

# Automated Mass Spectrometry Sample Prep C18 Clean-Up

PhyNexus has an automated process to desalt or “clean up” proteins/peptides using C18 PhyTip® columns in order to streamline sample preparation for mass spectrometry analysis up to 96 samples at a time. When performing a reverse phase purification using a manual pipette, back pressure while aspirating solutions can inconvenience the operator and cause inconsistent results.

PhyTip columns perform the clean-up with the consistency, ease, and scalability that go hand in hand with automation. With the high capacity and versatility in protein binding, the C18 PhyTip column is the optimal choice in developing a fluid MALDI or LC-MS workflow.

- Bind a wide range of protein/peptide sizes
- High absolute binding capacity
- High recovery, low peptide loss
- Compatible with common mass spectrometry solvents
- Compatible with major automation liquid handling robots

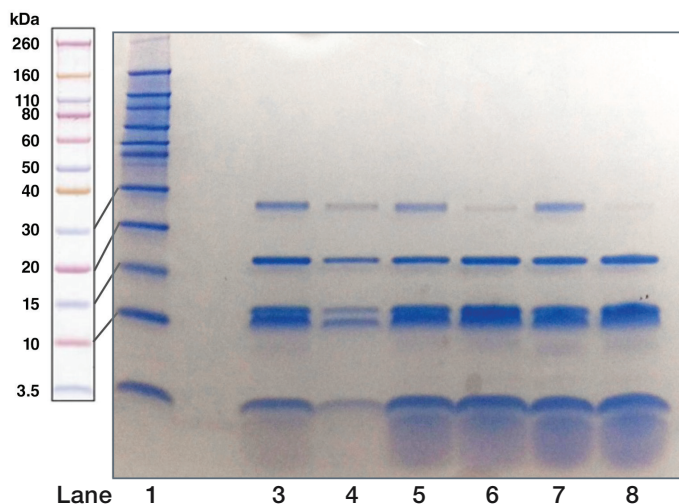
C18 Tip	Capacity (µg*)	Recovery	Range
<b>PhyTip Column</b>	<b>19.3</b>	<b>~85%</b>	<b>&lt;50 kDA</b>
ZipTip	8.33	~85%	<50 kDA
OMIX Tip	8.00	~85%	No Data

Figure 1. Specs of C18 tips

When comparing the capacity of some of the leading C18 tips, PhyNexus C18 PhyTip columns bind more than double the amount of protein per µL of resin while exhibiting a comparable recovery and range.

\*Capacity is measured in µg of protein per µL of resin

## C18 PhyTip Columns Bind a Wide Range of Proteins/Peptides

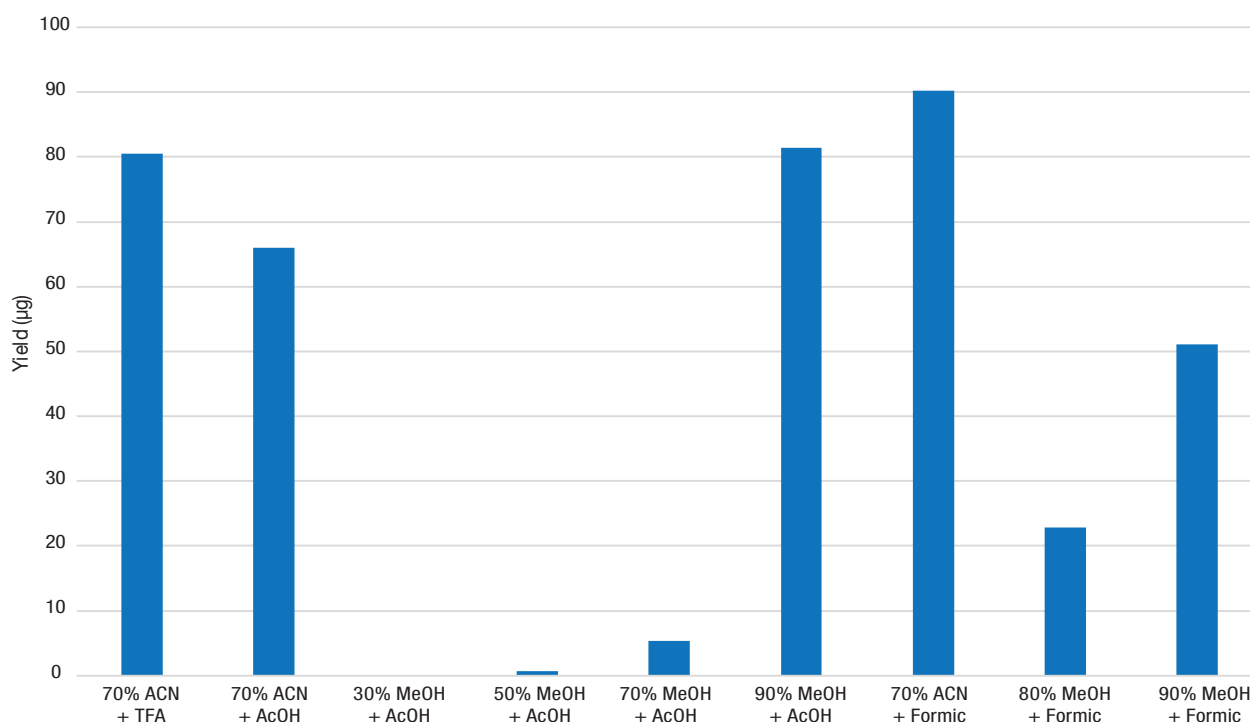


Lane	Sample
Lane 1	Novex high range protein ladder (Thermo)
Lane 3	Unprocessed low range protein ladder
Lane 4	1:2 dilution of unprocessed protein ladder
Lanes 5, 7	C18 PhyTip (300 Å) recovery of protein ladder
Lanes 6, 8	C18 PhyTip (90 Å) recovery of protein ladder

Figure 2. PhyTip columns recover full range of protein markers (1kDa–26.6 kDa)

A low molecular weight protein marker was processed by 90Å and 300Å C18 PhyTip columns. 5 µL of each recovered sample was run on a 16% tricine protein gel and stained with SimplyBlue SafeStain.

## Elute in Many Common Mass Spectrometry Buffers



**Figure 3. Optimization of elution solvent**

Different combinations of solvents and acids can be tested in one experiment to optimize recovery in a solvent compatible with your downstream assay or MS analysis and optimal for your target peptide/protein.

For example, formic acid is preferred over TFA in the mobile phase when preparing a sample for ESI-MS to prevent interference with the electrospray. In this example, both methanol and acetonitrile were tested with varying ion pairing agents against the C18 PhyTip columns.

## C18 PhyTip Columns

Tip Volumes	Resin Volumes
200 (µL)	5 (µL)
300 (µL)	10 (µL)
500 (µL)	20 (µL)
1000 (µL)	40 (µL)
	80 (µL)
	160 (µL)
	320 (µL)

To learn more, visit  
[www.phynexus.com/c18](http://www.phynexus.com/c18)

PhyTip columns can be used with all major 8, 12, and 96-channel automation liquid handling robots including Agilent Technologies, Beckman Coulter, Dynamic Devices, Hamilton, Perkin Elmer, Tecan, and PhyNexus MEA

