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Development of a Targeted Urine Proteome Assay for Kidney Diseases

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Abstract

Human urine is the least invasive and most readily available bio fluid whose proteome has been shown to change in response to disease or drug treatment. Urine is thus very amenable to quantitative proteomics and is a logical sample choice for identifying protein biomarkers for kidney diseases. In this study potential biomarkers were identified initially by using a multi-proteomics workflow to compare urine proteomes of kidney transplant patients who exhibited immediate versus delayed graft function. To comprehensively interrogate the urine proteome two “bottom up”, mass spectrometric-based discovery approaches, iTRAQ and Label Free Quantitation (LFQ), were complemented by Differential Fluorescence Gel Electrophoresis (DIGE) analyses of intact urine proteins from kidney transplant recipients who received a deceased donor kidney. Differentially expressed proteins in the two patient groups were identified, and corresponding stable isotope-labeled internal peptide standard (SIS) peptides were synthesized for scheduled multiple reaction monitoring (MRM). The Targeted Urine Proteome Assay (TUPA) was then developed by identifying those peptides for which there were at least 2 transitions for which interference in a urine matrix across 156 MRM runs was less than 30%. This resulted in a final assay that monitors 224 peptides corresponding to 167 quantifiable proteins.

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Conflict of Interest Statement

The authors declare there are no conflicts of interest.

Keywords

Chronic kidney disease; Polycystic kidney disease; Targeted Proteomics; Urine

Introduction

Kidney disease can result from a wide variety of insults including acute kidney injury (AKI), diabetes, hypertension, medications, and autoimmune diseases. Currently, the impact of these disparate conditions on kidney function is assessed by changes in serum creatinine levels which reflect only glomerular filtration rates, not structural kidney disease. This limitation is a major obstacle to implementing successful interventions to either prevent the onset of kidney diseases or to delay their progression to chronic kidney disease (CKD) and/or end-stage renal disease (ESRD). An ideal kidney disease biomarker would allow the identification of kidney disease before enough structural injury accrues to impair filtration, at a time when early intervention might have the greatest impact. In addition, such a biomarker would predict progression to CKD and, potentially, serve as a pharmacodynamic outcome for therapeutic trials.

A major reason behind the current deficiency of such non-invasive biomarkers for kidney injury is the presence of a vast spectrum of kidney diseases that have disease-specific underlying pathogenic mechanisms and exhibit predilections for distinct compartments within the kidney. Diseases such as diabetes predominantly affect the glomerulus and thus the presence of abnormal levels of albuminuria is used for their detection and monitoring. However, albuminuria is not specific for diabetic nephropathy and can be seen in any form of glomerular injury. In contrast, polycystic kidney disease primarily affects the tubules and is diagnosed by detection of tubular cysts on radiological imaging. In both cases the biomarker (albuminuria and cyst imaging) is seen only after significant, non-reversible anatomical changes have occurred.

For these reasons better non-invasive biomarkers are needed to accurately identify the underlying disease pathogenesis, monitor response to therapy, and predict patients at greatest risk for progression to CKD. Much of the current work in kidney disease biomarkers has focused on changes in excretion of urinary proteins such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), Interleukin -18 (IL-18) [1] and Insulin-like growth factor-binding protein 7 [2]. However, due to the wide variety of diseases that affect the kidney, it is unlikely that a single biomarker, or even small group of biomarkers, will provide sufficient diagnostic accuracy across such a broad spectrum of disorders. Therefore, we have chosen to develop a proteomic approach in order to simultaneously quantify multiple proteins from a single biological specimen. We have focused these efforts on the urinary proteome since changes in urinary protein concentrations can reflect either abnormal filtration due to glomerular disease, abnormal reabsorption or secretion due to tubular diseases, or abnormal secretion from interstitial inflammatory cells.

To date there have been >1,500 urinary proteins and >5,000 urinary peptides identified in healthy and diseased individuals [3, 4]. The approaches for detecting these proteins often

involve initial separation of proteins on 1D or 2-D gels followed by tryptic digestion and mass spectrometry of each individual gel fraction or spot. These techniques are laborious and time consuming, and provide only relative rather than absolute quantification of the resultant peptides. In the current study, we used discovery proteomics and literature searches to identify differentially expressed urinary proteins in the setting of kidney injury, and then synthesized and screened appropriate peptides for quantitative analysis of those proteins using multiple reaction monitoring (MRM).

