Carbon, TiO_2, and ZrO_2 Wall-Coated Trap'nTips™ for Online & Offline Phosphopeptide Analysis

The design of carbon, TiO_2, and ZrO_2 wall-coated pipette tips facilitates phosphopeptide enrichment and manual aspiration through the pipette tip followed by expulsion of purified solution for immediate nanobore column injection or direct loading into an offline nanospray emitter. As previously demonstrated, these substrate-specific resins effectively concentrate, desalt, and enhance the MS signal of phosphopeptides from trypic digests[1]. The novel design of the Trap'nTip™ eliminates geometric constraints of sample preparation, pipette tip-coupling, and pressurized back-loading associated with conventional practice (See Technical Note PT-6).

FIGURE 1 Carbon-, TiO_2-, and ZrO_2-coated Trap'nTips™

Product Description

The Trap'nTip is comprised of a standard gel-loader pipette tip containing one of three phosphopeptide-specific sorbents adsorbed to the inner wall. New Objective offers Trap'nTips for phosphopeptide analysis containing titania (TiO_2), zirconia (ZrO_2), and carbon sorbents.

Trap'nTip™ Conditioning and Sample Loading

Prior to the application, Trap'nTips coated with phosphopeptide-specific sorbents require a conditioning step. The complete preparation and sample-loading procedure is delineated below:

1. Conduct 5 aspriation/expulsion cycles of HPLC-grade water with a 0.5-10 µL Eppendorf® Single-Channel Research Pipette.
2. Load phosphopeptide analyte onto the Trap'nTip using 10 aspiration/expulsion cycles of 10 µL each.
3. Wash loaded samples via ten 10 µL aspiration/expulsion cycles of HPLC-grade water.
4. Aspirate 2 µL aqueous solution containing 50 mM NH_4HCO_3 and 50 mM triethylamine (TEA) to elute analyte from the Trap'nTip.
5. Expel eluent from the Trap'nTip into a clean vial.
6. Add 2 µL 50 mM TEA in CH_3OH to vial containing eluted sample.
7. Mix eluent with CH_3OH by centrifugation

Loading an Offline GlassTip™ Using the Trap'nTip™

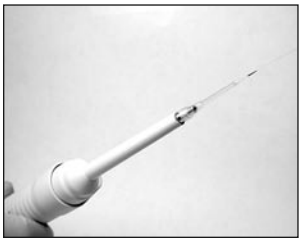

Figure 2 Trap'nTip™ fitted onto 10 µL pipette

Figure 3 Sample loaded into the Trap'nTip

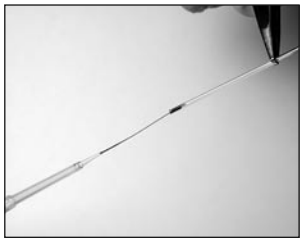

Figure 4 Insert the Trap'nTip into the distal end of the PicoTip®

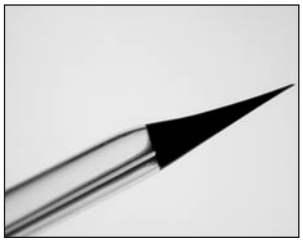

Figure 5 Capillary action draws the sample into the tip-end of the PicoTip emitter

Online Chromatography of Phosphopeptides Using the Trap'nTip™

The following procedure successfully separates phosphopeptide analytes for online analysis.

1. Conduct 5 aspriation/expulsion cycles of HPLC-grade water using a 0.5-10 µL Eppendorf® Single-Channel Research Pipette.
2. Load phosphopeptide analyte onto the Trap'nTip using 10 aspiration/expulsion cycles of 10 µL each.
3. Wash loaded samples through ten 10 µL aspiration/expulsion cycles of HPLC-grade water.
4. Aspirate 10 µL aqueous solution containing 250 mM NH_4HCO_3 (pH 9) to elute analyte from the Trap'nTip.
5. Expel eluent from Trap'nTip into a vial.
6. Conduct 9-10 additional eluent aspiration/expulsion cycles through the Trap'nTip
7. Deliver sample to injection port using conventional method (10 µL syringe, autosampler, etc.)

MassPREP™ standards were obtained from Waters. The first standard was a combined enolase digest/phosphopeptide mixture, and the second standard was a phosphopeptide standard. Both solutions contained phosphopeptides listed in Table 1.

