Scaling-up low input spatial proteomics using Evosep Eno on the timsUltra AIP

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Introduction

Characterizing the phenotypic diversity of cells derived from heterogeneous tissues at the resolution of individual cells requires analyzing a high number of samples to distinguish cell types and subtypes effectively. Advances in ultra-high sensitivity MS combined with miniaturized sample preparation and fast chromatography enables proteome depth and sample throughput, crucial factors for large clinical cohort studies. Here, we assess performance, scalability, and reproducibility of cellenONE based sample preparation, fast standardized peptide separation with the Evosep Eno system coupled to ultra-high sensitivity mass spectrometry on the timsUltra AIP.

Methods

K562 digest dilutions, single FaDu cells (Head and Neck squamous cell carcinoma), single HeLa cells (cervical cancer), and laser micro-dissected human FFPE tonsil tissue of T-cell, B-cell, and squamous cell epithelium were benchmarked. K562 digest was loaded on Evotips at 250 pg to 50 ng. FaDu and HeLa cells were isolated into single and 10 cell mini-bulks in proteoCHIPs EVO96 followed by lysis and digestion using cellenONE X1 Neo. Human FFPE tonsil tissue (30,000 µm²) was collected into proteoCHIPs EVO96 and prepared using cellenONE X1. Sample acquisition in dia-PASEF mode was performed on timsUltra AIP at 500, 300, 200, 100, 60 and 30 SPD and Whisper Zoom 120, 80, and 40 SPD. Data analysis was performed in directDIA using Spectronaut 21.

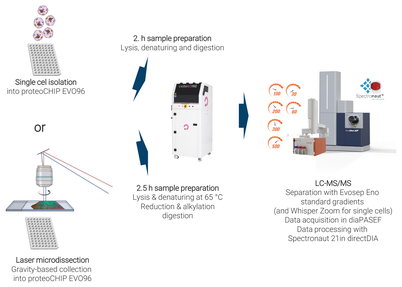
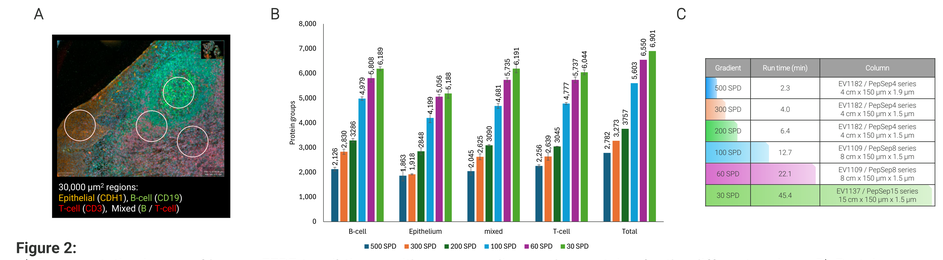


Figure 1:
Single cell and deep visual proteomics workflow representation from either single cell isolation into the prpteoCHIP EVO96 or region of interest selection of immunofluorescence characterized human FFPE tonsil tissue, laser microdissection (LMD) and gravity-based sample capture into proteoCHIPs EVO96, tissue lysis and digestion inside the cellenONE dispensing robot, sample cleanup and transfer onto Evotips Pure, LC-MS/MS based acquisition with peptide separation on Evosep Eno 500, 300, 200, 100, 60, 30 SPD standard gradients and peptide detection in dia-PASEF mode on a timsUltra AIP, and data processing in directDIA using Spectronaut 21.

Results



Gradient	Run time (min)	Column
500 SPD	2.3	EV1182 / PepSep4 series 4 cm x 150 µm x 1.9 µm
300 SPD	4.0	EV1182 / PepSep4 series 4 cm x 150 µm x 1.5 µm
200 SPD	6.4	EV1182 / PepSep4 series 4 cm x 150 µm x 1.5 µm
100 SPD	12.7	EV1109 / PepSep8 series 8 cm x 150 µm x 1.5 µm
60 SPD	22.1	EV1109 / PepSep8 series 8 cm x 150 µm x 1.5 µm
30 SPD	45.4	EV1137 / PepSep15 series 15 cm x 150 µm x 1.5 µm

Figure 2:
A) Representative image of human FFPE tonsil tissue with corresponding marker proteins for the different regions. B) Protein group identification rates across the different tissue areas (30k µm² each) run at 500 – 30 SPD using Evosep Eno processed with Spectronaut 21 in directDIA per gradient speed. C) Table summarizing key Evosep Eno gradient properties.

