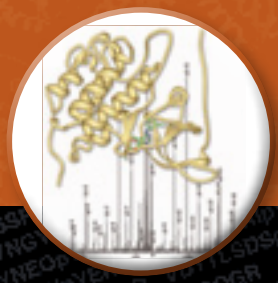

PTMScan®
Proteomics Kit Protocol

UNPARALLELED PRODUCT QUALITY, VALIDATION, AND TECHNICAL SUPPORT

Cell Signaling TECHNOLOGY®

Pharma Services Department ■ ptmscan@cellsignal.com **Web** ■ **www.cellsignal.com/proteomics**

A. Solutions and Reagents

Reagents Not Included:

1. HEPES (Sigma, H-4034)
2. Sodium pyrophosphate (Sigma, S-6422)
3. β-glycerophosphate (Sigma, G-9891)
4. Urea, Sequanal Grade (Thermo Scientific, 29700)
5. Sodium orthovanadate (Sigma, S-6508)
6. Iodoacetamide (Sigma, I-6125)
7. Dithiothreitol (American Bioanalytical, AB-00490)
8. Trypsin-TPCK (Worthington, LS-003744)
9. Trypsin (Promega, V5113)
10. Lysyl Endopeptidase, LysC (Wako, 129-02541)
11. Trifluoroacetic acid, Sequanal Grade (Thermo Scientific, 28903)
12. Acetonitrile (Thermo Scientific, 51101)
13. Sep-Pak® Classic C18 columns, 0.7 ml (Waters, WAT051910)
14. Burdick and Jackson Water (Honeywell, AH365-4)

NOTE: Prepare solutions for cell lysis, Sep-Pak purification, and IAP enrichment with Milli-Q or equivalent grade water. Prepare solutions for subsequent steps with HPLC grade water (Burdick and Jackson water).

Stock Solutions:

1. **200 mM HEPES/NaOH, pH 8.0:** Dissolve 23.8 g HEPES in approximately 450 ml water, adjust pH with 5 M NaOH to 8.0, and bring to a final volume of 500 ml. Filter through a 0.22 µM filter (as used for cell culture), use for up to 6 months.
2. **Sodium pyrophosphate: Make 50X stock (125 mM, MW = 446):** 1.1 g/20 ml. Store at 4°C, use for up to one month.
3. **β-glycerophosphate:** Make 1000X stock (1 M, MW = 216): 2.2 g/10 ml. Divide into 100 µl aliquots and store at -20ºC.
4. **Sodium orthovanadate (Na_3VO_4):** Make 100X stock (100 mM, MW = 184): 1.84 g/100 ml. Sodium orthovanadate must be depolymerized (activated) according to the following protocol:
   a. For a 100 ml solution, fill up with water to approximately 90 ml. Adjust the pH to 10.0 using 1 M NaOH with stirring. At this pH, the solution will be yellow.
   b. Boil the solution until it turns colorless and cool to room temperature (put on ice for cooling).
   c. Readjust the pH to 10.0 and repeat step 2 until the solution remains colorless and the pH stabilizes at 10.0 (usually it takes two rounds). Adjust the final volume with water.
   d. Store the activated sodium orthovanadate in 1 ml aliquots at -20°C. Thaw one aliquot for each experiment; do not refreeze thawed vial.
5. **Dithiothreitol (DTT):** Make 1.25 M stock (MW = 154): 19.25 g/100 ml. Divide into 200 µl aliquots, store at -20ºC for up to one year. Thaw one aliquot for each experiment.
6. **Trypsin-TPCK:** Store dry powder for up to 2 years at -80ºC. Parafilm cap of trypsin container (Worthington) to avoid collecting moisture, which can lead to degradation of the reagent. Prepare 1 mg/ml stock in 1 mM HCl. Divide into 1 ml aliquots, store at -80ºC for up to one year.
7. **Lysyl Endopeptidase (LysC):** Store dry powder up to 2 years at -80ºC. Parafilm cap of LysC container to avoid collecting moisture, which can lead to degradation of the reagent. Prepare 5 mg/ml stock in 20 mM HEPES pH 8.0. Divide into single use aliquots, store at -80ºC for up to one year.

Cell Lysis and Protein Digestion

A. Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalent grade water.

1. **Urea Lysis Buffer:** 20 mM HEPES pH 8.0, 9 M urea, 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate.