Peptide	Sequence	$[M+H]^+$	$[M+2H]^{2+}$
T18 1P	NCPLpY K	813.3912	407.1995
T19 1P	HLADL pSK	863.4028	432.2053
T43 1P	VNQIG pTLSES IK	1368.6776	684.8428
T43 2P	VNQIG TLpSEpS IK	1448.6439	724.8259

TABLE 1

Figure 6 displays scans of the enolase digest (Figure 6A) and phosphopeptide standard (Figure 6B) prior to Trap'nTip purification. Figures 6C and 6D display the enolase digest after treatment with the TiO_2 and ZrO_2 Trap'nTips. Due to trace-level presence, the T43 2P phosphopeptide is not visible in the latter two figures.

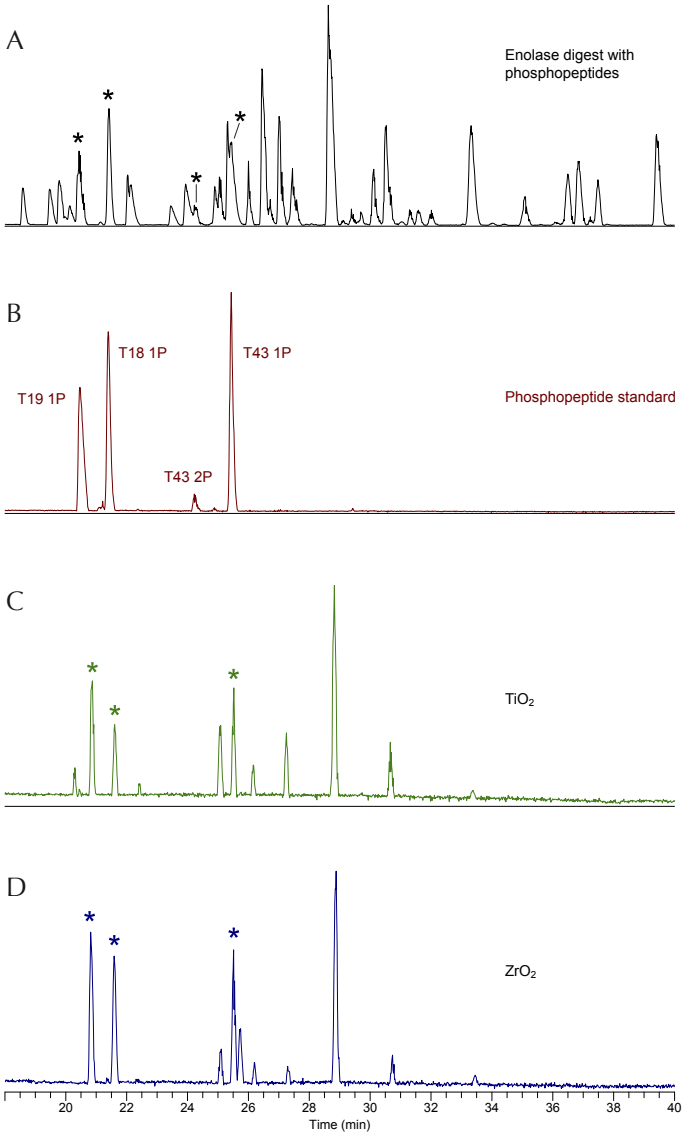

FIGURE 6

References

1. Toher, C.J.; Perala, A.W.; Shukla, A.K.; Valaskovic, G.A.; Oetting, A.A.; Shukla, M.M. "Offline Nano-ESI Phosphopeptide Analysis with Carbon, TiO_2, and ZrO_2 Wall-Coated Trap'nTips". Poster presented at Association of Biomolecular Research Facilities Conference, Long Beach, CA, 2006.
2. Toher, C.J.; Perala, A.W.; Shukla, A.K.; Valaskovic, G.A.; Oetting, A.A.; Shukla, M.M., Marshall-Waggett, C.J. "Online and Offline Nanoelectrospray Analysis of Phosphopeptides Purified by TiO_2, ZrO_2, and Carbon Wall-Coated Pipette Tips". Poster presented at American Society for Mass Spectrometry, Seattle, WA, 2006.