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INSTRUCTIONS

PepFrag™ MALDI Sequencing kit

Product Number **P 5011**

INTRODUCTION

Mass spectrometry is becoming more useful tool for *de novo* sequencing of peptides due to the speed and greater sensitivity provided by this method. Peptide mass fingerprinting (PMF) using MALDI-TOF spectrometry has become a major tool for identifying proteins in proteomics research. However, peptide sequence analyses from one or more peptides, provides amino acid sequence information for more unambiguous identification[1] and informative characterization of post-translational modification(PTM) of proteins (eg. protein phosphorylation)[2].
The major drawback of mass spectrometry in this context is the complexity of the obtained tandem mass spectra, due to the various types of ions present, and thus resulting in the time-consuming and ambiguous interpretation. However, the use of sulfonation reagent on N-terminal amino group prior to mass spectrometric analysis has shown to diminish this problem in ESI and MALDI-MS[3,4]. Moreover, this modification greatly improve the fragmentation efficiency and generate major y-type ions then facilitating *de novo* sequencing of peptides using MALDI-PSD and enabling straightforward interpretation of spectrum.
This PepFrag ™ MALDI Sequencing kit provides the optimized procedures and reagents for *de novo* sequencing of peptides and phosphorylation site determination of protein in conjunction with the use of Phos-Pep™ Phosphopeptide Enrichment Kit.

(Technical bulletin http:// www.genomine.com)

Kit contents **50 Reactions**

Guanidination Reagent (O-methylisoureahydrogen sulfate salt solution)	0.4ml
Guanidination Buffer (Ammonium hydroxide solution)	1ml
Sulfonation Reagent (4-Sulfophenyl isothiocyanate)	2 × 2mg
Sulfonation Buffer	1ml

Additional Materials Required

- C18 microtip (eg ZipTip milipore)
- Ultrapure water

Preparation of Materials

Guanidination Solution
Mix Guanidination Reagent to Guanidination buffer ratio of 1:2 just prior to use.
Sulfonation solution
Dissolve 2mg Sulfonation Reagent with 1ml Sulfonation Buffer and divide into aliquot.

Storage: Upon dissolving the sulfonation reagent with sulfonation buffer, store the aliquot of this solution at -20°C; store other reagents at RT

Procedure Summary

1. In-gel guanidination (optional)
2. Trypsin digestion
3. Sulfonation
4. Cleaning of modified peptides

In gel guanidination of lysine side chains

1. Wash gel slice twice with deionized water

2. Add 10~15µl Guanidination Solution(mixture of Guanidination reagent and Guanidination buffer)
3. Incubate the gel slice for 15min at 60°C
4. Remove Guanidination solution (if necessary, destain the Coomassie or silver staine at this step) and wash the gel slice with 100µl of deionized water twice for 10min.
5. Add acetonitrile for dehydration of gel slice and follow user's own protocol for trypsin digestion.

Comments: Guanidination process could be omitted if you would like to perform pH controlled sulfonylation on α-amino group without protection of ε-amino group of lysine side chain.

Trypsin digestion

Alternatively, following trypsin digestion condition will be recommended for subsequent optimum sulfonization reaction.

1. Add 50 µl of acetonitrile to dehydrate the gel pieces. After 10-15 min remove the solvent and dry the gel slices in a rotatory evaporator.
2. Re-swell the gel pieces with 5 microliters of 50 mM ammonium bicarbonate containing sequencing grade trypsin (Promega modified) at a concentration such that a substrate to enzyme ratio of 10:1 has been achieved. After 5-10 minutes, add 15 microliters of additional ammonium bicarbonate buffer to cover the gel pieces.
3. Incubate 6 hrs to overnight at 37°C. (During incubation, the gel piece must be wet)

Sulfonation of N-terminal α-amino group of trypsin digest(pH controlled sulfonation)

1. Add 3ul sulfonation solution to 10µl trypsin digest and mix well with brief vortexing for a few seconds.
2. Incubate the reaction mixture at 55°C for 30 min.
3. Load the peptide solution onto C18 microcolumn (eg.ZipTip C18) and wash the column with deionized water then elute peptides with 50% Acetonitrile/0.2% TFA for mass spectrometry. (Alternatively elute the peptide with appropriate matrix solution containing 50% acetonitrile/0.2%TFA directly onto the target plate for MALDI-TOF mass analysis)

Optimization of Results

For phosphorylation site determination, when peptide solution contains high salt, dilute the solution below 100mM of salt prior to enrichment (using PhosPep™) of phosphopeptides to obtain the better result.

References

1.Thomas, K. et al, Electrophoresis,21,2252-2265 (2000)

2. Application Note
www.genomine.com/

3. Gevaert K. et al, Electrophoresis, 22, 1645-1651 (2001)

4. Yong Ho,K. et al, Proteomics, 4, 1684-1694

Related Products Product Code
PhosPep Phosphopeptide Enrichment kit P5010

PhosPro Phosphoprotein enrichment kit P5012