   NOTE: The Urea Lysis Buffer should be prepared prior to each experiment. Aliquots of the Urea Lysis Buffer can be stored in the -80°C freezer for up to 6 months.

   NOTE: Dissolving urea is an endothermic reaction. Urea Lysis Buffer preparation can be facilitated by placing a stir bar in the beaker and by using a warm (not hot) water bath on a stir plate. 9 M urea is used so that upon lysis, the final concentration is approximately 8 M. The urea lysis buffer should be used at room temperature. Placing the urea lysis buffer on ice will cause the urea to precipitate out of solution.
2. **DTT solution, 1.25 M** (see stock solutions for preparation)
3. **Iodoacetamide solution:** Dissolve 95 mg of iodoacetamide (formula weight = 184.96 mg/mmol) in water to a final volume of 5 ml. After weighing the powder, store in the dark and add water only immediately before use. The iodoacetamide solution should be prepared fresh prior to each experiment.

B. Preparation of Cell Lysate, Suspension Cells

1. Grow approximately 1-2 x 10^8 cells for each experimental condition (enough cells to produce approximately 10-20 mg of soluble protein).

   NOTE: Cells should be washed with 1X PBS before lysis to remove any media containing protein contaminants. Elevated levels of media-related proteins will interfere with the total protein determination.
2. Harvest cells by centrifugation at 130 rcf (g), for 5 min at room temperature. Carefully remove supernatant, wash cells with 20 ml of cold PBS, centrifuge and remove PBS wash and add add 10 ml Urea Lysis Buffer (room temperature) to the cell pellet. Pipet the slurry up and down a few times (do not cool lysate on ice as this may cause precipitation of the urea).

   NOTE: If desired, the PTMScan™ protocol may be interrupted at this stage. The harvested cells can be frozen and stored at -80°C for several weeks.
3. Using a microtip, sonicate at 15 W output with 3 bursts of 15 sec each. Cool on ice for 1 min between each burst. Clear the lysate by centrifugation at 20,000 rcf (g) for 15 min at 15ºC or room temperature and transfer the protein extract (supernatant) into a new tube.

   NOTE: Centrifugation is performed at 15°C or room temperature to prevent urea from precipitating out of solution. Centrifugation should be performed in an appropriate container rated for at least 20,000 rcf (g).

C. Peparation of Cell Lysate, Adherent Cells

1. Grow 1-2 x 10^8 cells for each experimental condition (enough cells to produce approximately 10-20 mg of soluble protein). The cell number corresponds to approximately THREE to TEN 150 mm culture dishes (depending on the cell type), grown to between 70-80% confluence.

   NOTE: Cells should be washed with 1X PBS before lysis to remove any media containing protein contaminants. Elevated levels of media-related proteins will interfere with the total protein determination.
2. Take all 150 mm culture dishes for one sample, remove media from the first dish by decanting, and let stand in a tilted position for 30 seconds so the remaining medium flows to the bottom edge. Remove the remainder of the medium at the bottom edge with a P-1000 micropipettor. Wash each dish with 5 ml of cold PBS. Remove PBS as described above.
3. Add 10 ml of Urea Lysis Buffer (at room temperature) to the first dish, scrape the cells into the buffer, let the dish stand in tilted position after scraping the buffer to the bottom edge of the tilted dish. Remove the medium from the second dish as above. Transfer the lysis buffer from the first dish to the second dish using a 10 ml pipette, then tilt the first dish with the lid on for 30 sec and remove remaining buffer from the dish and collect. Scrape cells from the second dish and repeat the process until the cells from all the dishes have been scraped into the lysis buffer. Collect all lysate in a 50 ml conical tube.

   NOTE: DO NOT place Urea Lysis Buffer or culture dishes on ice during harvesting. Harvest cells using Urea Lysis Buffer at room temperature. During lysis, the buffer becomes viscous due to DNA released from the cells.
4. The yield will be approximately 9-12 ml lysate after harvesting all the culture plates.

   NOTE: If desired, the PTMScan™ protocol may be interrupted at this stage. The cell lysate can be frozen and stored at -80°C for several weeks.
5. Using a microtip, sonicate at 15 W output with 3 bursts of 15 sec each. Cool on ice for 1 min between each burst. Clear the lysate by centrifugation at 20,000 rcf (g) for 15 min at 15ºC or room temperature and transfer the protein extract (supernatant) into a new tube.