Materials and Methods

The following provides a brief overview of the experimental procedures with a more detailed description in Supporting Information.

Discovery Analyses

Urine from 4 patients with immediate graft function (IGF) and 5 patients with delayed graft function (DGF) following kidney transplantation was collected, centrifuged and frozen as described in Supporting Methods (see Supporting Table 1 for patient characteristics). Aliquots of the 4 IGF and 5 DGF urines were then pooled separately and discovery difference gel electrophoresis (DIGE) [5] [6], isobaric tags for relative and absolute quantitation (iTRAQ) [7] and label free quantitation (LFQ) [8] analyses performed to identify differentially expressed proteins as described in Supporting Methods. Individual LFQ analyses were carried out on those samples for which there was sufficient remaining material (4 of the IGF samples and 3 of the DGF samples). In addition to analyzing undepleted samples, discovery analyses were also carried out on pooled samples after depletion of either 14 highly abundant plasma proteins (HAPS, removes 95% of serum protein mass including albumin, IgG, α 1-Antitrypsin, IgA, IgM, transferrin, haptoglobin, α 2-macroglobulin, fibrinogen, complement C3, α 1-acid glycoprotein (orosomucoid), HDL (apolipoproteins A-I and A-II), LDL (mainly apolipoprotein B) or both HAPS and moderately abundant plasma proteins (MAPS, removes an additional 4% of moderately abundant proteins leaving 1% of the original protein amount enriched for low abundance proteins (LAP)(Supporting Figure 1)). Amino acid analysis was used to determine accurate total protein concentrations of the pooled urine samples used for DIGE, iTRAQ, and LFQ discovery analyses. Samples destined for iTRAQ and LFQ analyses were subjected to Lysyl endopeptidase and then trypsin digestion as described in Supporting Information.

Extended Multiple Reaction Monitoring (xMRM)

To identify optimal peptides for quantification of the differentially expressed proteins detected in our discovery studies, tryptic digests of 25 human urine samples (Supporting Table 1) from normal controls (n=5), transplant (n=11), and other kidney disease patients (n=9) were analyzed on an AB SCIEX 5600 TripleTOF hybrid triple quadrupole TOF mass spectrometer interfaced with a Waters nanoACQUITY UPLC® system running AB Sciex Analyst® 1.5 software (research version). Protein identification data obtained from the 5600 TripleTOF was filtered at 1% FDR (false discovery rate, decoy using reversed protein sequences) and then further filtered to include peptides with MASCOT scores greater than or equal to the identity score. The identity score was calculated as: $-10 * (\log (p/\#matches))$

where p is the probability threshold and #matches is the number of precursor matches. For each protein, the MS/MS spectra with the highest MASCOT scores were chosen and all y- and b-ions were matched to an insilico fragmentation and then sorted by peak height intensity. From there the list was filtered so that each protein had two peptides and each peptide was distinct within a combined Human Taxonomy SwissProt database blast search. Peptides containing methionine residues were excluded in all cases except one. This protein, S10A6, had only one non-methionine peptide (LQDAEIAR) that had an acceptable MASCOT score and so the methionine-containing peptide, LMEDLDR, was included for TUPA analysis.

The peptides were then rank ordered based on their number of occurrences in the Yale Protein Expression Database (YPED). The peptides that occurred most frequently in YPED were then used to synthesize 400 corresponding “heavy” idiotypic peptides (2 peptides/protein for 198 proteins, one peptide corresponding to the BIN1 protein, and three peptides for the S10A6 protein in order to include peptides $\pm$ oxidized Met) as internal quantitative standards. A scheduled LC-MRM assay was then built on an AB SCIEX 5500 Q-TRAP that has nearly identical front end quadrupoles (Q0, Q1, and Q2), MS/MS peptide fragmentation patterns, and that was equipped with the same Waters nanoACQUITY UPLC system as the AB SCIEX TripleTOF 5600, allowing us to assign precise retention times to each peptide identified from each protein and to import this information into YPED [9] where bioinformatic tools selected two peptides per protein and two MRM transitions per peptide for the TUPA MRM assay.

