# A complete and automated sample preparation strategy forhigh-throughput and standardized proteomics, applied to a clinical cohort of patient plasma samples

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Towards standardized workflows with integration of peptide loading on Evotips with a fully automated end-to-end protocol on the Opentrons platform starting from protein.



Highlights

Towards clinical proteomics by increasing throughput to 500 SPD.

## **Complete, and automated sample loading**

#### Simplified sample loading in one step

We have developed a simple and automation friendly protocol using the Opentrons OT-2 for sample loading on Evotips, which makes use of a layered sandwich approach with defined airgaps between the layers. By using the Opentrons-2 pipette to apply pressure a perfectly loaded Evotip is prepared every time ready for injection on the Evosep One.

## High-throughput plasma proteome profiling

#### **Opentrons deck layout for protein aggregation capture**

A magnetic rack and the Evotip loading module were used as special labware on the Opentrons for the end-to-end workflow based on PAC digestion. Hydroxyl beads (Resyn Biosciences) were used for aggregation. The total time to process 96 samples rom protein to peptides loaded on Evotips were 2.5 hours.



#### **Clinical applicability to patients with systemic sclerosis**

To demonstrate clinical applicability, we tested a sample cohort of 96 plasma samples collected from 48 patients diagnosed with the incurable autoimmune disease, systemic sclerosis, where diagnosis in an early phase is still difficult due to lack of symptoms, and compared these to plasma samples from 48 healthy control individuals.



Workflow for robust and reproducible single cell analysis

### **Excellent sensitivity and reproducibility**

The presented data features that the automated loading protocol is a viable solution for a robust and reproducible loading of Evotip Pure. Sensitivity results using the Whisper 40SPD method on an Bruker TimsTOF Pro 2 show similar proteome coverage of an average of 3,100 quantified proteins from 1ng peptide input, and 7,000 proteins quantified from 50ng peptide input, when using the automated loading protocol compared to manually loaded Evotips.





#### Faster chromatography with up to 500 samples per day

Automated sample preparation workflows require faster chromatography. We have developed a robust method with 500 samples analyzed per day with two minutes gradient time. For high throughput analysis of complex samples, highest peak capacity in the shortest time is a relevant measure, which is achieved by the fastest method.



When comparing robot-to-robot reproducibility under 10% difference in identified precursors are observed when comparing two different Opentrons-2 platforms.



Robustness of three boxes loaded on the OT-2

A high degree of robustness is observed when using the automated loading protocol as under 5% coefficient of variation in each box is observed.



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