Figure 3:
A) Pearson correlation matrix of protein abundance profiles of the same cell type niche samples across all 6 Evosep Eno standard gradients.. B) Display of the first two components of PCAs performed per standard gradient; C) Display of the first two components of PCAs performed on all proteins identified in every sample across all six standard gradient; D) Display of the first two components of PCAs performed across all samples and gradients after data imputation using the downshifted normal distribution approach. E) Volcano plot representation of statistical analysis performed after data imputation by downshifted normal distribution per standard gradient comparing A) epithelial niche (orange) against T-cell niche (red) B) B-cell niche (light green) against T-cell niche (red) and C) epithelial (orange) niche against B-cell niche (light green). Cell type specific marker proteins highlighted; CD3D, CD3E, CD3G, CD4, CD5, CD8A, and CD99 used for T-cells, CD19, CD72, and CD79B used for B-cells and CDH1, Keratin 4, Keratin 13, and MUC21 used for epithelium. F) Box plot representation of log2(protein abundance) of CD3D, CD5 and CD19 across the 6 standard gradients for T-cell niche (red), B-cell/T-cell mixed niche (violet) and B-cell niche (light green); no data imputation.

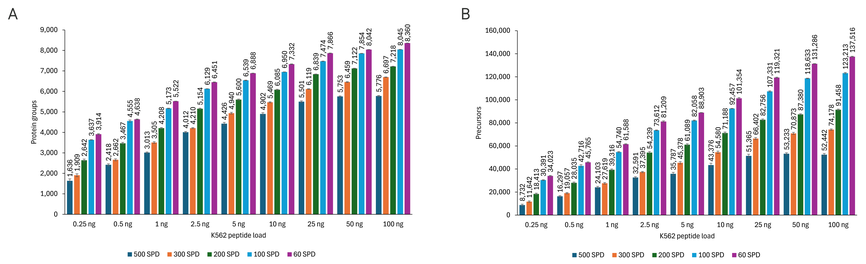


Figure 4:
Protein group (A) and precursor (B) identification rates of K562 digest dilution series from 100 ng to 0.25 ng run at 500 to 60 SPD using an Evosep Eno and a timsUltra AIP processed with Spectronaut 21 in directDIA per gradient speed and load, no matching from higher loads. Excellent sensitivity was achieved for 250 pg loaded on Evotips and run at the various standard methods. 1,600 protein groups with 8,700 precursors were identified at 500 SPD increasing to 3,900 protein groups with 34,000 precursors at 60 SPD. The highest load (100 ng) identified at 500 SPD ~5,800 protein groups with ~53,000 precursors and ~8,400 proteins groups with 138,000 precursors at 60 SPD.

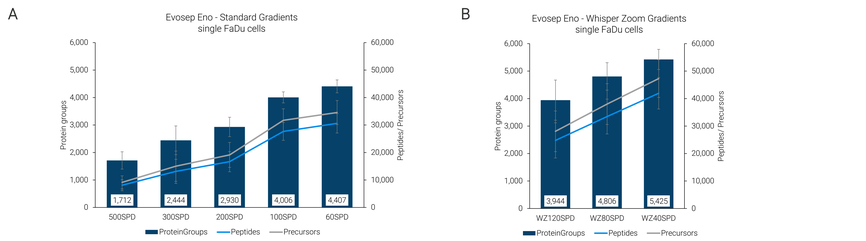


Figure 5:
A) Protein groups, peptide and precursor identification rates of single FaDu Head and neck squamous cell carcinoma (HNSCC) cells analyzed A) standard gradients at 500, 300, 200, 100 and 60 SPD and B) in Evosep Whisper Zoom 120, 80 and 40SPD using the Evosep Eno and a timsUltra AIP with data processing in Spectronaut 21 in directDIA per gradient speed

References

[1] P. Skowronek, Matthias Mann et al. Mol Cell Proteomics, 2022, 21, 9, 100279

Further reading

Application Note, Bruker Daltonics, LCMS-206, 1815135, 2023
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Conflict of Interest
CK, POS, DH are employees of Bruker;
AS was employee of Cellenion;
DBJ, NB are employees of Evosep.

Conclusion

- Accurate and consistent differentiation of epithelial and immune cell rich regions in human FFPE tonsil tissue (30,000 µm²) at all standard gradient speeds (**500 – 30 SPD**) on the **new Evosep Eno** on the **timsUltra AIP**
- timsUltra AIP enables deep proteome profiling from limited FFPE tissue material and preserving spatially distinct features even at 2.5 min run times, with cell type specific marker proteins detected at all gradient speeds
- Excellent proteome coverage from single FaDu cells at **Whisper Zoom 120 – 40 SPD** and **standard gradients at 500 – 60 SPD** on the **timsUltra AIP.**

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timsUltra AIP

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