The MRM assay, which was developed using a previously described workflow [10], monitored five transitions from each of six “light” peptides from albumin, useful for estimating the relative amount of this typically most abundant urinary protein, resulting in 1,630 transitions for the combined “light” and “heavy” TUPA extended MRM (xMRM) assay (Supporting Table 3). Except where indicated in Supporting Table 3, the MRM method used peak windows of 5 min and a cycle time of 2.5 sec. In the case of hydrophilic peptides that elute before 20 minutes, the peak window was extended to 400 seconds to allow for the higher variability in the retention times of these peptides. The xMRM assay continuously monitors the primary (1) transition while the secondary transition (2) is only monitored when the primary transition reaches above the threshold value that was set at a value of 200 to be above the noise level. Additionally, the xMRM assay was run with the “timeslip” feature that extends the scheduled MRM window by an additional half window (2.5 minutes) if the peak area threshold is greater than 200 at the end of the first window. All transitions were weighted equally. The resulting LC-xMRM data was processed and quantitated using Multiquant™ 2.1 software (research version). The SignalFinder™ 2 algorithm (research version) was used to integrate and score peak groups.

Statistical Analysis

iTRAQ analyses were carried out in duplicate on undepleted, pooled samples from 4 IGF and 5 DGF samples. The peptides identified were subjected to Cyclic Lowess normalization to compensate for any differences between iTRAQ labels. After normalization the technical replicates were averaged together to enable pair-wise comparisons. From these data the

protein level fold-changes were calculated based on the mean of the peptide fold-changes observed for each protein with each protein level fold change being calculated from the mean of 3.8 peptide level fold changes that in turn resulted from the analysis of an average of 6.2 spectra/peptide or an average of 23.5 data points/protein.

2D DIGE was carried out as described in Supporting Methods. Amersham Biosciences DeCyder software was used to quantify the gel images and spots with >1.5 fold change in Cy5/Cy3 ratio were chosen for MALDI-TOF/TOF analysis and MS/MS-based protein identification.

LFQ analyses were carried out in triplicate as described in Supporting Methods. The LC-MS/MS data was processed with Progenesis LC-MS software (Nonlinear Dynamics, LLC, version 3.1.4003) with protein identification carried out using the Mascot search algorithm (Matrix Science, version 2.4.0). The Progenesis LC-MS software performs feature/peptide extraction, chromatographic/spectral alignment (one run is chosen as a reference for alignment), data filtering, and statistical analysis. A normalization factor for each run was calculated to account for differences in sample load between injections as well as differences in ionization. The normalization factor was determined by calculating a quantitative abundance ratio between the reference run and the run being normalized, with the underlying assumption being that the quantitative value should equal 1 for most proteins. The experimental design was setup to group multiple injections from each run. The algorithm then calculated the tabulated raw and normalized abundances, maximum fold change, and Anova *p* values for each feature in the data set. The MS/MS spectra (i.e., a combined list of all LC-MS/MS runs in the experiment) were exported as .mgf (Mascot generic files) for database searching. The Mascot search results were exported as .xml files using a significance cutoff of *p*<0.05 and FDR of 1%, which were then imported into the Progenesis LC-MS software, where search hits were assigned to corresponding features or peptides. In the case of the individual LFQ analyses, the protein-level fold change was calculated as the average IGF/DGF fold change of peptides from each protein.

Literature Search

A literature search was performed to identify differentially expressed proteins in the setting of acute kidney injury using peer-reviewed research published between January 1966 and March 2014 on MEDLINE (Pubmed) using the Evidence-Based Health Informatics Nephrology Filters developed by the Health Information Unit, McMaster University (http://hiru.mcmaster.ca/hiru/HIRU_Hedges_Nephrology_Filters.aspx). Using the “All Nephrology” content search with a “Sensitive (broad)” filter, we inputted search terms including permutations of *urine*, *acute kidney injury*, *acute renal failure*, *kidney transplant*, and *urine proteomics*. The search was limited to human studies.

Data Sharing and Dissemination

Data from this study are permanently archived in YPED [9]. Data in YPED are stored in MIAPE compliant formats [11] that utilize the current HUPO Proteomics Standards Initiative modules (<http://www.psdev.info/miape>) and their associated XML markup languages (e.g. mzML format for storing mass spectrometry data). In addition, all raw data,

MRM methods, and MultiQuant results have been uploaded to PeptideAtlas [12] with identifier PASS00616 (<http://www.peptideatlas.org/PASS/PASS00616>), Username: PASS00616 and Password: VS9473yc.

