3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ProteoMass™ Guanidination Kit

Product Code **MS0100**
Store at Room Temperature

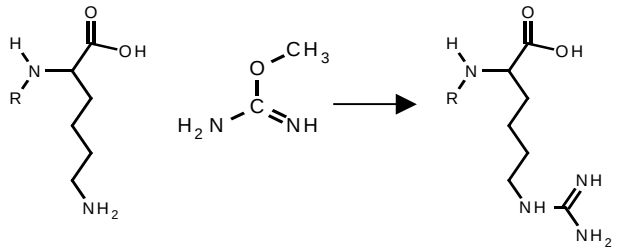
TECHNICAL BULLETIN

Product Description

The ProteoMass™ Guanidination Kit provides a complete set of reagents for guanidination of tryptic peptides. The results of this derivatization are enhanced signal and increased sequence coverage when analyzing samples by MALDI-TOF Mass Spectrometry (MS).

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 higher 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, the lysine-containing peptides can be guanidinated to convert the ε-amine side chain to a homoarginine group (see Figure 1).

Figure 1.
Guanidination Reaction



C-terminal lysine | O-Methylisourea | Homoarginine

O-Methylisourea is considered specific for the reaction at the ε-amine of lysine residues. However, the reaction may also occur at N-terminal amines, primarily at glycine residues. The procedure and reagents provided with this kit maximize the reaction at lysine residues and minimize the reaction at N-terminal glycines.

Guanidination results in a theoretical monoisotopic mass increase of 42.0218 Da ($C_1H_2N_2$) for lysine containing peptides. In the case of missed cleavages or if a proline residue adjacent to a lysine prevents cleavage, multiple guanidination events may occur on a single peptide.

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.

Components

Sufficient reagents are supplied to perform at least 96 guanidination reactions.

O-Methylisourea hemisulfate (Product Code 45,559-8)	8 vials
Base Reagent (Product Code B 1060) 2.85 M NH_4OH	5 ml
Stop Solution (Product Code S 0444) 10% TFA	10 ml
Control Peptide (Product Code C 1865)	1 vial

The Control Peptide contains one C-terminal glutamine peptide and one C-terminal lysine peptide.

LTGPSNQ ($[M+H]^+$ = 716.3579 Da)

TNEIVEEQYTPQSLATLESVFQELGK
($[M+H]^+$ = 2953.4682 Da)

Precautions and Disclaimer

This product is for laboratory research use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Guanidination Reagent - Add 0.6 ml of deionized water to one vial of O-Methylisourea hemisulfate (Product Code 45,559-8). Mix well to dissolve.

Control Peptide Solution - Prepare the solution by adding 100 µl of buffer to the vial of the Control Peptide (Product Code C 1865). An appropriate buffer would be one used for tryptic digestion (e.g. 10–50 mM ammonium bicarbonate, not supplied).

The Base Reagent and Stop Solution are supplied ready-to-use.

Storage/Stability
The kit should be stored at room temperature. The prepared Guanidination Reagent is stable for at least 2 weeks at room temperature. This reagent may be stored frozen for extended usage.

The Control Peptide Solution may be stored at 2–8 °C for up to two weeks, or in the freezer for at least one month.

Procedure
Sample Preparation
1) Prepare a sample of the proteins of interest using a solubilization reagent, such as the Protein Extraction Reagent Type 4 (Product Code C 0356), resulting in a sample solution with a pH greater than 9. Protein samples should have a concentration of approximately 5–10 mg/ml.
2) Reduce and alkylate the protein sample. This may be performed by using the ProteoPrep™ Reduction and Alkylation Kit (Product Code PROT-RA). Alternatively, reduce the protein sample using tributylphosphine (TBP, Product Code T 7567) at a final concentration of 5 mM with a 30 minute incubation at room temperature. Then, alkylate the sample by adding iodoacetamide (Product Code A 3221) to a final concentration of 15 mM with further incubation for 1 hour at room temperature.
3) Fractionate the protein sample by SDS-PAGE. Alternatively, reduce levels of denaturants and excess reagents by dilution, dialysis, or gel filtration. For these procedures, a buffer suitable for tryptic digestion such as 10 - 50 mM ammonium bicarbonate, pH 8.5 (Product Code A 6141) should be utilized.
4) Digest the protein sample with trypsin. It is recommended to use Proteomics Grade Trypsin (Product Code T 6567) following the directions supplied. For in-gel digestion of the protein sample, the Trypsin Profile IGD Kit (Product Code PP0100) is recommended. For solution digestion, a ratio between 1:100 to 1:20 (w/w) of trypsin to protein is recommended. The digest should be incubated at 37 °C for 2–18 hours.
5) If desired, the digestion reaction may be stopped by adjusting the reaction pH to < 3 with an appropriate acid (HCl).

