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INNOVATIVE SAMPLE PREPARATION TECHNOLOGY ENABLING HIGH SENSITIVE MALDI MS ANALYSIS

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INTRODUCTION

This work presents a simple and robust sample preparation technology for MALDI-MS analysis by using a novel MALDI target plate, the Waters® Micromass® MassPREP™ PROtarget™ Plate. The PROtarget Plate is a uniquely-designed MALDI target plate that enables sample preparations directly on the target. A large volume (up to 10 µL) of diluted sample is placed on the target plate and dries down with focusing to a confined region coated by a membrane having peptide binding property. The region is then subsequently washed to remove contaminants that are contained in sample and may interfere with MALDI analysis, whereas analytes are selectively adsorbed onto the membrane and enriched.

The poster will demonstrate that:

- Significant concentration of very dilute sample can be achieved by applying an increased sample volume to the MassPREP PROtarget Plate.
- Contaminated samples can be directly analyzed by a MALDI mass spectrometer after on-target cleanup, and overall sensitivity is not affected by the cleanup.
- Micro-scale chemical reactions can be performed *in-situ* on the MALDI target plate.
- The sample preparation of protein digests coming from in-gel digestion of 2D electrophoresis gel spots has significantly improved the sensitivity.

EXPERIMENTAL

1. Description of the Target Plate

   On a regular stainless steel plate, a thin layer of Teflon® is coated

   A sample well of 0.6 mm is created on the Teflon (Figure 1)

   The sample well is then coated by a thin film of a hydrophobic polymer that has peptide/protein binding properties

2. Sample Preparation Method

   Sample solutions are prepared in 30% acetonitrile (v/v), 0.1% TFA (v/v)

   Deposit up to 10 µL of sample (Figure 2), wait for sample to dry completely

   Add 5 µL of washing solution (0.1% TFA) and wait 3 min, remove washing solution, and repeat this step two more times

   Wait for sample to dry completely

   Add 1 µL matrix solution (CHCA 0.5 mg/ml, 90% ACN, 0.1% TFA (v/v))

   Wait for matrix to dry and analyze by MALDI-TOF MS (Waters Micromass MALDI-R)

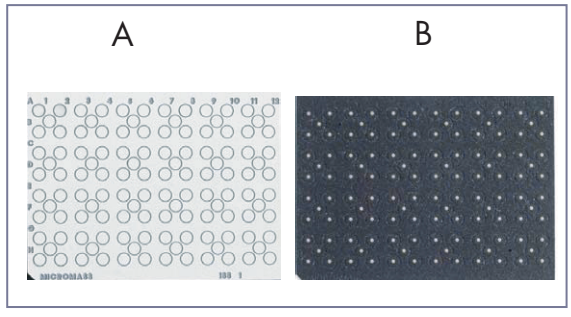

Figure 1. Standard stainless steel plate (A) and MassPREP PROtarget Plate (B)

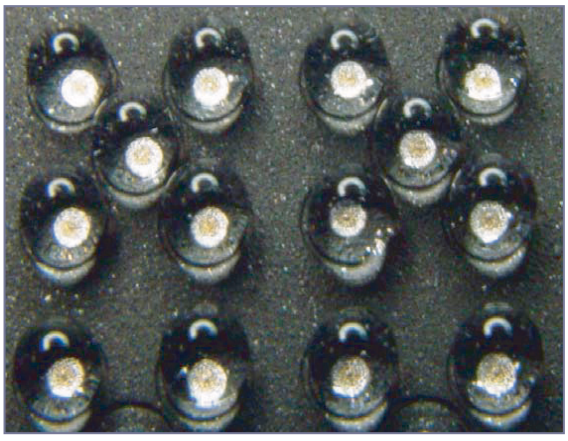

Figure 2. The sample volume capacity on MassPREP PROtarget Plate. The volume of each droplet shown in the picture is 10 µL.

RESULTS

1. Limit of Detection and Sample Focusing of MassPREP PROtarget Plate

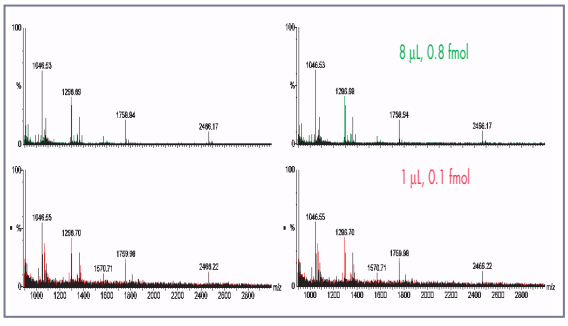

Figure 3. The sample focusing effects and limit of detection of MassPREP PROtarget Plate. Different aliquots of the same peptide mixture were added to the target. The concentration of applied sample was 0.1 fmol/µL.