Ethical Considerations

All the patients who provided urine samples for this study signed an informed consent document approved by the Yale Human Investigations Committee.

Results

Discovery Proteomics

The multi-quantitative proteomics approach depicted in Figure 1 was used to identify differentially expressed urinary proteins from 4 IGF versus 5 DGF hospitalized kidney transplant recipients. Urine proteomes in these two groups of patients were first interrogated using pooled, undepleted urine protein as the starting material. iTRAQ and LFQ analyses of Lysyl endopeptidase/trypsin digested samples and DIGE analysis of intact proteins was then performed. The combined results of the three analyses identified and relatively quantified 256 unique proteins, with 134 of these changed by two-fold or more between the two patient groups. LFQ provided the greatest number of candidate proteins (144 proteins identified with 89 exhibiting 2 fold change), followed by iTRAQ (59 with 2 fold change) and DIGE (14 with 2 fold change) (Figure 2A). Only 6 of the differentially expressed proteins were identified by all three analyses, AMBP (alpha-1-microglobulin/bikunin precursor), PGBM (basement membrane-specific heparin sulfate proteoglycan core protein), PTGDS (prostaglandin D2 synthase), RET4 (retinol-binding protein 4), APOA1 (apolipoprotein-A1) and HBB (hemoglobin subunit B). The remaining 122 identified proteins were not found to change significantly between IGF and DGF patients in any of the three analyses. LFQ analysis of 4 individual IGF and 3 individual DGF urine samples identified 79 differentially expressed proteins of which only 28 were also identified as differentially expressed in the pooled LFQ analysis (Figure 2B).

Since abundant proteins such as albumin can decrease the sensitivity of mass spectrometry for detecting low abundance proteins, discovery analyses were also performed after depletion of the top 14 HAPS or after depletion of both HAPS and MAPS in the same pooled urine samples. LFQ following HAPS depletion enabled the identification of 189 differentially expressed proteins in DGF versus IGF urine samples, 154 of which were not identified as significantly changing in the undepleted, pooled LFQ analyses (Figure 2C). Combined HAPS/MAPS depletion revealed an additional 38 low abundance proteins as differentially expressed that had not been identified in undepleted or HAPS depleted samples (Figure 2C). Thus LFQ analysis of HAPS combined with HAPS/MAPS depletion identified 192 proteins as differentially expressed that were not identified in the same undepleted samples. Finally, DIGE analysis of the HAPS/MAPS depleted pooled samples identified 14 proteins as differentially expressed in DGF versus IGF, with 9 of those proteins not having been detected by DIGE analysis of the undepleted samples (Figure 2D). *In toto*, depletion of HAPS and MAPS proteins allowed us to identify 235 differentially expressed

proteins of which 159 were not identified by analyses of the undepleted samples (Figure 2E).

Taken together these discovery analyses provided relative expression levels of 514 proteins, of which 332 (65%) were found to be changed by 2-fold or more in one or more of the analyses (Supporting Table 2).

Development of TUPA Panel

Optimized MRM Selection from 5600 TripleTOF—From the discovery analyses and literature searches (as described in Methods section), we compiled a list of 416 proteins (332 from discovery analyses and 84 from literature searches) for potential inclusion into TUPA. Since the peptide MS/MS fragmentation intensity patterns in tandem MS discovery data from Thermo Scientific Orbitrap Velos mass spectrometers using either Ion-Trap (CID) or Higher-Energy Collisional Dissociation (HCD) spectra are very different from those obtained on the AB SCIEX 5500 QTRAP mass spectrometer that is needed for the MRM assay [10], we performed 31 LC-MS/MS Information Dependent Acquisition (IDA) runs on an AB Sciex Triple TOF 5600 using 25 urine samples (the 9 used for discovery + 16 additional urines (Supporting Table 1)). This provided us with a urine proteome spectral library from which to search for the best peptides and fragmentation ions corresponding to the 416 proteins of interest (Supporting Figure 2). We were able to identify suitable peptides for 200 of the 416 proteins (198 proteins with 2 peptides, 1 protein with 2 peptides as well as a methionine-oxidized version of one of these peptides, and 1 protein with only 1 peptide), of which 116 came from the original discovery analyses and 84 from the literature search (Table 1). As described in Materials and Methods, for each of these 200 proteins/400 peptides we synthesized the corresponding SIS synthetic peptide for internal quantification of the corresponding urinary protein.