   NOTE: Lysate sonication fragments DNA and reduces sample viscosity. Ensure that the sonicator tip is submerged in the lysate. If the sonicator tip is not submerged properly, it may induce foaming and degradation of your sample (refer to the manufacturer's instruction manual for the sonication apparatus).

D. Reduction and Alkylation of Proteins

1. Add 1/278 volume of 1.25 M DTT to the cleared cell supernatant (e.g. 36 µl of 1.25 M DTT for 10 ml of protein extract), mix well and place the tube into a 55ºC incubator for 30 min.
2. Cool the solution on ice briefly until it has reached room temperature (tube should feel neither warm nor ice-cold by hand).
3. Add 1/10 volume of iodoacetamide solution to the cleared cell supernatant, mix well, and incubate for 15 min at room temperature in the dark.

E. Protease Digestion

Protease Digestion Reference Table:

No.	PTMScan Kit Description	Recommended Protease Treatment
5563	PTMScan® Phospho-Akt Substrate Motif mAb 2 (RXRXXS*/T*) Kit	LysC*
5561	PTMScan® Phospho-Akt Substrate Motif mAb 1 (RXXS*/T*)	LysC*
5564	PTMScan® Phospho-AMPK Substrate Motif (LXRXXS*/T*)	LysC*
5565	PTMScan® Phospho-PKA Substrate Motif (RRXS*/T*)	LysC*
8803	PTMScan® Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit	Trypsin
5636	PTMScan® Phospho-Tyrosine Mouse mAb (P-Tyr-100)	Trypsin
5567	PTMScan® Phospho-T*PP Motif (T*PP) XP® Kit	Trypsin
5566	PTMScan® Phospho-ST*P Motif (ST*P) XP® Kit	Trypsin
4652	PTMScan® Phospho-MAPK/CDK Substrate Motif (PXS*P and S*PXK/R) Kit	Trypsin
5562	PTMScan® Ubiquitin Remnant Motif (K-ε-GG) Kit	Trypsin

* For LysC-digested material, there is a second digestion performed after the StageTip purification of enriched peptides (see the protocol after StageTip Purification).

Please visit http://www.cellsignal.com/services/ptmscan_kits.html for an updated version of the table

NOTE: Alternative proteases such as GluC, chymotrypsin, and others can be used in addition to the protease treatments outlined above to expand the coverage of modified peptides from each Motif Antibody. When considering the use of additional protease treatments it should be compatible with the respective Motif Antibody by not cleaving residues within the designated sequence motif. Protease treatments that generate larger proteolytic peptides may not be ideal if the resulting peptides do not ionize well in the mass spectrometer.

1. Dilute 3-fold with 20 mM HEPES pH 8.0 to a final concentration of 2 M urea, 20 mM HEPES, pH 8.0. For example, for 10 ml of lysate add 30 ml 20 mM HEPES pH 8.0.

F. Trypsin Digestion

1. Add 1/100 volume of 1 mg/ml trypsin-TPCK (Promega) stock in 1 mM HCl and digest overnight at room temperature with mixing.
2. Analyze the lysate before and after digest by SDS-PAGE to check for complete digestion.
3. Continue through the Sep-Pak, IAP, and StageTip protocols prior to LC-MS analysis of enriched peptides.

G. LysC Digestion

1. Prepare 5 mg/ml stock solution of LysC in 20 mM HEPES pH 8.0. Aliquot for single use and store at -80°C.
2. Add LysC solution to peptides at 1:250 (w:w). For 20 mg sample, use 20 mg ÷ 250 = 80 µg x 1 µl/5 µg = 16 µl LysC and digest overnight at room temperature.
3. Analyze the lysate before and after digest by SDS-PAGE to check for complete digestion.
4. Continue through the Sep-Pak, IAP, and StageTip protocols before conducting the **SECONDARY DIGESTION** with trypsin (see end of protocol, Trypsin Digestion of Enriched LysC Peptides).

Sep-Pak® C_{18} Purification of Lysate Peptides

NOTE: Purification of peptides is performed at room temperature on 0.7 ml Sep-Pak columns from Waters Corporation, WAT051910.

