Introduction
In proteomics research, trypsin is commonly used for protein digestion to produce peptides with molecular masses in the optimal range for MS analysis. Tryptic peptides contain either arginine or lysine at the C-terminus. Because of the basicity of the arginine side-chain, these peptides undergo preferential ionization and are therefore more efficiently detected in MALDI-TOF MS. To reduce this bias and enhance overall ionization, lysine residues can be guanidinated to convert the ε-amine side-chain to a homoarginine group as shown in Figure 1. Following guanidination, increased MS peak intensity is observed for lysine-containing peptides. This results in enhanced ability to identify proteins by providing a larger number of candidates for peptide mass fingerprinting.

Optimized guanidination method
The ProteoMass Guanidination Kit (Product Code MS0100) provides a complete set of reagents for quick and efficient guanidination. This includes a base reagent, O-methylisourea, a stop solution and a control peptide. The reaction scheme is shown in Figure 2. For this method, the researcher has only to provide a tryptically digested protein sample. This sample can be a protein that has been digested from a 1-D or 2-D gel piece or a complex tryptic digest, e.g. from a cell extract. To enable the guanidination reaction, a base reagent (ammonium hydroxide) is first added to the digest to ensure the reaction pH is optimal. The guanidination reagent (O-methylisourea) is then added and the mixture is incubated at 65 °C for 30 minutes. Finally, the reaction is terminated by the addition of a stop solution (trifluoroacetic acid). The sample is then ready to be analyzed by MALDI-TOF MS. A control peptide is supplied in the kit for verification of the reaction. Compared to traditional procedures for guanidination that require at least two hours, the ProteoMass Guanidination Kit provides a fast yet efficient method of peptide modification.

Specificity is an important aspect to the guanidination method. O-Methylisourea is considered specific for modification of the ε-amine of lysine residues. This reaction, however, may also occur at N-terminal amines, primarily at glycine residues. The procedure and reagents provided in this kit maximize reaction at lysine residues and minimize the reaction at N-terminal glycines. The specificity of our guanidination procedure provides an additional level of confidence when performing database searches for modified peptides.

Increased sensitivity and sequence coverage
Guanidination results in a theoretical monoisotopic mass increase of 42.0218 Da (C,H,N) for lysine-containing peptides. This is demonstrated in the MALDI-TOF mass spectrum of the control peptide in Figure 3. This spectrum represents an equimolar mixture of the peptide before and after guanidination. The mass of the peptide increased by 42 Da following guanidination. More importantly, the signal-to-noise ratio improved for the guanidinated peak by a factor of 2.75. This demonstrates the ability of the guanidination event to enhance ionization of the modified peptide and consequently improve the level of detection.

Application Notes
- Identify more proteins with greater accuracy and confidence
- Increase throughput and save time – only 30 minutes for complete guanidination
- Compatibility – for use with tryptic digests of 1-D or 2-D PAGE bands as well as complex protein samples
The ProteoMass Guanidination Kit has been used to successfully increase sequence coverage for a variety of proteins. The sequence coverage for a number of model proteins before and after guanidination is shown in Figure 4. In some cases the improvement in sequence coverage is modest; however, in other cases, guanidination was critical for protein identification. The guanidination method is therefore a proven and indispensable tool to the researcher for confident protein identification.

Convenience with performance
The ProteoMass Guanidination Kit provides a convenient format for fast and easy modification of peptide samples. No weighing of reagents is required and one kit supplies sufficient reagents for up to 96 guanidination reactions. The ProteoMass Guanidination Kit is a complete set of reagents for peptide modification to facilitate enhanced analysis of proteins by MALDI-TOF MS, leading to the generation of improved proteomic data.

Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Unit</th>
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<tbody>
<tr>
<td>MS0100</td>
<td>ProteoMass™ Guanidination Kit</td>
<td>1 kit</td>
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Kit Components:
- O-Methylisourea,
- Base reagent (ammonium hydroxide),
- Stop solution (trifluoroacetic acid),
- Control Peptide