Transition Peak Interference—To assess TUPA MRM assay performance we first subjected the 400 SIS synthetic peptide surrogates for the 200 TUPA proteins to triplicate MS/MS analysis in 0.1% TFA solvent so that the average transition 1/transition 2 peak area ratio could be determined for each peptide. Six of the 400 SIS peptides were excluded by this analysis as these peptides failed to provide a measurable ratio, leaving 394 peptides that still represented the original 200 TUPA proteins (192 proteins with 2 peptides, 7 proteins with one peptide, 1 protein with 2 peptides as well as a methionine-oxidized peptide) (Supporting Table 4).

To identify possible interference of these peptides in urine matrix, the 394 SIS peptides were then added as a mixture into 52 human urine samples from kidney transplant recipients and subjected to 156 MRM runs. The average transition 1/transition 2 peak area ratio for the heavy SIS peptides were calculated for each run, and the average percent deviation from the corresponding ratios in 0.1% TFA solvent were determined across the 156 runs (Supporting Table 4). A 30% deviation across runs was set as the cut-off for acceptable interference based on guidelines established by the NIH Workshop for Tier 2 targeted proteomics assays in biomedical research and identification of interference-free transitions by Percy et al [13,

14]. Using these criteria, 224 of the original 400 SIS peptides were judged to be interference-free.

The 224 interference-free SIS peptides correspond to 167 of the original 200 TUPA proteins (Table 2). Of these 167 proteins, 111 have one qualified SIS peptide, 55 have 2 qualified SIS peptides, and one has 3 qualified SIS peptides (S10A6). Since the NIH Workshop on Targeted Peptide Measurements concluded that one interference-free transition is acceptable for protein quantitation [13], our current TUPA can potentially quantify the level of expression of 167 proteins in human urine. As one measure of how well this assay performs, during the triplicate LC-MRM analyses of the 52 human urine samples, 111 of the 167 (66%) SIS qualified proteins had one or more corresponding “light” peptides whose transitions were similarly qualified by meeting the 30% deviation criterion (Supporting Table 5, summarized in Table 2).

Discussion

Recent investigations into biomarkers of kidney injury have identified multiple markers capable of early detection of structural injury as well as associating with outcomes. While such results are promising, individual markers studies to date still lack in sensitivity and specificity. One of the primary reasons for this is likely the fact that such tests treat kidney disease as a homogenous entity. In reality, different diseases and insults affect the kidney in very disparate manners. While the end-stage of most renal diseases is similar (diffuse fibrosis), different diseases typically arrive at this common destination via very different pathways. Currently, by the time chronic kidney disease (CKD) has been detected, the disease is often irreversible and resistant to therapy. Knowledge, early in the course of a disease, of the specific pathophysiologic processes at work could allow for application of targeted and efficacious treatments. Distinguishing and identifying the disease pathways involved in the numerous diseases that affect the kidney will require a large panel of biomarkers able to tease out subtle mechanistic differences.

To this end, we have developed a library of quantifiable proteins using urine samples from patients undergoing deceased donor kidney transplantation. These samples were chosen to develop the library since deceased donor kidneys undergo significant cold ischemia time and thus the urine that is made following transplantation is predicted to contain a large number of proteins involved in kidney injury and repair.

To compile a robust list of potential biomarkers we carried out a comprehensive, multi-proteomics analysis that determined the relative expression in urine of 514 proteins in IGF and DGF patients. The discovery DIGE, iTRAQ, and LFQ analyses described in this study identified 134 potential biomarker proteins that were differentially expressed by two-fold or more in pooled, undepleted urine samples from kidney transplant patients with DGF versus IGF. The majority of these were identified as differentially expressed by only one of the three technologies (112 of the 134), with only 22 proteins identified by 2 technologies and only 6 identified by all three approaches. Of these 112 proteins that were identified by only one technology, 67 were identified by LFQ, 40 by iTRAQ, and 5 by DIGE. While these data suggest that LFQ might be the most useful of the three technologies, we note that the

reproducibly quantify the *same* proteins in repetitive discovery LC-MS analyses, cross-reactivity and lack of specific antibodies for many human proteins) of other MS/proteomic and immunological methods to identify and quantify protein biomarkers in the relatively large numbers of samples needed to validate potential biomarkers. Importantly, the multilaboratory study by Addona et al [18] demonstrates that LC-MRM “assays can be highly reproducible within and across laboratories and instrument platforms”.

