

Protein Identification: The PROTEINEER™ Strategy

INTRODUCTION

Proteomics projects frequently include an initial screening step that aims to identify large numbers of proteins. Here, sample throughput and success rate (i.e. the number of positively identified proteins) are key issues. The task typically is performed via automated MALDI-TOF MS peptide mapping, utilising ABgene® Protein Purification Plates to digest and extract relevant protein samples. From sample to knowledge, the PROTEINEER™ system from Bruker Daltonics comprises an integrated hardware and software toolbox. The system automates sample processing from the 2D gel through to successful protein identification and characterisation.



GEL PLUG SELECTION

2D gel imaging, spot detection and spot selection are performed by the Bruker PROTEINEER™ spill robot. Gel pieces are identified manually or automatically using spotlists generated from an image analysis package, and then cut out and deposited into ABgene® Protein Purification Plates.

DIGESTION AND PREPARATION

Proteolytic fragments for mass spectrometric analysis are generated by Bruker's PROTEINEER™ dp (digest & prep) robotic workstation by "in-gel digestion" of the gel plugs in the ABgene® Protein Purification Plate. Designed for quick and easy digestion/extraction from SDS-PAGE gels, the 96-well plate features two 250µm laser-cut holes at the base of each conical well. The wells are designed to retain liquids dispensed into the wells during incubation steps, enabling sequential de-staining, proteolytic digestion and shrinkage of the gel plugs. During this procedure, liquids are filtered to waste at the appropriate times by applying positive air pressure from the top of each well (plates can be processed using vacuum or centrifuge). No manual interaction is required for the subsequent sample preparation for mass spectrometric analysis: after the digest step, samples are prepared directly onto

SCOUT MTP or AnchorChip MALDI targets. The new Prespotted AnchorChip targets provide guaranteed performance on a single-use MALDI target with pre-spotted matrix and prepared with calibrants. The dp workstation supports various preparation procedures and clean-up steps. For electro-spray ionization-MS analysis, a fraction collector can collect samples from the 96-well plate.



FINGERPRINT SCREENING

Following digestion and preparation, MALDI-TOF peptide mapping is performed on the FLEX series of MALDI-TOF mass spectrometers in a fully automated procedure. All acquisition parameters are kept in one central spreadsheet, together with sample preparation parameters and search results. A crucial factor for highest success rate in protein identification is the quality (most important: sensitivity) of MALDI-TOF data. As the ABgene® Protein Purification Plate eliminates the need for manual pipetting

Dr. Simon May¹, Dr. Holger Nagel²



from the wells (pure protein is automatically eluted through the 250µm holes), it facilitates consistent and reproducible peptide digests, helping to ensure maximum sensitivity as provided by the FLEX series MALDI-TOF/TOF MS.

MS/MS SCREENING

Samples which cannot be analysed sufficiently using the mass fingerprint can be examined further using MALDI-TOF/TOF MS. For this optional second screening, the same prepared samples can be used. Identifications can be obtained from the fragment ion spectrum (MS/MS) of selected precursors, again in a fully automated mode.

DETAILED CHARACTERISATION

For in-depth analysis, samples can be analysed by nano liquid chromatography coupled with electro-spray ionization tandem MS/MS on the Bruker Daltonics esquire or HCT ion trap, coupled to an Ultimate nanoLC (LC Packings) or an alternative nanoLC/ CapLC system. This provides an extraordinary sensitivity (low to sub-fmole range) and information content.

CONCLUSION

The PROTEINEER™ system from Bruker Daltonics provides a convenient, reproducible and accurate solution for expression proteomics. Together with the ABgene® Protein Purification Plate, the system enables end-users to isolate, purify and identify large numbers of proteins. Hands-on time is minimised with the use of automated protocols throughout, while knowledge generation and validation is achieved by sophisticated bioinformatics tools like BioTools™ and ProteinScape™.

CAT. NO.	DESCRIPTION	QUANTITY
AB-1151/C	Protein Purification Plate (centrifuge-compatible)	25 plates
AB-1151/V	Protein Purification Plate (vacuum-compatible)	25 plates
AB-0765	0.8ml Storage Plate (for sample collection)	50 plates
For further information, please visit www.abgene.com .		

¹Business Development Manager, ABgene® ²Director CARE Products, Bruker Daltonics®, Fahrenheitstrasse 4, D-28359 Bremen, Germany