Guanidination Procedure
Samples in the concentration range of 0.075–2 mg/ml of reduced, alkylated, and tryptically digested proteins are recommended for this guanidination procedure.

This procedure may be used to prepare the following three samples for MALDI-TOF MS analysis:
(1) Guanidinated sample (G)
(2) Unguanidinated sample for comparison (U)
(3) Guanidinated control peptide for verification of the reaction (C)

If desired, a sample of unguanidinated control peptide can be analyzed by following a similar procedure to the unguandinated sample (U).

1. Pipette 10 µl of the tryptic peptide sample into two separate tubes, labeled G and U.
2. To a third tube labeled C, pipette 10 µl of the Control Peptide Solution.
3. To ensure that the pH is optimal for the guanidination reaction, pipette 10 µl of the Base Reagent (Product Code B 1060) into each of the three tubes. Mix well by vortexing.
4. To the tubes labeled G and C, add 10 µl of the Guanidination Reagent. Mix well by vortexing.
5. To the tube labeled U, add 10 µl of deionized water. Mix well by vortexing.
6. Incubate all three tubes for 30 minutes at 65 °C in a water bath or heat block.
7. To acidify and stop the guanidination reaction, add 30–60 µl of the Stop Solution (Product Code S 0444) to each tube. Mix well by vortexing.
8. Store samples at 2–8 °C until ready for analysis by MALDI-TOF MS.
9. For an optimal signal to noise ratio, remove excess reagents by purifying the guanidinated peptides with a reverse phase microextraction device such as a ZipTip® pipette tip. This step is optional.
10. Analyze sample by MALDI-TOF MS.

Results

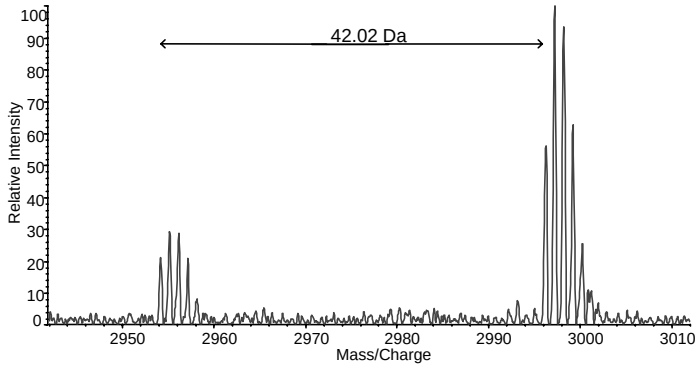
The Control Peptide contains one C-terminal lysine peptide and one C-terminal glutamine peptide. Following guanidination, the lysine containing peptide will have a theoretical monoisotopic mass increase of 42.0218 Da ($C_1H_2N_2$).

The peptides are as follows:

Sequence	**$[M+H]^+$ (Monoisotopic)**
Prior to guanidination	
LTGPSNQ	716.3579 Da
TNEIVEEQYTPQSLATLESVFQELGK	2953.4682 Da
Following guanidination	
LTGPSNQ	716.3579 Da
TNEIVEEQYTPQSLATLESVFQELG**K***	2995.4900 Da

The MALDI-TOF mass spectrum of the control peptide is shown in Figure 2. This spectrum represents an equimolar mixture of the peptide before and after guanidination. The mass of the guanidinated peptide is increased by 42 Da, and the signal to noise ratio increases for the guanidinated peak by a factor of 2.75.

Figure 2.
MALDI-TOF mass spectrum of the control peptide TNEIVEEQYTPQSLATLESVFQELGK.



This spectrum represents a 1:1 mixture of guanidinated and unguanidinated peptide.

References

1. Brancia, F.L. et al., Improved matrix-assisted laser desorption/ionization mass spectrometric analysis of tryptic hydrolysates of proteins following guanidination of lysine-containing peptides. Rapid Commun. Mass Spectrom., **14**, 2070-2073 (2000).
2. Hale, J. E. et al., Increased Sensitivity of Tryptic Peptide Detection by MALDI-TOF Mass Spectrometry is Achieved by Conversion of Lysine to Homoarginine. Anal. Biochem., **287**, 110-117 (2000).
3. Beardsley, R.L., and Reilly, J.P., Optimization of Guanidination Procedures for MALDI Mass Mapping. Anal. Chem., **74**, 1884-1890 (2002).

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