We took advantage of the Yale Protein Expression Database (YPED, [9, 26]) that contains 340,449 distinct human peptides from 19,327 proteins to choose the best two peptide surrogates for each target protein by rank ordering the peptides for each protein based on their number of occurrences in YPED. The implicit assumption for rank ordering is that peptides that occur most frequently in YPED are the most likely to be isolated in high yield and to ionize well. Finally, the two most intense Y or B ions were chosen from each prototypical spectrum. As noted above, two keys to the success of this strategy are choosing two mass spectrometers that give nearly identical peptide fragmentation spectra and equipping both platforms with identical nanobore HPLC systems to ensure easy transference of fragmentation patterns and peptide elution times from the discovery to the targeted MS systems. The importance of rigorously determining the level of transition interference is highlighted by the finding that only 734 (46%) of the 1,600 transitions in the initial version of the TUPA assay met the criteria that we set for both the heavy and corresponding light peptides of having Signal/Noise >3 and transition peak area ratios (for the two transitions selected from each targeted peptide) in urine that differed by less than 30% from the corresponding ratios for the heavy SIS peptides in 0.1% TFA. In addition to interference from other peptides, another major cause of “light” transitions failing to meet these criteria is low abundance of the parent proteins. This is evident in Supporting Tables 4 and 5 where 88% (138/156) of the LC-MRM runs for SIS transitions had Signal/Noise above 3 as compared to only 62% of the corresponding “light” transitions.

The initial goal of this work was to use multi-proteomics analyses to identify proteins that are differentially expressed in kidney transplant patients who have DGF versus IGF. Since the maladaptive repair that occurs in DGF leads to inflammation and fibrosis which may progress to CKD, we reasoned that urine from DGF patients provides an opportunity for identifying urgently needed biomarkers for the development of CKD and disease progression. As a result of the findings reported in this study, a refined TUPA that meets the “best practices” for targeted peptide MRM assays [13] can now be brought to bear on kidney disease research that will enable the robust, high throughput quantitation of potential biomarkers for DGF specifically and, more generally, for abnormal repair and progression to CKD. Such an assay, with the ability to quantitate hundreds of proteins on a single run, could revolutionize mechanistic understanding of kidney disease. In addition, TUPA has the ability to bring into clinical practice an extraordinarily robust test involving specific diagnostic thresholds for hundreds of proteins. With the potential ability to quantify 167 proteins and the demonstrated ability to quantify 111 proteins (based on interference-free “light” and matching SIS transitions in urine matrix), TUPA is among the largest extant single injection LC-MRM assays with internal SIS peptides (see Supporting Table 6 for a list of other large published MRM assays) and can be utilized far beyond the specific setting of

DGF. Indeed, it may be that such an approach can move the diagnosis of kidney disease beyond invasive biopsies.

Going forward, multiple approaches may enable discovery analyses to reach deeper into the urine proteome to uncover additional biomarkers for kidney diseases. These include the use of Hexapeptide Combinatorial Ligand Libraries (CPLL) to equalize protein concentrations by depleting highly abundant and enriching for low abundant proteins [27] and the elegant differential trypsin digestion procedure of Fonslow et al [28] that exploits the abundance-dependent Michaelis-Menten kinetics of trypsin digestion to selectively digest and deplete abundant proteins. Lastly, the increased acquisition rate of new mass spectrometers should greatly increase the depth of coverage of discovery analyses of the human urine and other complex proteomes [29, 30].

