Ultra-high sensitivity for targeted proteomics using Evosep-MRM at the single cell level

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Highlights

- The Evosep One combined with the Agilent 6495C represents a highly sensitive and robust high throughput platform for targeted proteomics applications.
- Reproducible quantification of HeLa proteins at concentrations down to 62 pg peptide load.
- Detection of markers of cell cycle progression and tumorigenesis in individual HEK293 cells.

Whisper "out-of-the-box" nano-flow

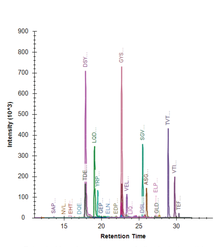
High sensitivity with Whisper Flow Technology

Whisper™ Flow Technology supports robust nanoflow chromatography providing ultra-high sensitivity, which is required when starting material is low. Using a dilution series of HeLa (62.5 pg, 125 pg, 250 pg 500 pg, 1 ng, 10 ng, 100ng HeLa in triplicates), we benchmarked the performance of the Whisper 40 SPD method with Evosep-MRM on an Agilent 6495C against the standard 60 SPD method.

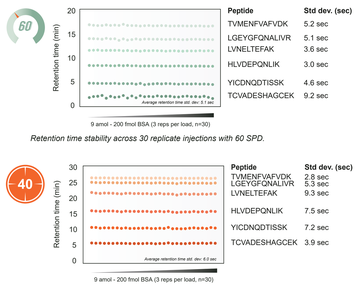
Standard proteomics workflow

Evosep-MRM using Whisper

We designed an assay targeting 59 peptides, covering 31 proteins utilizing 177 transitions in total. Retention time stability was excellent with an average retention time standard deviation of 5-6 seconds with both methods based on six BSA peptides.



Extracted ion chromatograms of all selected peptides.



Peptide	Std dev. (sec)
TVMENFVAFVDK	5.2 sec
LGEYGFQNALIVR	5.1 sec
LVNELTEFAK	3.6 sec
HLVDEPQNLIK	3.0 sec
YICDNQDTISSK	4.6 sec
TCVADESHAGCEK	9.2 sec

Retention time stability across 30 replicate injections with 60 SPD.

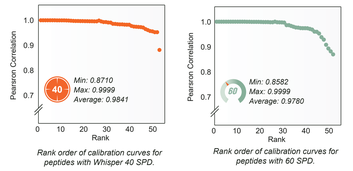
Peptide	Std dev. (sec)
TVMENFVAFVDK	2.8 sec
LGEYGFQNALIVR	5.3 sec
LVNELTEFAK	9.3 sec
HLVDEPQNLIK	7.5 sec
YICDNQDTISSK	7.2 sec
TCVADESHAGCEK	3.9 sec

Retention time stability across 30 replicate injections with Whisper 40 SPD.

Boost in performance with Whisper

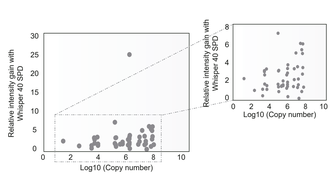
Assay quality check

Both the Whisper 40 SPD and the standard 60 SPD method provided excellent linear curves spanning three orders of magnitude with Pearson correlations above 0.99. From the 51 succesful calibration curves, the Whisper method provided the best overall linear performance and a median 4 fold gain in intensity compared to 60 SPD.

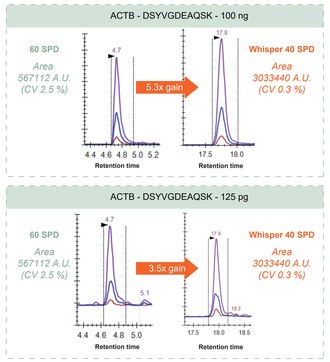


Rank order of calibration curves for peptides with Whisper 40 SPD.

Rank order of calibration curves for peptides with 60 SPD.



