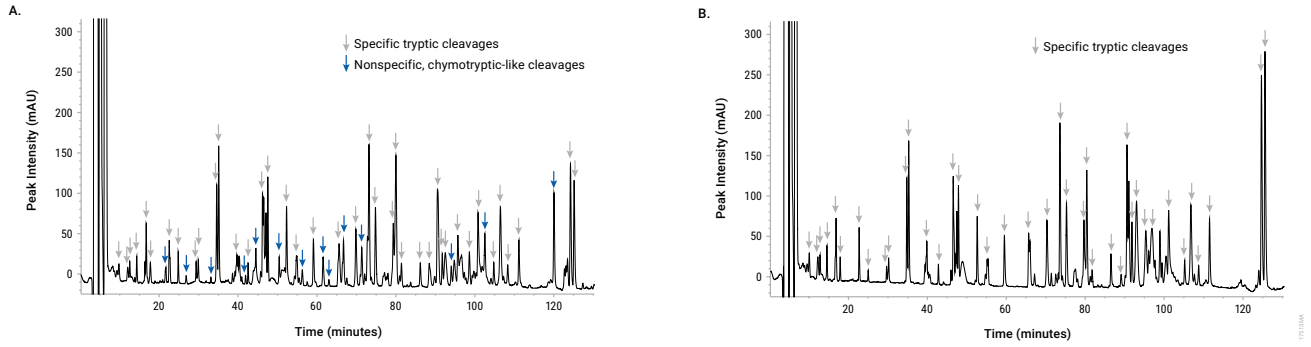


Figure 1. Trypsin Platinum, Mass Spectrometry Grade, cleavage specificity. Panitumumab (Vectibix®) was used as a model protein substrate. Digestion reactions used a 1:10 trypsin:protein ratio, and the digested peptides were analyzed with RP-HPLC-UV. The peptide peaks were assigned with LC-MS to differentiate between specific and nonspecific peptides. The analysis showed that MS grade trypsin from vendor T contains prominent nonspecific proteolytic activity (Panel A). Close analysis indicated a chymotryptic-like pattern of the generated nonspecific cleavages (data not shown). In contrast, Trypsin Platinum generated specific tryptic cleavages only (Panel B).

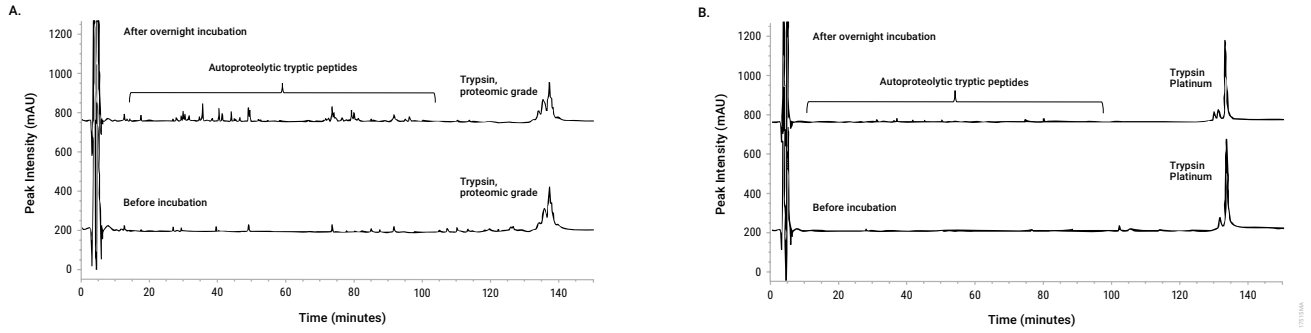


Figure 2. Trypsin Platinum, Mass Spectrometry Grade, autoproteolytic resistance. Proteomic Grade trypsin from vendor S and Trypsin Platinum were incubated at conventional digestion conditions. Specifically, the trypsin products were reconstituted in 100mM Tris-HCl (pH 8)/2mM $CaCl_2$ and incubated overnight at 37°C. Autoproteolytic products were then analyzed with RP-HPLC-UV. Fresh, nonincubated aliquots of each trypsin were analyzed as a control. Proteomic Grade trypsin demonstrated prominent autoproteolysis, whereas autoproteolysis of Trypsin Platinum was reduced to a negligible level (compare Panels A and B).

Rapid-Digestion Trypsin

Rapid-Digestion Trypsin is designed to shorten protein digestion times to 60 minutes versus the typical 4–18 hours required for trypsin digestion (Figure 3). Rapid-Digestion Trypsin and Trypsin/Lys-C Mix are available. 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 protein digestions.

Trypsin digestion with these kits follows a simple protocol that is both fast and efficient (Figure 4). The protocol is flexible, can accommodate a large range of sample volumes and protein concentrations, and requires no special equipment or off-line desalting. The entire sample preparation procedure is performed in as little as 60 minutes.

- **Reproducible Data:** Steamlined workflow.
- **Obtain Data in One Day:** Faster digestion time.
- **Flexibility:** Use with different volumes and protein concentrations.

