# Micro-Adsorptive Sample Preparation for Mass Spectrometry


## Millipore Corporation, Life Science Division, Danvers, MA, 01985



## ABSTRACT

Advancements in Mass Spectrometry (MS) instruments over the last 5 years have reduced the amount of sample required and increased the molecular weight analysis range. As the result of these improvements, MS has emerged as a major analysis technique for biomolecules in the areas of proteomics and functional genomics. Subsequently, there has been a significant increase in the number of samples that need to be devoid of contaminants, such as salts and detergents. Elimination of these contaminants from proteins, peptides and oligonucleotides is critical because of ionization suppression. In many cases, sample preparation has become the bottleneck in the acquisition of high quality mass spectra. To address this problem, we present data on the use of micro-adsorptive pipette tips (ZipTip®) containing chromatographic media ($C_{18}$, $C_4$, SCX and metal chelate) for sample clean-up prior to MALDI-TOF MS. Due to the liquid handling capability of the small pipette tip format (10 µl), it is well suited for low volume applications. The devices can be used for simple desalting or fractionation at the micro-scale. Furthermore, adsorbed solutes can be eluted and spotted directly onto a target plate. Several peptide and protein applications are presented. Including the use of ZipTip containing metal chelate media for the capture of ß-casein phosphopeptides or the purification of a histidine rich fusion protein. Excellent mass spectra were obtained on a variety of samples.


## INTRODUCTION

Analysis of biomolecules using matrix-assisted laser description/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has developed into an enabling technique for biochemical analyses. With MALDI-TOF MS, researchers can rapidly and accurately determine the mass of peptides, proteins and oligonucleotides. When this analytical method is combined with specific enzymatic cleavage (trypsin digests) or other biospecific interaction, MALDI-TOF MS has been shown to be a powerful tool in the identification of proteins. Currently, MALDI-TOF MS is the analytical method of choice for proteome characterization and is emerging as a competitive technology for SNP analysis. Although analysis by MALDI affords the opportunity to characterize biomolecules at low concentration and high precision, spectral quality is often diminished by solution components that are common in biological samples. These include salts, buffers, detergents, chaotropic agents, etc. In order to obtain good data, such substances must be removed from the sample prior to analysis. The sample preparation need can be solved by a number of approaches. However, it is very difficult to work with microliter and less volumes. The most common method for sample preparation, at this scale, is the use of miniature chromatography columns that are "home-packed" pipette tips. Millipore has developed a process using polymer technology that can immobilize chromatography beads in very small volumes. The first product from this technology was ZipTip$_{C18}$ which consists of a P10 pipette tip containing 0.6 µl of $C_{18}$ resin immobilized on the distal end. This device provides a ready-made and convenient means of conducting sample preparation at the microliter scale. Data are presented on the usage of ZipTips containing $C_{18}$, $C_4$, SCX and immobilized metal chelate (IMAC) resins for the sample preparation of peptides and proteins. Desalting was achieved by binding the biomolecules to the immobilized adsorbent, washing away the impurities with water and then eluting in ca 2 µl directly onto the MALDI target. The effectiveness of sample preparation was clearly demonstrated by the acquisition of high quality MS spectra with strong signal to noise ratios.

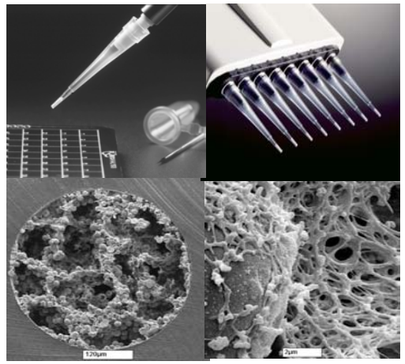

**Figure 1**: Top: Picture of devices. Lower Left: Cross-section of ZipTip pipette tip. Note the large channels that allow good flow and liquid/bead contact. The structure is similar to an expanded chromatography bed. Lower Right: High magnification of polymeric scaffold. The polymer "netting" holds the beads in place.

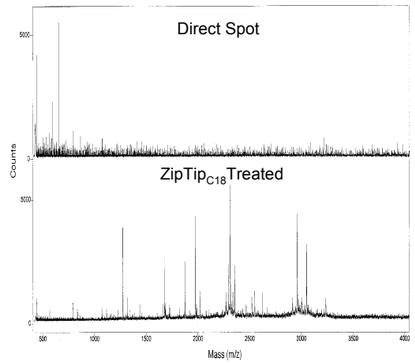


**Figure 2:** MALDI MS Spectra of a Tryptic peptide digest from an in-gel 2D digest. The top spectra represents a contaminated sample prior to sample clean-up. The lower spectra represents the sample after using a ZipTip$_{C18}$ prior to MALDI TOF MS analysis.

