

Efficient peptide desalting on novel C18 StageTips with a broad capacity range for LC-MS/MS proteomic studies in food matrices

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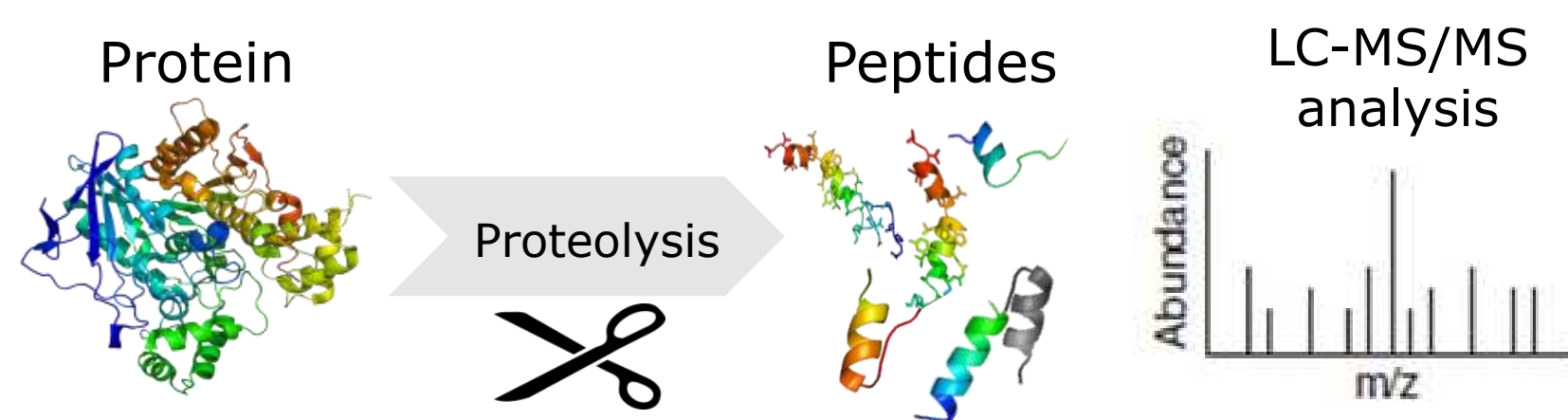
Objective

Protein analysis in food matrices



Method

Bottom-up workflow for proteomics



Challenge

Salts in digestion buffers

- Generate ion-suppression
- Limit peptide detection
- Impact proteins identification

- Salts must be efficiently removed before LC-MS/MS analysis
- All peptides must be retained to preserve all useful information about the sample

Solution

Peptide desalting with AttractSPE®Disks Tips C18

Among a large diversity of C₁₈ powders, a C₁₈ with a wide spectrum of interactions was selected to interact with the broadest range of peptides. Small sorbent particles embedded in a monolithic disk were used to combine high capacity and small dead volume.

These C₁₈ disks were tested into home-made SPE tips (called Stage Tips) to evaluate performances in desalting tryptic digests before peptides analysis by nanoLC-MS/MS.



Principle of AttractSPE® Disks

AttractSPE®Disks are soft, thin, uniform and mechanically stable membranes for the extraction, purification and concentration of analytes in proteomics studies.

AttractSPE®Disks membranes are SPE sorbents able to efficiently capture analytes and to easily release them for analysis, if required.

