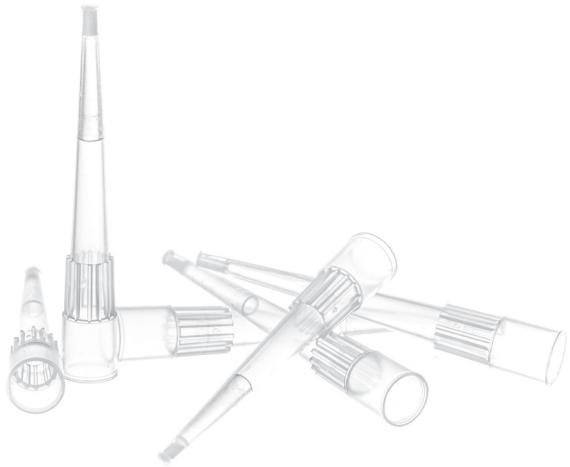
# Automated Mass Spectrometry Sample Prep

## Sample Desalting by Reversed Phase PhyTip® Columns

Biotage 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 preforming 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

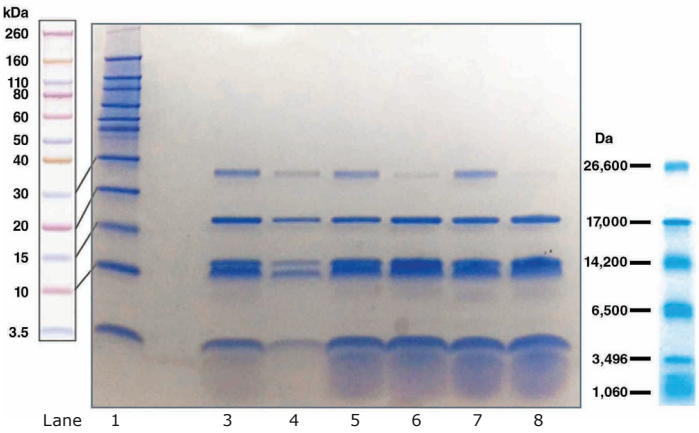


| C18 Tip | Capacity (µg*) | Recovery | Range |
|---|---|---|---|
| **PhyTip Column** | **19.3** | **~85%** | **<50 kDA** |
| ZipTip® | 8.33 | ~85% | <50 kDA |
| OMIX® Tip | 8.00 | ~85% | No Data |

*Capacity is measured in µg of protein per µL of silica.

**Figure 1. Specifications 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 silica while exhibiting a comparable recovery and range.

## 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 |
| Lane 5, 7 | C18 PhyTip (300 Å) recovery of protein ladder |
| Lane 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.



PhyNexus Now part of Biotage®

## Elute in Many Common Mass Spectrometry Buffers

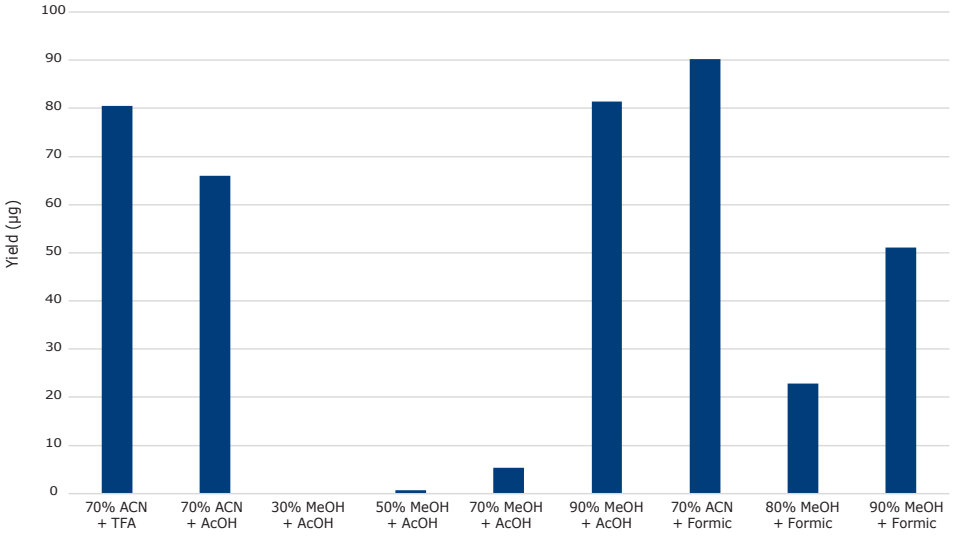
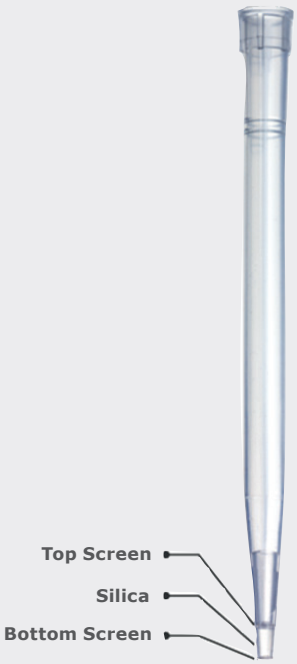



**Figure 3. Optimization of elution solvent.** Different combinations of solvents and acids can be tested in one experiment to optimize recovery in a solvent compatible with your downstream assay or MS analysis and optimal for your target peptide/protein. For example, formic acid is preferred over TFA 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.




### Reversed Phase PhyTip® Columns

Reversed phase PhyTip® columns are silica columns packed in a pipette tip between two thin insert screen.

### Available Reversed Phase Silicas

| C4 (300Å) | C18 (90Å) | C18 (300Å) |
|---|---|---|

### Silica Bed Volumes

Available with a many different silica bed volumes for processing of a vast range of sample volumes and concentrations.

| 5 µL | 10 µL | 20 µL | 40 µL | 80 µL | 160 µL | 320 µL |
|---|---|---|---|---|---|---|

### Robotics Compability

Available for all major liquid handling systems

- Hamilton
- Tecan
- Beckman Coulter
- Opentrons
- Dynamic Devices
- Perkin Elmer
- Agilent Technologies
- Caliper
- MEA 2

**To learn more, visit**: www.biotage.com/reversed-phase-phytip-columns

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