2. Removal of SDS (0.1%) Contained in Peptide Samples

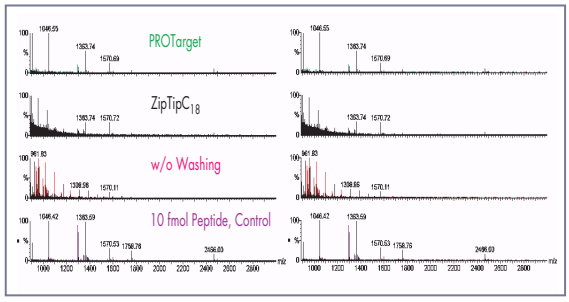

Figure 4. Examples of MALDI spectra from samples processed by MassPREP PROtarget Plate and ZipTip™ C_{18}. The peptide standard mixture solution (10 fmol/µL) contains 0.1% SDS.

3. Guanidination and Purification of Tryptic Peptides

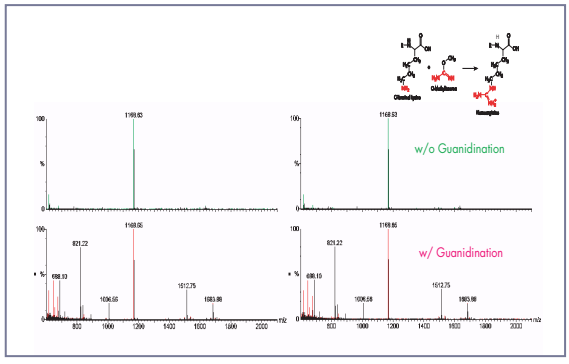

Figure 5. Mass spectra from 300 fmol Cytochrome C tryptic digest without guanidination and with guanidination. The guanidination reaction mixture was prepared according to a published method[1], and was directly applied to the MassPREP PROtarget Plate. Upon drying, the sample was washed with 0.1% TFA three times to remove excess amount of o-methylisourea (0.5 M) in the reaction mixture.

4. Identifications of Unknown Yeast Cytosol Proteins Separated by 2D Gel Electrophoresis

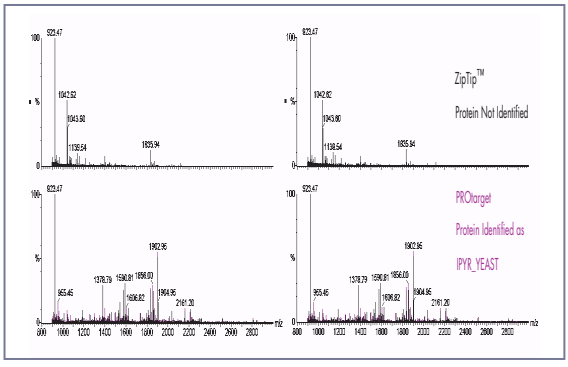

Figure 6. MALDI spectra from one of excised gel spots(#55). A number of spots (circled in the 2D map) were excised and digested using the Waters Micromass MassPREP™ Station, giving 25 µL of extract. For comparison, same volume (5 µL) of extract was processed, in parallel, using the MassPREP PROtarget Plate or ZipTip™.

CONCLUSIONS

- A novel target plate, the MassPREP PROtarget Plate, has been developed to allow the whole sample preparation procedure to be directly performed on the MALDI target plate, thus greatly reducing sample losses in the sample preparation process. The unique design of the MassPREP target allows both desalting and concentrating of applied samples accomplished on the target.
- The MassPREP PROtarget Plate significantly enhances the sensitivity of the MALDI-TOF-MS analysis allowing limits of detection in the sub-femtomole range.
- The MassPREP PROtarget Plate is robust and very simple to use. With a robotic liquid handling system, the whole procedure is amenable to automation.
- The peptide/protein-binding surface of the MassPREP PROtarget Plate can be regenerated for repeated usage.

REFERENCE

1. Beardsley, R. and Reilly, J. *Anal. Chem.* 74, 1884-1890 (2002)

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