NOTE: Sep-Pak® C18 purification utilizes reversed-phase (hydrophobic) solid-phase extraction. Peptides and lipids bind to the chromatographic material. Large molecules such as DNA, RNA, and most protein, as well as hydrophilic molecules such as many small metabolites are separated from peptides using this technique. Peptides are eluted from the column with 40% acetonitrile (MeCN) and separated from lipids and proteins, which elute at approximately 60% MeCN and above.

NOTE: About 20 mg of protease-digested peptides can be purified from one Sep-Pak column. Purify peptides immediately after proteolytic digestion.

A. Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalent grade water. Organic solvents (trifluoroacetic acid, acetonitrile) should be of the highest grade. All percentage specifications for solutions are vol/vol.

1. **20% trifluoroacetic acid (TFA):** add 10 ml TFA to water to a total volume of 50 ml.
2. **Solvent A (0.1% TFA):** add 5 ml of 20% TFA to 995 ml water.
3. **Solvent B (0.1% TFA, 40% acetonitrile):** add 400 ml of acetonitrile (MeCN) and 5 ml of 20% TFA to 500 ml of water, adjust final volume to 1 L with water.

B. Acidification of Digested Cell Lysate

NOTE: Before loading the peptides from the protein digest on the column, the digest must be acidified with TFA for efficient peptide binding. The acidification step helps remove fatty acids from the digested peptide mixture.

1. Add 1/20 volume of 20% TFA to the digest for a final concentration of 1% TFA. Check the pH by spotting a small amount of peptide sample on a pH strip (the pH should be under 3). After acidification, allow precipitate to form by letting stand for 15 min on ice.
2. Centrifuge the acidified peptide solution for 15 min at 1,780 rcf (g) at room temperature to remove the precipitate. Transfer peptide-containing supernatant into a new 50 ml conical tube without dislodging the precipitated material.

C. Peptide Purification

NOTE: Application of all solutions should be performed by gravity flow.

1. Connect a 10 cc reservoir (remove 10 cc plunger) to the **SHORT END** of the Sep-Pak column.
2. Pre-wet the column with 5 ml 100% MeCN.

   NOTE: Each time solution is applied to the column air bubbles form in the junction where the 10 cc reservoir meets the narrow inlet of the column. These must be removed with a gel-loader tip placed on a P-200 micropipettor, otherwise the solution will not flow through the column efficiently. Always check for appropriate flow.
3. Wash sequentially with 1 ml, 3 ml, and 6 ml of Solvent A (0.1% TFA).
4. Load acidified and cleared digest (from **Section B**).

   NOTE: In rare cases, if the flow rates decrease dramatically upon (or after) loading of sample, the purification procedure can be accelerated by gently applying pressure to the column using the 10 cc plunger after cleaning it with organic solvent. Again make sure to remove air bubbles from the narrow inlet of the column before doing so. Do not apply vacuum (as advised against by the manufacturer).
5. Wash sequentially with 1 ml, 5 ml, and 6 ml of Solvent A (0.1% TFA).
6. Wash with 2 ml of 5% MeCN, 0.1% TFA.
7. Place columns above new 15 or 50 ml polypropylene tubes to collect eluate. Elute peptides with a sequential wash of 3 x 2 ml of Solvent B (0.1% TFA, 40% acetonitrile).
8. Freeze the eluate on dry ice (or -80°C freezer) for 2 hr to overnight and lyophilize frozen peptide solution for a minimum of 2 days to assure TFA has been removed from the peptide sample.

   NOTE: The lyophilization should be performed in a standard lyophilization apparatus. **DO NOT USE a SPEED-VAC apparatus at this stage of the protocol.**

   NOTE: The lysate digest may have a much higher volume than the 10 cc reservoir will hold (up to 50-60 ml from adherent cells) and therefore the peptides must be applied in several fractions. If available a 60 cc syringe may be used in place of a 10 cc syringe to allow all sample to be loaded into the syringe at once.

   NOTE: Lyophilization, the digested peptides are stable at -80°C for several months (seal the closed tube with parafilm for storage). The PTMScan™ procedure can be interrupted before or after lyophilization. Once the lyophilized peptide is dissolved in IAP buffer (see next step), continue to the end of the procedure.

