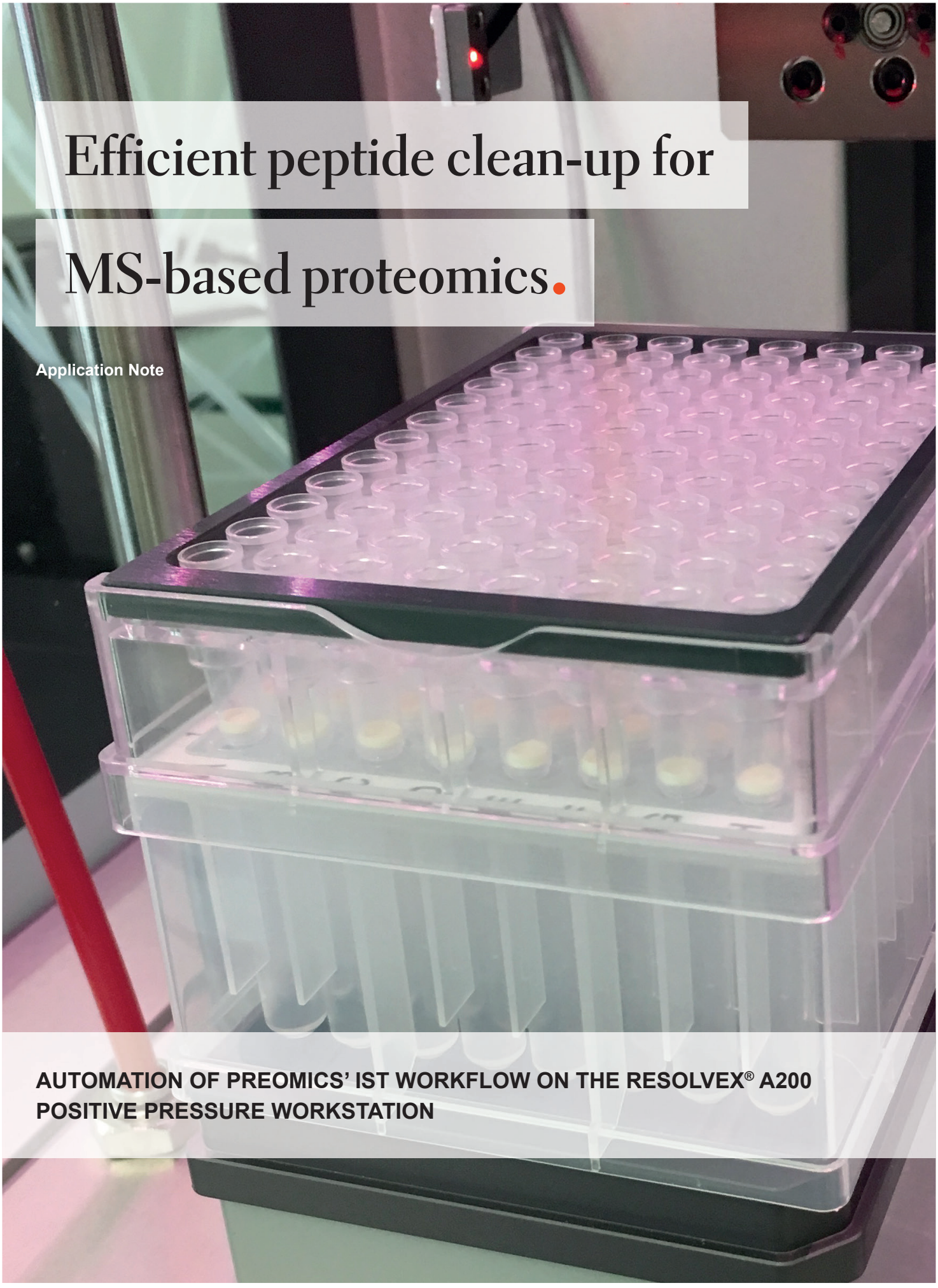
Efficient peptide clean-up for MS-based proteomics.

Application Note



AUTOMATION OF PREOMICS' IST WORKFLOW ON THE RESOLVEX® A200 POSITIVE PRESSURE WORKSTATION

INTRODUCTION

Mass spectrometry (MS) has emerged as the leading technology for the identification and quantification of protein populations in numerous sample types. This technology has become much faster and increasingly sensitive over the last decade, broadening the scope of the research questions that it can address. Process speed and efficiency have created a demand for simplified sample preparation approaches, leading to the development of off-the-shelf sample preparation kits, such as PreOmics' iST technology.

Many labs still do not have the throughput to justify investing in a full, end-to-end automation system to perform automated sample preparation and clean-up, which has remained a largely manual process, making it a tedious and time-consuming task. This application note assesses the suitability of the Resolvex A200, together with the PreOmics iST Kit, as an efficient alternative for automated sample clean-up.