Sorbent particles are tightly packed with no void space

No need of frits
Reduced dead volume

Minimized diffusion distance between particles

Small elution volumes

Thin membrane, small bed volume

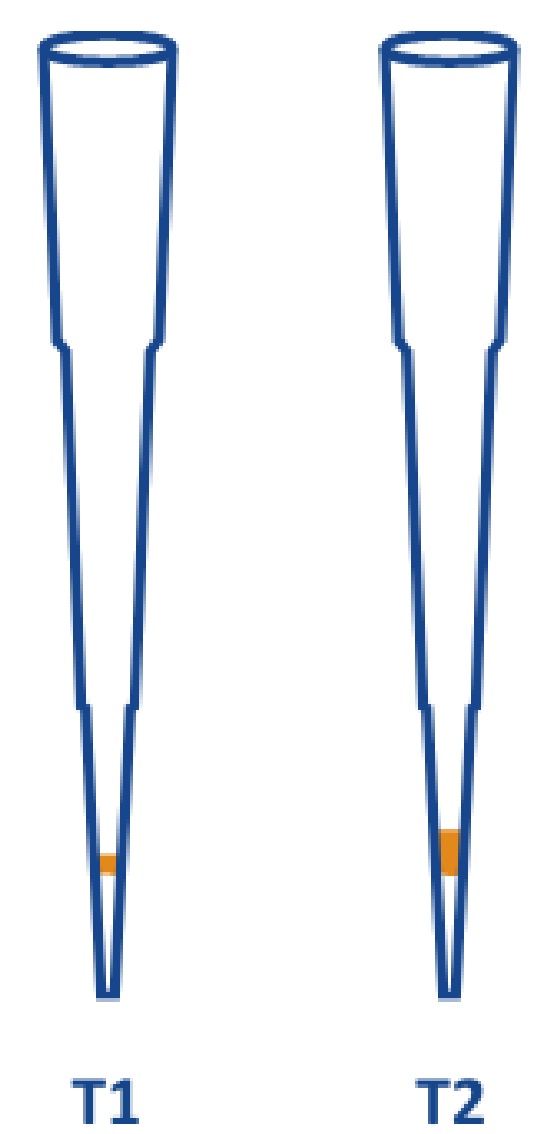
High sample recovery with excellent repeatability

Evaluation of C18 sorbent

Several new C18 Stage Tips, made with different thicknesses of AttractSPE®Disks C18 and therefore different capacities (T1 or T2), were evaluated for peptide desalting. These AttractSPE®Disks Tips C18 are particularly adapted for centrifugation or positive pressure assays.

T1: A layer of C18 sorbent with a thickness equivalent to ONE layer of SPE disks (around 0.6mm)

T2: A layer of C18 sorbent with a thickness equivalent to TWO layers of SPE disks (around 1.2mm)



Protocol of use for AttractSPE®Disks Tips C18

Processing step*	Operation	Centrifuge – time and speed
1 - Conditioning	100µl 70% ACN 0.1% FA	1min – 2,000 RPM (800 x g)
2 - Equilibration	100µl 0.1% FA	1min – 2,000 RPM (800 x g)
3 - Loading of sample	1.1ng, 11ng, 110ng, 1µg, 5µg or 10µg HeLa Digest in 100µl 0.1% FA	1min – 2,000 RPM (800 x g)
4 - Washing	100µl 0.1% FA	1min – 2,000 RPM (800 x g)
5 - Elution	100µl 40% ACN 0.1% FA	1min – 2,000 RPM (800 x g)
6 - Evaporation	Speed Vacuum dried	
7 - Reconstitution	Samples resuspended in 5.5µl (with IRT) and 5µl (1,10 or 100ng) injected in LC-MS/MS	

*Same protocol used for all the AttractSPE®Disks Tips, regardless of their capacity

LC-MS/MS analysis of peptides

NanoLC conditions

Trap column: nanoViper Acclaim PepMap™ C18 (2cm x 75µm)

Column: Acclaim PepMap™ RSLC C18 (50cm x 75µm, 2µm)