Immunoaffinity Purification (IAP)

A. Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalent grade water. Trifluoroacetic acid should be of the highest grade. All percentage specifications for solutions are vol/vol.

1. **Materials Provided in the PTMScan Kit:** 10X IAP buffer; dilute with water to 1X buffer before use. Store 1X buffer up to one month at 4°C.

B. Procedure

1. Centrifuge the tube containing lyophilized peptide in order to collect all material to be dissolved. Add 1.4 ml IAP buffer. Resuspend pellets mechanically by pipetting repeatedly with a P-1000 micropipettor taking care not to introduce excessive bubbles into the solution. Transfer solution to a 1.7 ml Eppendorf tube.

   NOTE: After dissolving the peptide, check the pH of the peptide solution by spotting a small volume on pH indicator paper (The pH should be close to neutral, or no lower than 6.0. In the rare case that the pH is more acidic (due to insufficient removal of TFA from the peptide under suboptimal conditions of lyophilization), titrate the peptide solution with 1 M Tris base solution that has not been adjusted for pH. 5-10 µl is usually sufficient to neutralize the solution.
2. Clear solution by centrifugation for 5 min at 10,000 rcf (g) at 4°C in a microcentrifuge. The insoluable pellet may appear considerable. This will not pose a problem since most of the peptide will be soluable). Cool on ice.
3. Wash motif antibody-bead slurry sequentially, four times with 1 ml of 1X PBS and resuspend as a 50% slurry in PBS to remove the glycerol contaminating buffer.
4. Transfer the peptide solution into the microfuge tube containing motif antibody beads. Pipet sample directly on top of the beads at the bottom of the tube to ensure immediate mixing. Avoid creating bubbles upon pipetting.
5. Incubate for 2 hr on a rotator at 4°C. Before incubation, seal the microfuge tube with parafilm in order to avoid leakage.
6. Centrifuge at 2,000 rcf (g) for 30 sec and transfer the supernatant with a P-1000 micropipettor to a labeled Eppendorf tube to save for future use. Flow-through material can be used for subsequent IAPs.

   NOTE: In order to recover the beads quantitatively, do not spin the beads at lower g-forces than what is specified in this procedure. Avoid substantially higher g-forces as well, since this may cause the bead matrix to collapse. All centrifugation steps should be performed at the recommended speeds throughout the protocol.

   NOTE: If the cells were directly harvested from culture medium without PBS washing, some Phenol Red pH indicator will remain (it co-elutes during the Sep-Pak® C18 purification of peptides) and color the peptide solution yellow. This coloration has no effect on the immunoaffinity purification step.

   NOTE: All subsequent wash steps are at 0-4°C.

   NOTE: In all wash steps, the supernatant should be removed reasonably well. Avoid removing the last few microliters, except in the last step, since this may cause inadvertent carry-over of the beads.
7. Add 1 ml of IAP buffer to the beads, mix by inverting tube 5 times, centrifuge for 30 sec, and remove supernatant with a P-1000 micropipettor.
8. Repeat step 7 once for a total of TWO IAP buffer washes.

   NOTE: All steps from this point forward should be performed with solutions prepared with Burdick and Jackson or other HPLC grade water.
9. Add 1 ml chilled Burdick and Jackson water to the beads, mix by inverting tube 5 times, centrifuge for 30 sec, and remove supernatant with a P-1000 micropipettor.
10. Repeat step 9 two times for a total of THREE water washes. During the last water wash, the tube may need to be shaken while inverting in order to ensure efficient mixing.

    NOTE: After the last wash step, remove supernatant with a P-1000 micropipettor as before, then centrifuge for 5 sec to remove fluid from the tube walls, and carefully remove all remaining supernatant with a gel loading tip attached to a P-200 micropipettor.
11. Add 55 µl of 0.15% TFA to the beads, tap the bottom of the tube several times (do not vortex), and let stand at room temperature for 10 min, mixing gently every 2-3 min.

    NOTE: In this step, the post-translationally modified peptides of interest will be in the eluent.
12. Centrifuge 30 sec at 2,000 rcf (g) in a microcentrifuge and transfer supernatant to a new 1.7 ml Eppendorf tube.
13. Add 50 µl of 0.15% TFA to the beads, and repeat the elution/centrifugation steps. Combine both eluents in the same 1.7 ml tube. Briefly centrifuge the eluent to pellet any remaining beads and carefully transfer eluent to a new 1.7 ml tube taking care not to transfer any beads.