MATERIALS AND METHODS

Sample preparation for LC-MS analysis

HEK 293 cells were cultivated in high glucose DMEM, supplemented with 10 % FCS and 1 % penicillin-streptomycin (all Gibco). Cells were collected by scraping, washed twice with PBS by centrifugation, lysed in LYSE buffer (PreOmics) using strong ultrasonication (20 cycles, Bioruptor®, Diagnode), and incubated at 95 °C for 10 min. Protein concentrations were then determined by BCA assay (Thermo Fisher Scientific), normalized to a concentration of 1 mg/ml, then digested by incubation with DIGEST buffer (PreOmics) at 37 °C for 12 hours. Following digestion, samples were acidified by the addition of STOP buffer (PreOmics), split in 10 aliquots and purified using iST cartridges (PreOmics) in the following manner; 5 aliquots were prepared following the manufacturer's instructions using a fixed-angle centrifuge (Eppendorf) and 5 were prepared using the Tecan Resolvex A200 system. Finally, peptides were dried under vacuum and stored at -20 °C until further use.

LC-MS/MS analysis

Peptides were resuspended in 0.1 % aqueous formic acid solution, and 0.35 µg of peptides were analyzed by LC-MS/MS using an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer fitted with an EASY-nLC™ 1200 (both Thermo Fisher Scientific) and a custom-made column heater set to 60 °C. Peptides were resolved using a RP-HPLC column (75 µm × 36 cm) packed in-house with C18 resin (ReproSil-Pur® C18–AQ, 1.9 µm resin; Dr. Maisch) using a gradient separation approach with 0.1 % formic acid in water (A) and 80 % acetonitrile + 0.1 % formic acid in water (B), and a flow rate of 0.2 µl/min. The following gradient was used: from 5 % B to 12 % B over 5 min, to 35 % B over 65 min, to 50 % B over 20 min, to 95 % B over 2 min, followed by 18 min at 95 % B.

The mass spectrometer was operated in DDA mode, with a cycle time of 3 seconds. The data acquisition mode was set to obtain one high resolution MS scan in the Orbitrap at a resolution of 240,000 full width at half maximum (at 200 m/z, MS1), and a scan range from 375 to 1,600 m/z followed by MS/MS (MS2) scans in the linear ion trap at 'Rapid' scan rate. Maximum ion injection time was set to 50 ms (MS1) and 35 ms (MS2), with an AGC target of $1.0e^6$ and $1.0e^4$ respectively. Only peptides with charge state 2 to 5 were included in the analysis. The collision energy was set to 35 %, and one microscan was acquired for each spectrum. MIPS was set to 'Peptide', intensity threshold was set to $5.0e^3$ and the dynamic exclusion duration was set to 30 seconds.

Data analysis

The raw data was imported into the Progenesis QI software (v2.0, Nonlinear Dynamics) to extract peptide precursor ion intensities across all samples, by applying the default parameters. The resulting MGF files were searched against a forward and reverse decoy database of the predicted proteome from Homo sapiens (download date: 18/3/19, total of 41,616 entries) using MASCOT (v2.4.1) with the following search criteria: full tryptic specificity was required (cleavage after lysine or arginine residues, unless followed by proline); 3 missed cleavages were allowed; carbamidomethylation (C) was set as fixed modification; oxidation (M) and acetylation (protein N-term) were applied as variable modifications; mass tolerance of 10 ppm (precursor) and 0.6 Da (fragments).

Results from the database search were imported into Progenesis QI, and the final peptide measurement list containing the peak areas of all identified peptides was exported. The database search results were filtered using the ion score to set the false discovery rate to 1 % on both the peptide and protein level, based on the number of reverse protein sequence hits in the datasets. The relative quantitative data obtained was normalized and statistically analyzed using an in-house script (SafeQuant, PMID: 27345528).

Additionally, the raw files were converted to MGF using MSconvert (Proteowizard) and searched directly using MASCOT (v2.4.1), applying the parameters described above. The resulting identifications were converted to SF3 format, and analyzed using Scaffold (Proteome Software).

RESULTS

As shown in Figure 1, the results obtained from samples cleaned up using the Resolvex A200 are fully comparable to the data obtained by following the PreOmics protocol using a fixed-angle centrifuge (Eppendorf). The number of peptides and proteins identified is nearly identical, demonstrating very reproducible peptide clean-up with both approaches. Median CVs for both peptide and protein quantifications are even slightly lower using the Resolvex A200. In summary, preparation using the Resolvex A200 is fully compatible with PreOmics' iST Kits, offering improved reproducibility, increased scalability and greater convenience.

CONCLUSIONS

The peptide clean-up step of the PreOmics' iST Kit protocol can be performed seamlessly on the Resolvex A200 system with the pressure profiles provided. This maximizes workflow reproducibility and robustness, reducing processing times and enhancing analytical performance.