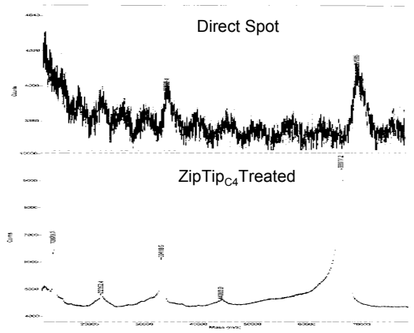


**Figure 3:** MALDI-TOF MS of BSA (200 ng) in 4M GuHCl (10 µl) before (top) and after ZipTip$_{C4}$ clean-up (bottom). Sample was bound to the tip out of 4M GuHCl, washed with water and eluted directly onto the MALDI MS target with 75% ACN in 0.1% TFA containing matrix.

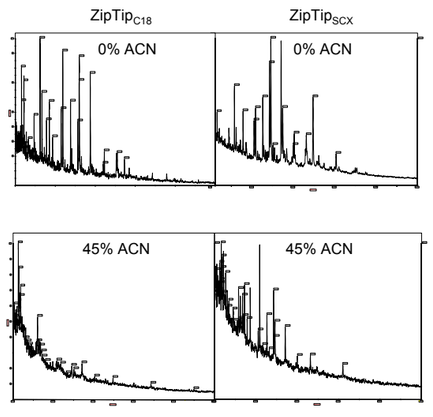


**Figure 4:** The MALDI TOF MS spectra on the left are of trypsin digest peptides from rabbit glycogen phosphorylase (200 fmol) dissolved in 0.1% TFA containing 0 or 45% ACN that have been prepared on ZipTip$_{C18}$. The spectra on the right are of the same samples prepared on ZipTip$_{SCX}$. Note that at 0% ACN the peptide coverage of both devices was very similar.

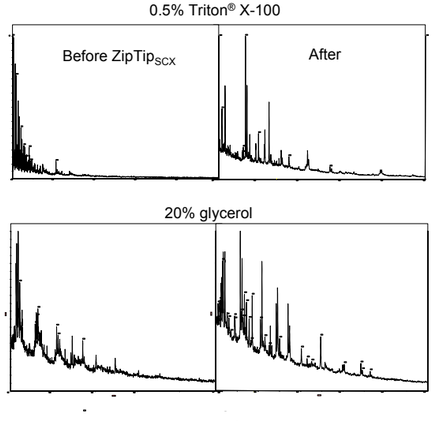


**Figure 5:** MALDI TOF mass spectra of glycogen phosphorylase tryptic peptides (200 fmol) contaminated with Triton X-100 or glycerol (left). The spectra on the right are of the same samples that have been prepared using ZipTip$_{SCX}$.

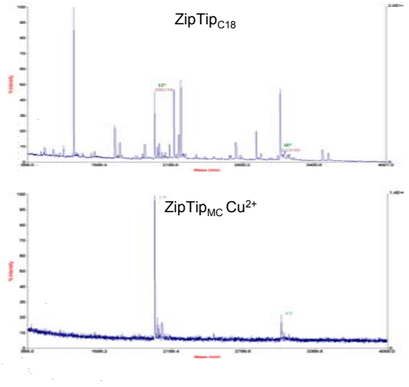


**Figure 6:** MALDI-TOF MS spectra of 1 pmol β-casein tryptic peptides (top panel) prepared on ZipTip$_{C18}$ prior to phosphopeptide enrichment using ZipTip$_{MC}$. MALDI-TOF MS spectra of eluted β-casein (bottom) phosphopeptides from a tryptic digest captured by ZipTip$_{MC}$ charged with $Cu^{2+}$.

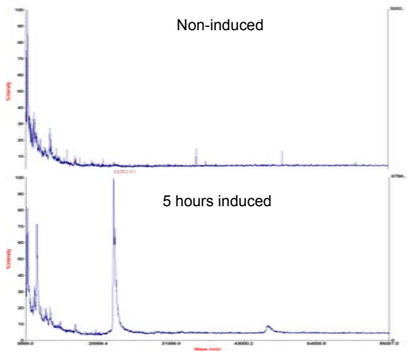


**Figure 7:** MALDI-TOF MS spectra histidine rich fusion protein from cell supernatants after enrichment with ZipTip$_{MC}$. The protein was expressed using QIAexpress® System following induction with 1 mM IPTG. An Aliquot was removed at 5 hours and the cell supernatant was prepared under denaturing conditions. Cell supernatant in denaturing buffer was bound to $Cu^{2+}$ charged ZipTip$_{MC}$.

## CONCLUSIONS

- ZipTip with $C_{18}$, $C_4$ were efficient at desalting peptides and proteins prior to MALDI-TOF MS analysis.
- Samples eluted from the $C_4$ and $C_{18}$ tips with approximately 2.0 µl of ACN/ matrix could be spotted directly onto the target.
- Strong Cation Exchange tips were effective at concentrating peptides from solutions containing acetonitrile and non-ionic detergent.
- Affinity capture of ß-casein phosphopeptides was demonstrated using ZipTip$_{MC}$ charged with $Cu^{+2}$.
- ZipTip$_{MC}$ charged with $Cu^{+2}$ was also effective in capturing a histidine rich fusion protein from cell supernatant following IPTG induction.