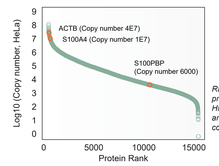
Median fold gain with Whisper 40 SPD compared to 60 SPD for all calibration curves.



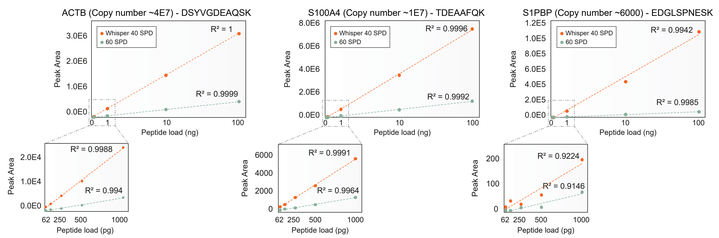
Extracted ion chromatogram for DSYVGDEAQSK peptide for 100 ng and 125 pg loads with 60 SPD and Whisper 40 SPD methods.

Sensitivity of targeted assay

Proteins in the assay were selected based on clinical relevance, thus among histones, cell cycle markers and S100 proteins. Furthermore, the copy number of the proteins selected representing a wide range of abundance in HeLa.



Rank order of HeLa protein copy numbers. Highlighted proteins are used for comparisons below.

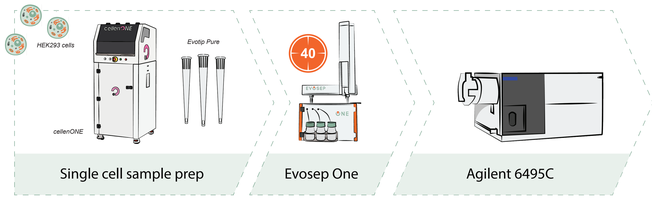


Calibration curves for HeLa dilution series for proteins ACTB, S100A4 and S1PBP with 60 SPD and Whisper 40 SPD methods.

Ultimate sensitivity for targeted proteomics in single cells

High sensitivity with Whisper Flow Technology

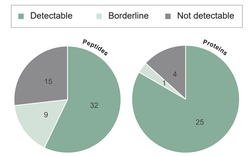
We challenged the assay by applying it to sorted single cells, where ultimate sensitivity is needed. HEK293 cells were sorted in triplicates, harvested and digested using the cellenONE and peptides were transferred to the Evotip.



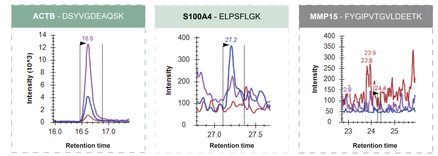
Single cell workflow for targeted proteomics

Single cell data quality

We were able to detect more than 70% of the selected peptides, which was impressive as the assay was not optimized to perform in the relevant matrix, corresponding to almost 90% of the selected proteins in the assay.



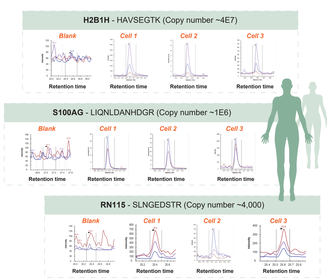
Overview of succesful detection of peptides and proteins in single cells.



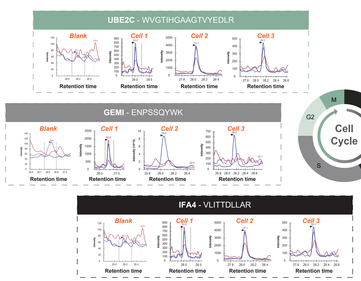
Extracted XICs representing 'detectable', 'borderline' and 'not detectable' peptides in single cells.

Relevant biological targets and cell cycle markers

Our selected protein targets include cell cycle markers and S100 proteins of clinical relevance. As these are detected, we believe interesting studies can be initiated from a larger number of single cells taking the heterogeniety into account.



Extracted XICs representing clinical relevant proteins in blank sample and single cells.



Extracted XICs representing cell cycle markers in blank sample and single cells.