Temperature: 40°C

Gradient: 3% to 29% buffer B over 91min at a flow rate of 300 nL/min

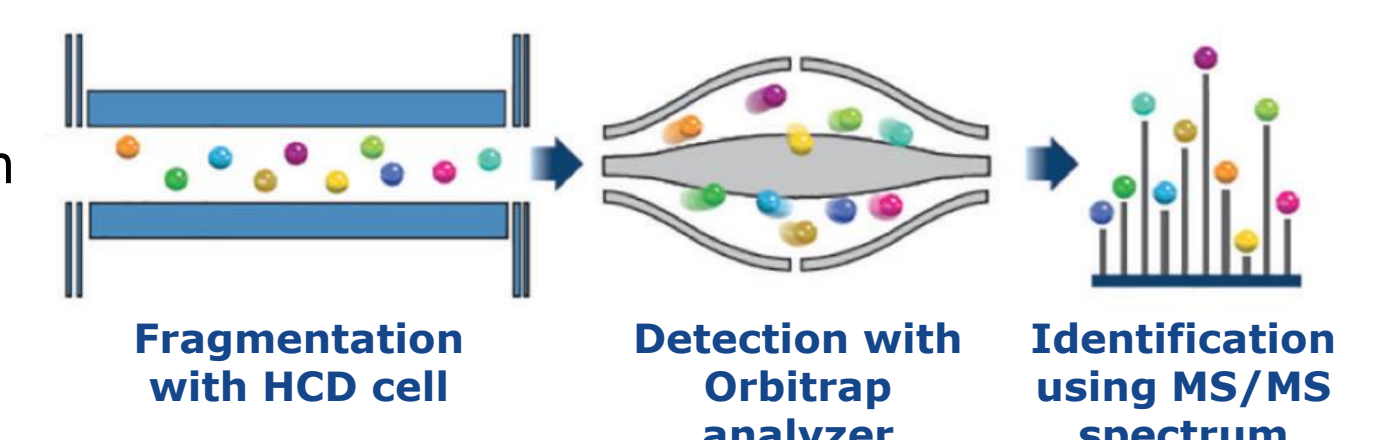
Buffer A: 2/98 ACN/H₂O in 0.1% FA

Buffer B: ACN in 0.1% FA