We believe the current work represents an important step towards the development of a clinically useful assay for kidney diseases based on MRM analyses of urine samples. The TUPA assay, which is among the largest LC-MRM assays yet reported for human proteins, should also be useful for supporting other areas of biomedical research that extend well beyond chronic and acute kidney injury and repair. Further work is required to expand the library and validate the utility of the identified differentially expressed proteins in other clinical settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AKI	acute kidney injury
SIS	stable isotope–labeled internal peptide standard
YPED	Yale Protein Expression Database
DGF	delayed graft function
IGF	immediate graft function

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Statement of clinical relevance

Kidney disease can occur in response to many distinct pathogenic processes. Typically these diverse diseases are identified and followed using non-specific markers of kidney dysfunction including serum creatinine and urine albumin. These clinical surrogates are frequently not dysregulated until there is substantial, irreversible structural damage of the kidney. The Targeted Urine Proteome Assay (TUPA) that we have developed opens the way for using a robust mass spectrometric technology, Multiple Reaction Monitoring (MRM), for quantifying and validating biomarkers from amongst 167 urinary proteins. This approach, while developed using differentially expressed urinary proteins from patients with delayed graft function or immediate graft function after kidney transplant, can be expanded to include differentially expressed urinary proteins in multiple kidney diseases. Thus TUPA could provide a single diagnostic assay to identify a wide range of kidney diseases, as well as giving important patient-specific data for prognosis and disease management.

Figure 1.

Workflow of Discovery Proteomics and MRM Interference Analysis used for TUPA Development. Individual and pooled urine samples from patients with delayed graft function (DGF) and immediate graft function (IGF) after kidney transplantation were used for DIGE, LFQ, and iTRAQ discovery analyses with or without depletion of highly and moderately abundant proteins (HAPS and HAPS/MAPS depletion) followed by multiple reaction monitoring (MRM) analyses of SIS and corresponding endogenous peptides in solvent and human urine matrix.

Figure 2.

Depiction of the relative contribution of the Discovery Proteomic Approaches to identifying proteins that change by 2-fold for inclusion in TUPA.

A) Relative contributions of LFQ, iTRAQ and DIGE in identifying 134 proteins changing by 2-fold or more in pooled, undepleted DGF versus IGF human urine. 122 additional proteins were detected by these methods that did not change significantly.

B) Relative comparison of LFQ analysis of undepleted individual urine samples versus those same pooled samples. Of the 89 proteins identified as changing by 2-fold in pooled samples,

28 were also identified in the individual samples. Individual LFQ analysis identified an additional 51 proteins as changing by 2-fold, but failed to identify 61 proteins that were detected in the pooled samples.

C) Relative contributions of LFQ analyses of undepleted pooled urines versus those same urines subjected to HAPS or HAPS/MAPS depletion. HAPS depletion identified 154 proteins as changing by 2-fold that were not identified in undepleted samples, but failed to identify 54 proteins that were identified in the undepleted samples. HAPS/MAPS depletion identified an additional 38 proteins that were not identified by the other technologies.

D) Relative contributions of DIGE analyses of undepleted pooled urines versus those same urines subjected to HAPS/MAPS depletion. DIGE analyses identified 23 proteins as changing by 2-fold, with both undepleted and depleted samples contributing distinct protein populations.

E) Combined contributions of all analyses of undepleted urine samples versus HAPS or HAPS/MAPS depleted samples to TUPA. While both approaches identified large numbers of proteins as being differentially expressed between patients with DGF and those with IGF, HAPS and/or HAPS/MAPS depletion contributed significantly more to the candidate list than did the undepleted analyses.

Figure 3.

Development of TUPA from discovery through testing in human urine matrix. Of the 416 proteins identified by discovery analyses and literature searches as potential candidates for inclusion in TUPA, 200 had spectra in the YPED database that included at least 1 peptide deemed suitable for SIS peptide synthesis and quantification. Analysis of these SIS peptides in 0.1% TFA solvent and human urine demonstrated that 224 peptides covering 167 target proteins were interference-free and useable for quantitation. Preliminary analysis of human urine has shown that the endogenous light peptide(s) from at least 111 of these 167 proteins can be detected and quantified.

