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Application Note

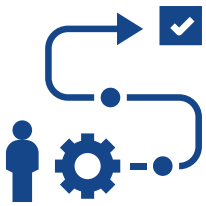
Estimation of the working range on BioSPE™ PurePep SPE Tips - Standard capacity

Introduction

Affinisep BioSPE™ SPE Tips (commonly referred as Stage-Tips) have demonstrated an excellent performance for many proteomic applications, ranging from peptide desalting to peptide fractionation or intact protein purification. The BioSPE™ SPE Tips are based on small particles densely packed and embedded in a soft and mechanically stable SPE membrane, with greatly reduced dead volume and high extraction capacity. The BioSPE™ SPE Tips 200µL are available with two capacities, called Standard (S) and High (H), corresponding to a maximal loading capacity of 15µg and 50µg, respectively.

In a previous application note, several Affinisep SPE Tips with different capacities were compared to other commercially available tips brands for peptide desalting. The results obtained demonstrated that Affinisep SPE Tips led to a minimal peptide loss and efficiently facilitated the identification of 97% of proteins present in the tryptic digest.

In this application note, the working range of the BioSPE™ PurePep SPE Tips – 200µL Standard capacity was determined for peptide desalting. Quantities of HeLa digest ranging from 1ng to 10µg were loaded on the SPE Tips. For 110ng and higher quantities of loading, 100ng of peptides were analyzed by LC-MS/MS to reduce any bias. All experiments were manually carried out.



Methods

HeLa Digest : Pierce™ HeLa Protein Digest Standard ; Cat : 88329) – 1 vial 20µg resuspended in 1ml of 1M Urea, 100mM Tris HCl ph 8.5 - 20ng/µl.
The amounts of samples 2, 4, 6, 7 were purified in 100µl of 1M Urea, 100mM Tris HCl pH 8.5.
The amounts of samples 8 and 9 were purified at 20ng/µl in 1M Urea, 100mM Tris HCl pH 8.5.
All assays were carried out in triplicate with BioSPE™ PurePep Tips 200µL Standard capacity, except assays 1, 3 and 5. Solutions of assays 1, 3 and 5 were prepared by dissolving HeLa Digest powder in 0.3% TFA.

Table 1 - Brief description of assays

Assay number for all graphs	Amount of HeLa digest desalted on BioSPE™PurePep SPE Tips 200µL Standard capacity	Amount of HeLa digest 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

Desalting Protocol

Abbreviation: ACN : Acetonitrile ; FA : Formic Acid

Table 2 – Description of general protocol for assays 1, 3 and 5. All assays were carried out manually.

Processing step	Operation	Centrifuge - time and speed
1 - Conditioning	100µl 70% ACN ; 0.1% FA	1min – 2000 RPM
2 - Equilibration	100µl 0.1% FA	1min – 2000 RPM
3 - Loading of the sample	X ng Hela Digest	1min – 2000 RPM
4 - Washing	100µl 0.1% FA	1min – 2000 RPM
5 - Elution	100µl 40% ACN 0.1% FA	1min – 2000 RPM
6 - Evaporation	Speed Vacuum dried	
7 - Reconstitution	Samples 2, 4, 6 were resuspended in 5.5µl (with iRT) and 5µl (1, 10 or 100ng) were injected and analyzed by LC-MS/MS. Samples 7, 8, 9 were resuspended in a volume to obtain 20ng/µl and 5µl (corresponding to 100ng) were injected and analyzed by LC-MS/MS	

LC-MS/MS Method

Chromatography was performed with an RSLCnano system (Ultimate 3000, Thermo Scientific) coupled online to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific). Peptides were trapped on a C18 column (75 µm inner diameter × 2 cm; nanoViper Acclaim PepMap™ 100, Thermo Scientific) with buffer A (2/98 ACN/H2O in 0.1% FA) at a flow rate of 3.0 µL/min over 4 min. Separation was performed on a 50 cm x 75 µm C18 column (nanoViper Acclaim PepMap™ RSLC, 2 µm, 100Å, Thermo Scientific) regulated to a temperature of 40°C with a linear gradient of 3% to 29% buffer B (100% ACN in 0.1% FA) at a flow rate of 300 nL/min over 91 min. MS full scans were performed in the ultrahigh-field Orbitrap mass analyzer in ranges m/z 375–1500 with a resolution of 120 000 at m/z 200. The top 20 most intense ions were isolated and further fragmented (normalized collision energy: 30%) via high energy collision dissociation activation, at a resolution of 15 000 and the normalized automatic gain control target set to 100%. Charge state from 2+ to 6+ were selected and dynamic exclusion was set to 40 s.

