

# Development and optimization of a method for automated peptide desalting on the DigestPro MSi robot using AttractSPE<sup>®</sup> C18 tips

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#### Introduction

Peptide desalting is a crucial step of sample preparation to perform bottom-up approach in proteomics analysis by LC-MS/MS. Indeed, salts present in the digestion buffers can generate ion-suppression that can limit the detection of peptides and greatly impact proteins identification. Throughout this study, we evaluated

AttractSPE<sup>®</sup> C18 Tips (Affinisep) compared to home-made StageTips (with Empore C18 SPE disks) starting from both 2 µg and 100 ng of protein digest using the DigestPro MSi robot (CEM, formerly Intavis). We also optimized the program on the robot to reduce the desalting time per sample.



## Quantification

### Comparison between AFF and HM tips

MaxQuant 2.1.3.0 without MBR + ProStaR 1.28.0 MaxQuant : min ratio = 1 | without normalization ProStaR filtering : at least 2 values out of 3 in at least one conditions



Steps	Classic method	FaST method
	(40min/samples)	(10min/samples)
Conditionning: dispense speed		
200 μl MetOH	0.1 mal /main	0 2mal /main
100 μl 50% ACN 0,5% acetic acid	0.1mL/min	0.2mL/min
200 μl 0,5% acetic acid		
Sample load: dispense speed		
68 μl 0,5% acetic acid	0.01 mL/min	0.05 mL/min
Sample wash: dispense speed	0.1mL/min	0.2mL/min
100 μl 0,5% acetic acid		
Elution: dispense speed	0.01 mL/min	0.1mL/min
80% ACN 0,5% acetic acid		
Elution: volumes	100+60µL	60+60µL



**High amount** : Peptides LFQ intensities of 200 ng Rat brain Digest (2 µg desalted) 

> $\rightarrow$ +4% peptide intensity with AFF StageTips

Boxplot of the differences in Log2 average peptide intensities between AFF and HM

HM-6 AFF-1 AFF-2 Euclidean complete clustering from ProStar

- > Almost **no difference** observed in terms of peptide intensities **when desalting high quantities (2µg)** with AFF or HM StageTips.
- **Low amount** : Peptides LFQ intensities of 10 ng HeLa Digest (100 ng desalted)



> FaST program was created with higher dispense speeds for each step on the Digest Pro MSi robot in order to reduce the processing time per sample from 40 min to 10min.

**Classic vs FaST desalting with 100 ng of HeLa Digest (10 ng injected)** 



Scatter plot of peptide intensities from Perseus

Euclidean complete clustering from ProStar

> When working with low amount of digest, AFF StageTips give 25% gain in peptide intensities compared to HM. Moreover, AFF and HM separate in two distinct groups on the clustering, with less missing values in the AFF group.

Scatter plot of peptide intensities from Perseus

between FaST and Classic program

> A small increase (11% gain) in peptide intensities was observed with the FaST program (10 min) compared to the classic program (40 min). The number of quantified proteins remains unchanged.

## Conclusion

This study shows no significant difference between AttractSPE<sup>®</sup> C18 Affinisep and home-made StageTips (Empore C18) when working with high amount of starting material (2µg). On the contrary, with low amount of starting material (100ng), AttractSPE<sup>®</sup> C18 tips give better results than home-made StageTips. Indeed, Affinisep StageTips show better hydrophilic peptide retention as well as 9% increase in the number of identified proteins and 25% increase in peptide intensities.

Moreover, the desalting program on the Digest Pro Msi robot has been optimized with great time saving, from 40 min down to 10 min. This shorter program did not show any loss in protein identification and quantification. On the contrary, a slight increase (11%) in peptide intensities was observed.

To conclude, the best configuration for peptide desalting on the Digest Pro MSi robot is based on the use of AttractSPE<sup>®</sup> C18 tips with the 10 min program.