MS/MS conditions (Orbitrap Exploris 480)

MS full scans in ranges m/z 375-1500

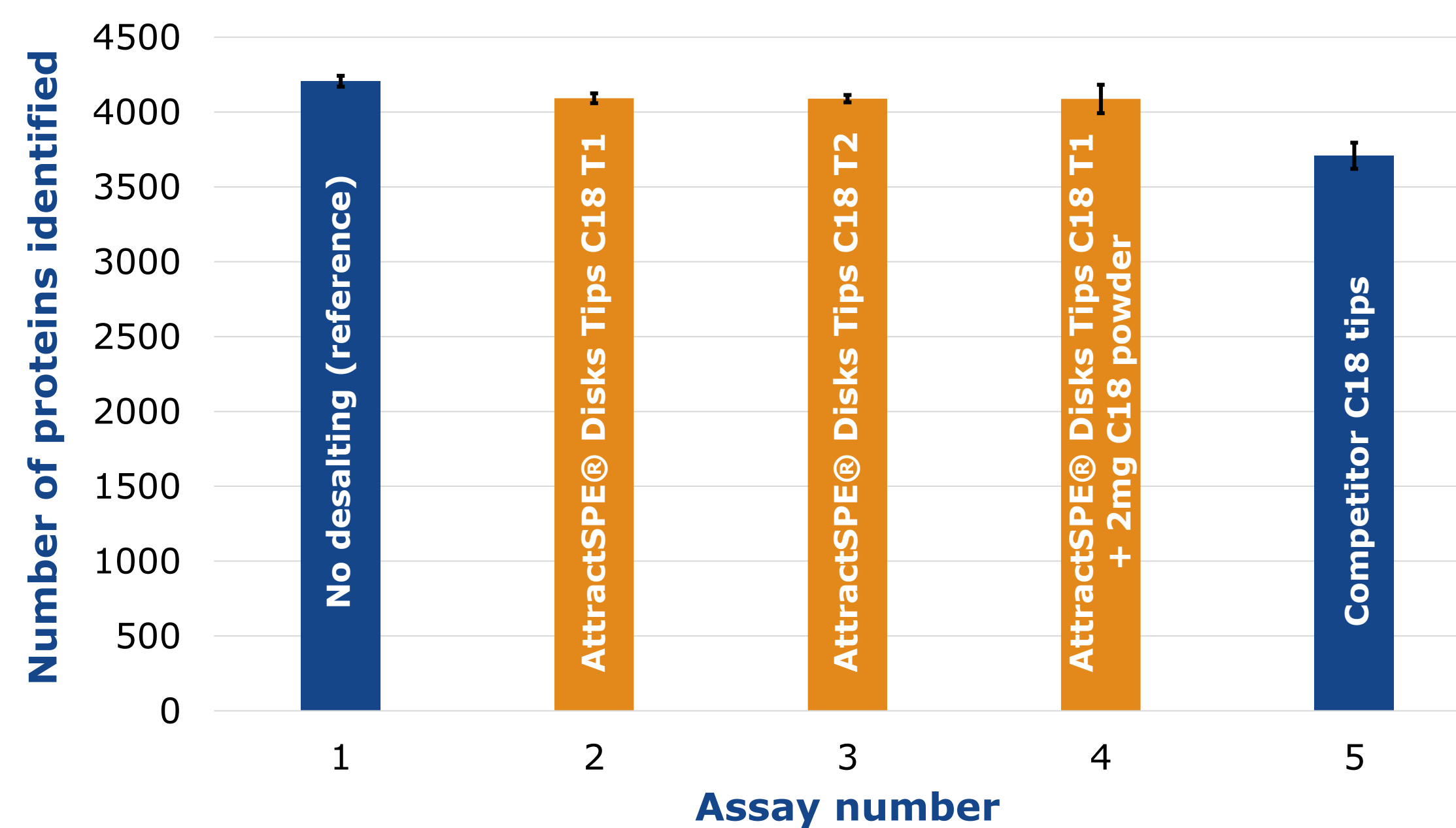
Top 20 most intense ions isolated and fragmented by HCD