Results and discussion

Data analysis: for proteins identification, data were searched against the Homo sapiens (UP000005640) UniProt database using Sequest HT through Proteome Discoverer (version 2.4) and resulting files were further processed using myProMS (PMID: 17610305, Institut Curie homemade web server): https://github.com/bioinfo-pf-curie/myproms).

PSM : Peptide spectrum matches

Figure 1: Peptides comparison

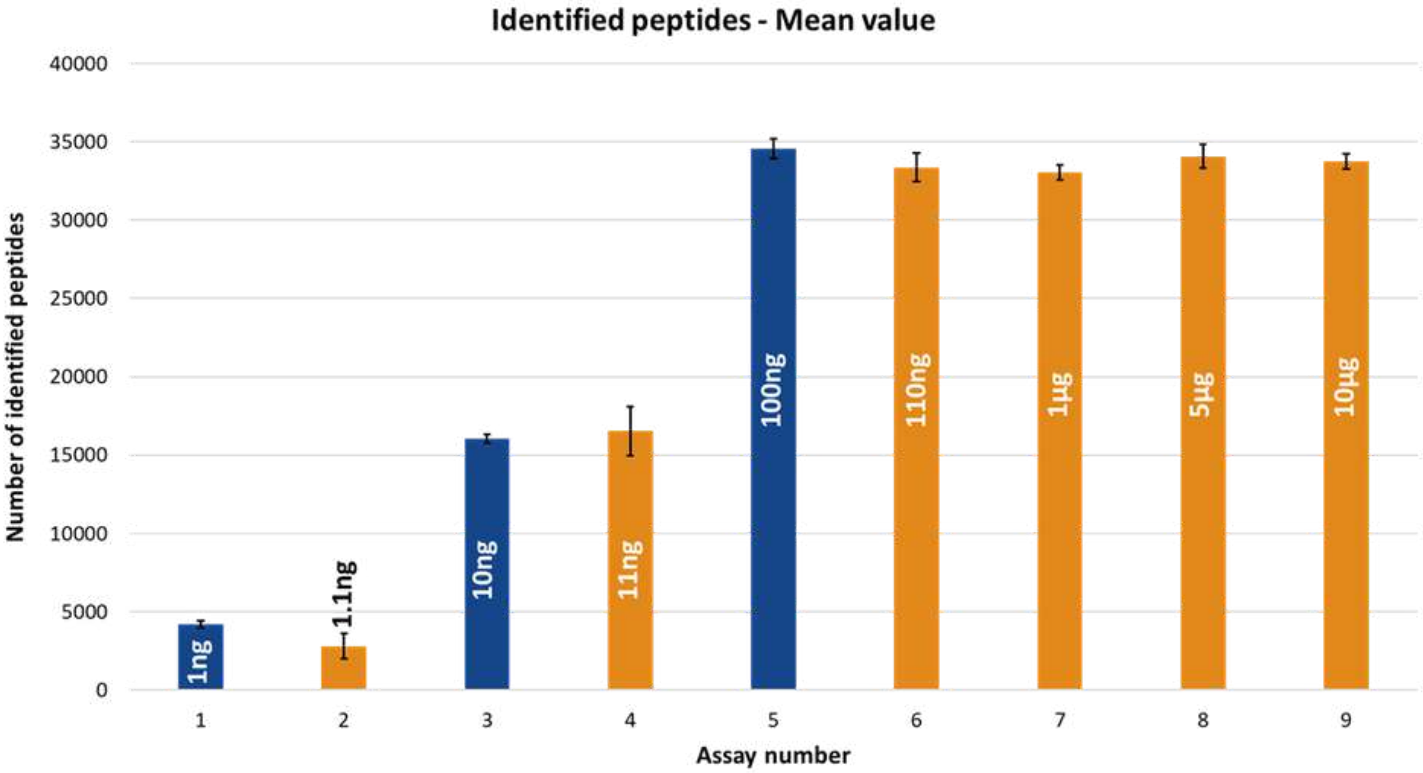


Figure 1 shows the identified peptides for each assay. Orange bars represent the peptides identified after desalting on the BioSPE™ PurePep SPE Tips. Blue bars are peptides identified for the reference solutions (direct injection of 1ng, 10ng or 100ng without pretreatment).

For all assays of 10ng and higher involving BioSPE™ PurePep SPE Tips, the number of identified peptides is very close to the reference sample. For assay with 1ng, the number of identified peptides remains very high despite of the very low quantity of peptides to be desalted. Loss of 1ng peptides can occur on consumable walls as well as during the evaporation and resolubilisation step, whereas the reference solution requires much less handling.