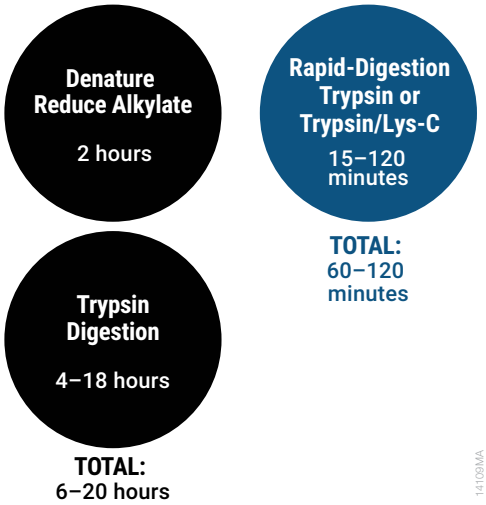


Figure 3. Comparison of standard vs rapid tryptic digest procedure.

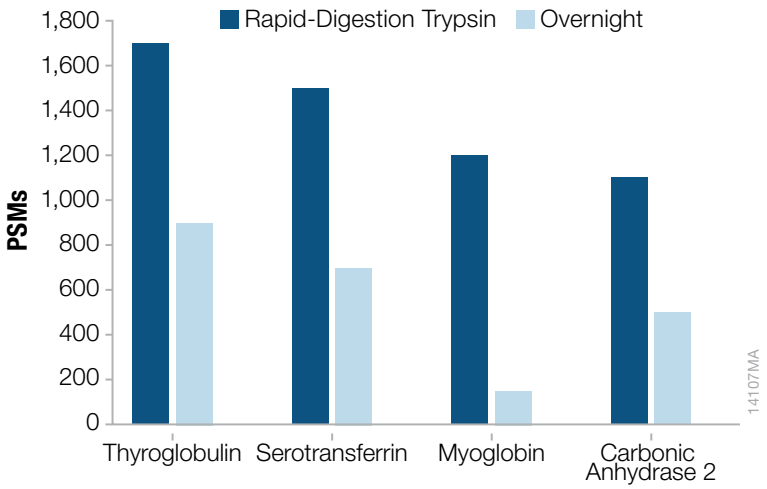


Figure 4. The increase in spectra noted for Rapid-Digestion Trypsin sample preparation (30-minute digestion) indicates more complete digestion than standard overnight digestion (16 hours using Trypsin Gold, Mass Spectrometry Grade).

Trypsin Gold, Mass Spectrometry Grade

Trypsin Gold, Mass Spectrometry Grade, is manufactured to provide maximum specificity. Lysine residues in the porcine trypsin 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 and lyophilized to yield Trypsin Gold, Mass Spectrometry Grade.

- **Consistent Performance:** Each lot is qualified by mass spectrometry to ensure compatibility with customer applications and instrumentation.
- **Pure:** TPCK treatment followed by affinity purification eliminates chymotrypsin activity for distinct and consistent data.
- **Stable:** Ensured stability for up to five freeze-thaw cycles, which minimizes wasted reagents.
- **Reliable and Customer-Proven:** Referenced in thousands of papers.

Sequencing Grade Modified Trypsin

Sequencing Grade Modified Trypsin is porcine trypsin modified by reductive methylation, rendering it resistant to proteolytic digestion. In enzymatic stability tests, modified trypsin was found to retain greater than two times the activity of unmodified trypsin. Trypsin is often used for in-gel or in-solution digestion of proteins. The digested peptides are purified and concentrated; then they are analyzed by mass spectrometry to determine their molecular weights.

- **Pure:** TPCK treatment followed by affinity purification.
- **Stable:** Ensured stability for up to five freeze-thaw cycles, which minimizes wasted reagents.
- **Reliable and Customer-Proven:** Referenced in thousands of papers.
- **Flexible:** Choose the format that is best for your experimental design and scope.

Immobilized Trypsin

Immobilized Trypsin provides a fast and convenient method for digesting a range of concentrations of purified protein or complex protein mixtures. Immobilized Trypsin is easily removed from the digested peptide solution using a spin column because the trypsin does not pass though the column frit.

- **Fast:** Digestions can be accomplished in as little as 30 minutes.
- **Scalable:** Easily adjustable protocol to accommodate various protein concentrations.
- **Easy-to-Use:** No shaking or water baths necessary.

Ordering Information

Product	Size	Cat.#
Trypsin Platinum, Mass Spec Grade	100µg	VA9000
Rapid Trypsin	100µg	VA1060
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Sequencing Grade Modified Trypsin	100µg	V5117
	100µg (5 × 20µg)	V5111
Sequencing Grade Modified Trypsin (Frozen)	100µg (5 × 20µg)	V5113
Immobilized Trypsin	2ml	V9012

For Research Use Only. Not for Use in Diagnostic Procedures.

Learn more about our complete offering of mass spectrometry reagents at:

www.promega.com/sampleprep

1. Fang, P. *et al.* (2015) Controlling nonspecific trypsin cleavages in LC-MS/MS-based shotgun proteomics using optimized experimental conditions. *Analyst* **140(22),** 7613–21.

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