Protein identification with AttractSPE®Disks Tips C18

Three AttractSPE® Disks Tips C18 with different capacities were tested for the desalting of 100ng of HeLa digest and were compared to a major competitor product (n=5 for each assay)

Assay number	Description
1	No purification (reference assay)
2	Desalting on AttractSPE®Disks Tips C18 200µL - T1
3	Desalting on AttractSPE®Disks Tips C18 200µL - T2
4	Desalting on AttractSPE®Disks Tips C18 200µL - T1 + 2mg powder AttractSPE® C18
5	Desalting on competitor C18 tips (100µL bed)



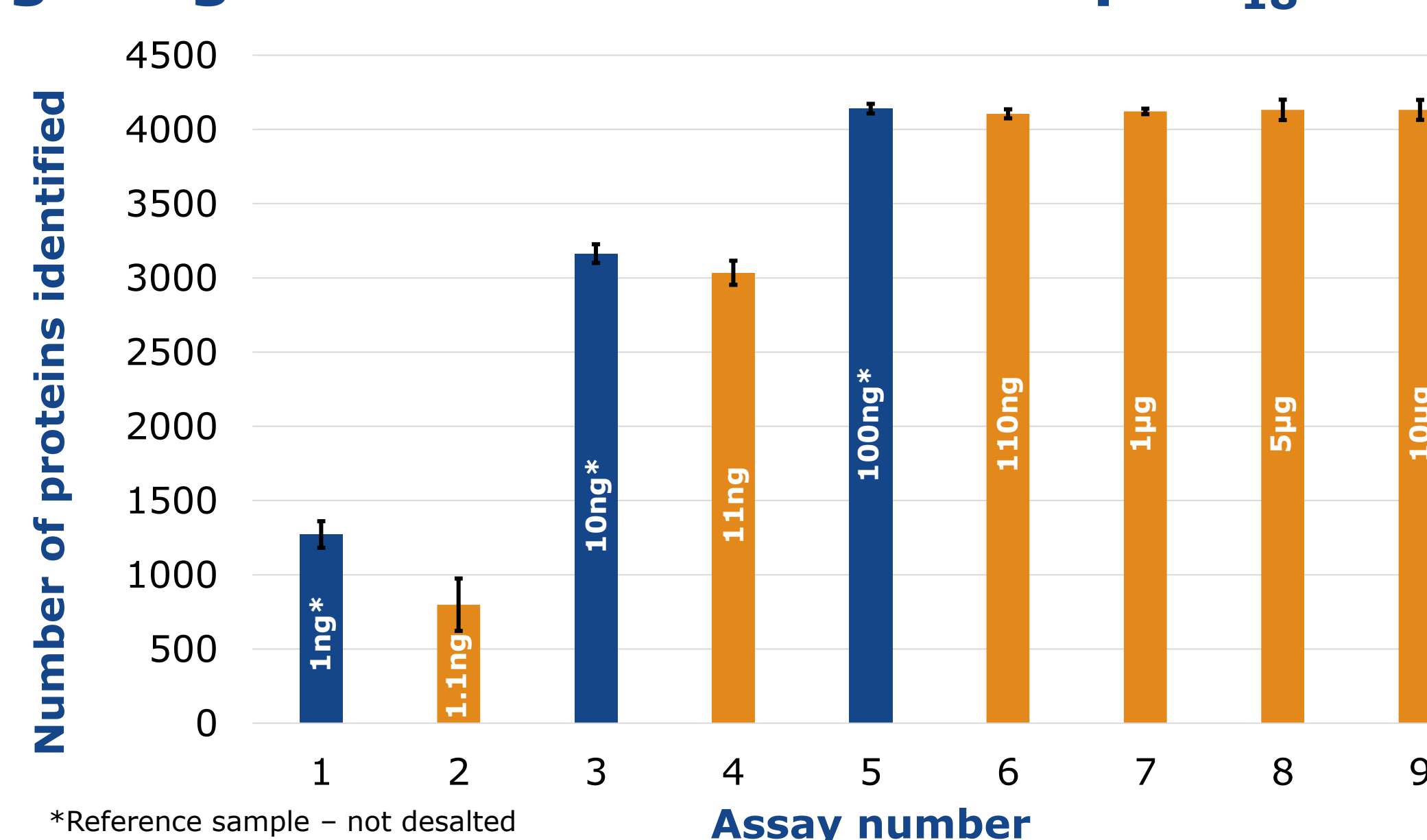
Results

- Identification of 97% of proteins after desalting of HeLa digest on AttractSPE®Disks Tips C18 (better results than competitor tips)
- Similar results obtained for the three AttractSPE®Disks Tips C18 : all retained peptides are released from the tips, regardless of the sorbent amount
- Good retention of peptides on the whole polarity range, from the most hydrophobic to the most polar
- Very low variability observed for intra- and inter-tips assays: reliability of AttractSPE®Disks Tips C18

Working range of AttractSPE®Disks Tips C18 T1

1ng to 10µg of HeLa digest were loaded on the AttractSPE®Disks Tips C18 T1 to determine their working range (n=3 for each assay)

Assay number	Amount of HeLa digest desalted	Amount analyzed by LC-MS/MS
1	-	1ng – not desalted as reference
2	1.1ng	1ng
3	-	10ng – not desalted as reference
4	11ng	10ng
5	-	100ng – not desalted as reference
6	110ng	100ng
7	1µg	100ng
8	5µg	100ng
9	10µg	100ng



*Reference sample – not desalted

Results

- Identification of 95% of proteins after desalting of HeLa digest amounts ranging from 10ng to 10µg on AttractSPE®Disks Tips C18 T1, with RSD < 3%
- No loss of performance for 10µg digest: maximal capacity of AttractSPE®Disks Tips C18 T1 not reached
- For 1ng of digest, the number of identified proteins remains very high despite the low amount of material: AttractSPE®Disks Tips C18 T1 could be used for single cell-like analysis

Conclusion

AttractSPE®Disks C18 sorbent is ideal for peptide desalting in proteomics and is available as Stage Tips, Spin columns, 96 well plates and SPE cartridges

- No loss of peptide
- High capacity
- Excellent repeatability
- Simplicity of use
- Broad range of use: from single cell to high peptide amounts