An excellent repeatability is observed for samples of 100ng and more with a RSD lower than 3%. The RSD progressively and logically increases for 10ng and then 1ng of peptides.

Figure 2: Protein comparison

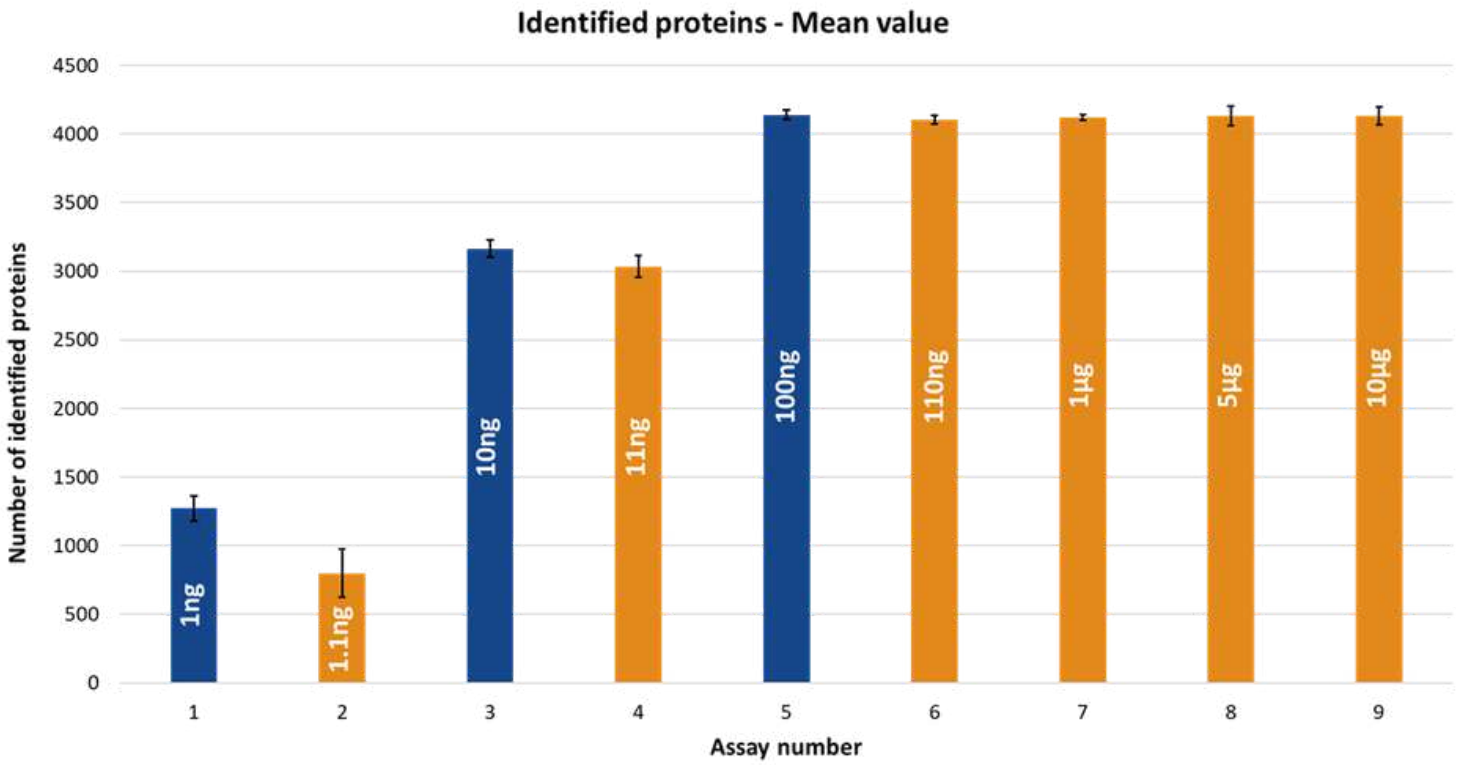


Figure 2 shows the number of proteins identified for each assay.
For all assays of 10ng and higher amounts involving BioSPE™ PurePep SPE Tips (orange bars), the number of identified proteins is very close to the reference sample (blue bar). For assay with 1ng, the number of identified proteins remains very high despite of the very low amount of materials.

Excellent repeatability is observed for samples of 10ng and more with a RSD lower than 3%. The RSD progressively and logically increases for 1ng of peptides due to the handling of a very small amount of material.