LEARN MORE

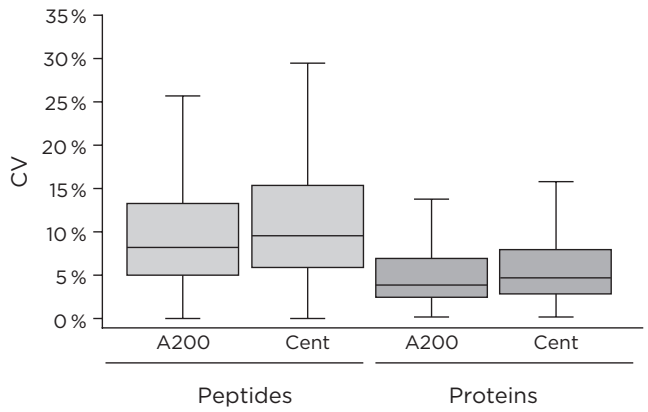
To learn more about Tecan's mass spectrometry sample preparation solutions, contact your sales representative or visit **www.tecan.com/resolvex**

To find out about PreOmics' range of innovative tools for mass spectrometry-based protein analysis, go to **www.preomics.com**

A

Analyte	A200	Centrifuge
Pep median CV (%)	8.22	9.61
Prot median CV (%)	3.99	4.64
PSM	58,226	58,242
Peptides	49,731	49,740
Proteins	5,246	5,247

B



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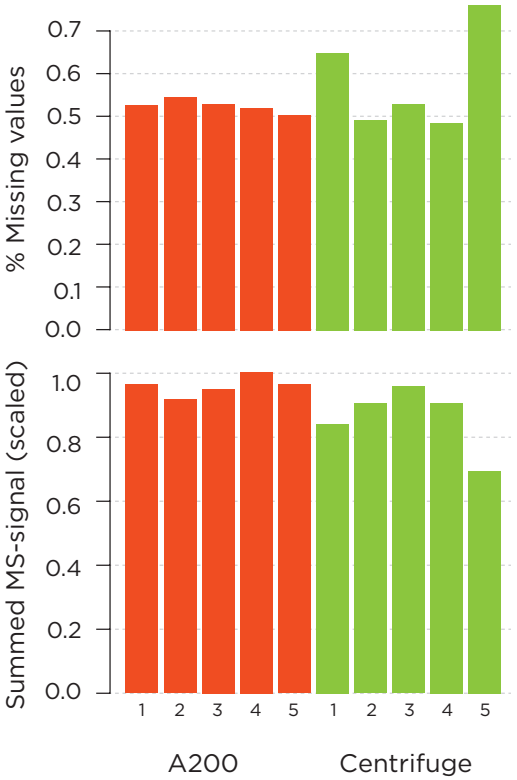

Figure 1: A/B) Results obtained using the Resolvex A200 or the standard centrifugation protocol are fully comparable, with the Resolvex A200 providing slightly lower CVs for peptide and protein quantification. C) % missing values are similar for both preparation methods, with the Resolvex A200 providing slightly better values for the summed MS-signals (PSM: peptide spectrum matches, Cent.: Centrifuge).

About the authors

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Dr Danilo Ritz joined the Proteomics Core Facility of the Biozentrum, University of Basel, as a staff scientist in 2018. He studied molecular biology at the University of Basel, and focused on the analysis of protein interaction networks using mass spectrometry during his PhD at the ETH Zürich. He then worked as a scientist in the biotechnology sector, applying MS-based techniques to the field of personalized medicine.

Manuel Bauer, Tecan Group

Dr Manuel Bauer joined Tecan Switzerland in 2013, and is Senior Market and Product Manager for the company's automated MS sample preparation offerings and the D300e Digital Dispenser. He studied biology at the University of Würzburg and the Free University of Berlin. Throughout his PhD at the ETH Zürich, he focused on systems biology, and used various MS-based proteomics techniques during his postdoc at the Biozentrum, University of Basel.

Fabian Hosp, PreOmics GmbH

Dr Fabian Hosp joined PreOmics in 2017, and is Head of Applications for PreOmics' automated sample preparation technologies. He studied molecular biology at the University of Heidelberg, and developed quantitative MS-based protein interaction screens during his PhD at the Max Delbrück Center for Molecular Medicine in Berlin. In his postdoc at the Max Planck Institute of Biochemistry, Martinsried, he employed MS-based proteomics for high throughput applications in the context of neurodegenerative diseases.

Alexander Schmidt, Biozentrum, University of Basel

Dr Alexander Schmidt joined the Biozenturm, University of Basel, in 2009, and is heading the Proteomics Core Facility. He studied analytical chemistry at the Friedrich-Alexander-University of Erlangen, and developed a quantitative proteomics isotope labeling approach during his PhD at the Max-Planck Institute of Biochemistry, Martinsried. In his postdoc at the ETH Zürich, he developed sensitive, targeted MS approaches, and applied them to several biological projects including diabetes, cancer and systems biology research.

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