Concentration and Purification of Peptides for LC-MS Analysis

NOTE: We recommend concentrating peptides using the following protocol by Rappsilber et. al. (Rappsilber, J., Ishihama, Y. & Mann, M. (2003) Stop and go extraction tips for matrix–assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal. Chem.* 75, 663–670).

NOTE: We recognize there are many other routine methods for concentrating peptides using commercial products such as ZipTip® (see link provided below) and StageTips (see link provided below) that have been optimized for peptide desalting/concentration. Regardless of the particular method, we recommend that the
method of choice be optimized for recovery and be amenable for peptide loading capacities of at least 10 µg.

StageTips: http://www.proxeon.com/productrange/sample_preparation_and_purification/stage_tips/index.html

ZipTip®: http://www.millipore.com/catalogue/item/ZTC18S096

Concentration and Purification of Peptides for LC-MS on StageTip

A. Solutions and Reagents

NOTE: Prepare solutions with HPLC grade water. Organic solvents (trifluoroacetic acid, acetonitrile) should be of the highest grade.

1. **Solvent C (0.1% trifluoroacetic acid, 50% acetonitrile):** add 0.1 ml trifluoroacetic acid to 40 ml water, then add 50 ml acetonitrile, adjust the final volume to 100 ml with water.
2. **Solvent D (0.1% trifluoroacetic acid):** add 0.1 ml trifluoroacetic acid to 50 ml water, adjust the final volume to 100 ml with water.
3. **Solvent E (0.1% trifluoroacetic acid, 40% acetonitrile):** add 0.1 ml trifluoroacetic acid to 30 ml water, then add 40 ml acetonitrile, adjust the final volume to 100 ml with water.

   NOTE: Organic solvents are volatile. Tubes containing small volumes of these solutions should be prepared immediately before use and should be kept capped as much as possible, because the organic components evaporate quickly.

B. Procedure

1. Equilibrate the StageTip by passing 50 µl of Solvent C through (once) followed by 50 µl of Solvent D 2 times.
2. Load sample by passing IP eluent through the StageTip. Load IAP eluent in 2 steps using 50 µl in each step.
3. Wash the StageTip by passing 55 µl of Solvent D through 2 times.
4. Elute peptides off the StageTip by passing 10 µl of Solvent E through 2 times, pooling the resulting eluent.

   NOTE: For enriched LysC peptides, a second digest with trypsin will be performed. Therefore, we recommend eluting the LysC peptides into a 0.5 ml Eppendorf tube in preparation for the trypsin digestion protocol, described below.
5. Dry down the StageTip eluent in a vacuum concentrator (Speed-Vac) and redissolve the peptides in an appropriate solvent for LC-MS analysis such as 5% acetonitrile, 0.1% TFA.

Trypsin Digestion of Enriched LysC Peptides

NOTE: Continued from **Section G** of Cell Lysis and Protein Digestion

NOTE: Trypsin digestion of enriched LysC peptides is recommended for all basophilic motif antibodies.

1. Prepare 1 M ammonium bicarbonate stock solution.
2. Prepare digestion buffer, 50 mM ammonium bicarbonate with 5% acetonitrile.
3. Dilute a stock solution of sequencing grade trypsin (Promega) with digestion buffer from 0.4 µg/µl to a final concentration of 25 ng/µl.
4. Resuspend the dried, LysC digested peptides generated from the StageTip concentration protocol above with 10 µl of trypsin solution (25 ng/µl, 250 ng total). Vortex 3 times to redissolve the peptides and microfuge the sample to collect peptides/trypsin solution at the bottom of the microfuge tube as the final step.
5. Incubate the solution at 37°C for 2 hr.
6. After trypsin digestion, add 1 µl of 5% TFA to the digest solution. Vortex to mix and microfuge to collect peptide solution at the bottom of the microfuge tube.
7. Transfer the acidified peptide solution to a newly conditioned StageTip, rinse the 0.5 ml Eppendorf tube with 40 µl of 0.1% TFA once and apply the rinse solution to the StageTip.
8. Perform the StageTip desalting of the peptide digest and elute the peptides into an HPLC insert. Dry purified peptides under vacuum prior to LC-MS analysis (as described above).