Figure 3: Peptides group abundances – Total signal

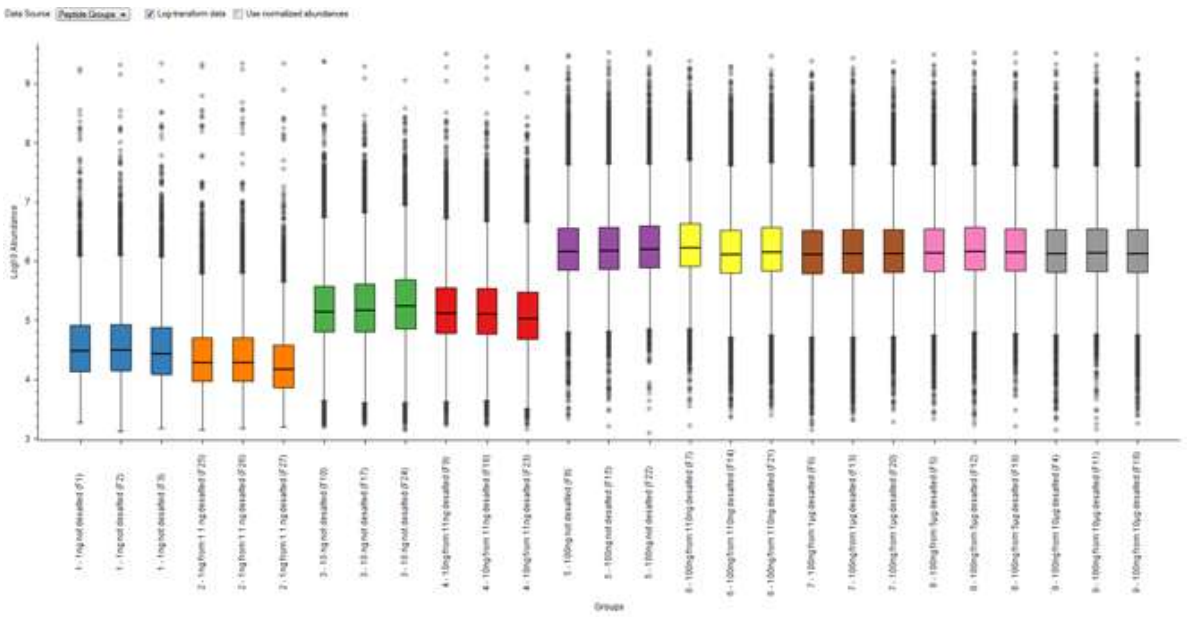


Figure 3 shows the summed peptides group abundances for each tip. Low variation is observed for each triplicates showing an excellent repeatability of each experiment and expressing a good reliability of the obtained results.

Conclusion

This application note explores the working range of peptide desalting with BioSPE™ PurePep SPE Tips – Standard capacity. 1ng to 10µg of peptides (a factor 10 000) were loaded and desalted on the SPE tips and compared to reference solutions of 1ng, 10ng and 100ng. When possible, 100ng of peptides were injected.

The results for 1ng were very good considering the process involved. Working with such a small amount of peptides is very intricate due to their potential loss on consumable walls and during the evaporation/resolubilisation step. This demonstrates that BioSPE™ PurePep SPE Tips – Standard capacity could be used for single-cell like analysis.

In the range 10ng to 10µg, performances of identified peptides and proteins were excellent with a very good repeatability and show that BioSPE™ PurePep SPE Tips – Standard capacity can be used as a desalting tool within this range. With 10µg of peptides, no loss of performance is observed meaning that this tool can also be used for much higher quantity of peptides.

In addition, BioSPE™ PurePep is available with an even wider working range with the high capacity SPE Tips, but also with different formats such as SPE spin columns for higher amounts of peptides and 96 SPE well plates for high throughput experiments.

Products used in this application note:

BioSPE™ PurePep – SPE Tips 200µL & spin adapters, Standard capacity, 96/pk

- KT-Adapt-Tips-PurePep.S.200.96

Related Products

BioSPE™ PurePep – SPE Tips 200µL & spin adapters, High capacity, 96/pk

- KT-Adapt-Tips-PurePep.H.200.96

BioSPE™ PurePep – SPE Micro Spin, Standard capacity, 96/pk

- µSpin-PurePep.S.96

BioSPE™ PurePep – SPE Mini Spin, Standard capacity, 96/pk

- Spin-PurePep.S.96

BioSPE™ PurePep – 96 SPE well plate for microelution & collection plates, Standard capacity, 1/pk

- KT-Coll-µ96W-PurePep.S.1

BioSPE™ PurePep – 96 SPE well plate & collection plates, Standard capacity, 1/pk

- KT-Coll-96W-PurePep.S.1

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We thank Normandy Region for their support!