Table 1**Proteins in the Targeted Urine Proteome Assay (TUPA)**

#	UniProtKB Entry Name	Recommended Name	Basis for Inclusion in TUPA ¹
1	I433E_HUMAN	14-3-3 protein epsilon	Lit
2	6PGD_HUMAN	6-phosphogluconate dehydrogenase	HAPS/LFQ
3	6PGL_HUMAN	6-phosphogluconolactonase	Lit
4	A1AG1_HUMAN	Alpha-1-acid glycoprotein 1	Lit
5	A1AG2_HUMAN	Alpha-1-acid glycoprotein 2	Lit
6	A1AT_HUMAN	Alpha-1-antitrypsin	HAPS/LFQ, HAPS/MAPS/LFQ
7	A1BG_HUMAN	Alpha-1 B-glycoprotein	HAPS/LFQ
8	A2AP_HUMAN	Alpha-2-antiplasmin	HAPS/LFQ
9	A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	LFQ
10	A2MG_HUMAN	Alpha-2-macroglobulin	LFQ, LFQ/Individual
11	A4_HUMAN	Amyloid beta A4 protein	Lit
12	AFAM_HUMAN	Afamin	HAPS/LFQ, LFQ/Individual
13	AGRIN_HUMAN	Agrin	iTRAQ, LFQ/Individual
14	ALBU_HUMAN	Serum albumin	HAPS/LFQ, HAPS/MAPS/LFQ
15	ALDOA_HUMAN	Fructose-bisphosphate aldolase A	HAPS/LFQ
16	ALDOB_HUMAN	Fructose-bisphosphate aldolase B	Lit
17	AMBP_HUMAN	Protein AMBP	DIGE, iTRAQ, LFQ, HAPS/MAPS/DIGE, HAPS/LFQ, LFQ/Individual
18	AMPN_HUMAN	Aminopeptidase N	Lit
19	ANAG_HUMAN	Alpha-N-acetylglucosaminidase	Lit
20	ANGT_HUMAN	Angiotensinogen	LFQ, HAPS/LFQ, HAPS/MAPS/LFQ
21	ANT3_HUMAN	Antithrombin-III	HAPS/LFQ
22	APOA1_HUMAN	Apolipoprotein A-I	DIGE, iTRAQ, LFQ
23	APOA2_HUMAN	Apolipoprotein A-II	iTRAQ, LFQ
24	APOA4_HUMAN	Apolipoprotein A-IV	DIGE, LFQ, HAPS/MAPS/DIGE, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
25	APOC2_HUMAN	Apolipoprotein C-II	LFQ, LFQ/Individual
26	APOD_HUMAN	Apolipoprotein D	Lit
27	APOE_HUMAN	Apolipoprotein E	LFQ, LFQ/Individual
28	APOH_HUMAN	Beta-2-glycoprotein 1	HAPS/LFQ
29	APOL1_HUMAN	Apolipoprotein L1	Lit
30	ASAH1_HUMAN	Acid ceramidase	Lit
31	B2MG_HUMAN	Beta-2-microglobulin	iTRAQ, LFQ, HAPS/LFQ, HAPS/MAPS/LFQ
32	B3AT_HUMAN	Band 3 anion transport protein	Lit
33	BGAL_HUMAN	Beta-galactosidase	Lit
34	BIN1_HUMAN	Myc box-dependent-interacting protein 1	Lit
35	BLVRB_HUMAN	Flavin reductase (NADPH)	iTRAQ, HAPS/LFQ
36	C1RL_HUMAN	Complement C1 r subcomponent-like protein	Lit
37	C4BPA_HUMAN	C4b-binding protein alpha chain	LFQ
38	CAD13_HUMAN	Cadherin-13	iTRAQ, LFQ/Individual

#	UniProtKB Entry Name	Recommended Name	Basis for Inclusion in TUPA ¹
39	CADH1_HUMAN	Cadherin-1	HAPS/LFQ
40	CADM4_HUMAN	Cell adhesion molecule 4	Lit
41	CAH2_HUMAN	Carbonic anhydrase 2	HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
42	CAH3_HUMAN	Carbonic anhydrase 3	Lit
43	CATA_HUMAN	Catalase	Lit
44	CATC_HUMAN	Dipeptidyl peptidase 1	Lit
45	CATD_HUMAN	Cathepsin D	HAPS/MAPS/LFQ
46	CBG_HUMAN	Corticosteroid-binding globulin	Lit
47	CD14_HUMAN	Monocyte differentiation antigen CD14	LFQ/Individual
48	CD44_HUMAN	CD44 antigen	Lit
49	CD59_HUMAN	CD59 glycoprotein	iTRAQ, LFQ, HAPS/LFQ, LFQ/Individual
50	CD5L_HUMAN	CD5 antigen-like	Lit
51	CERU_HUMAN	Ceruloplasmin	HAPS/LFQ
52	CFAB_HUMAN	Complement factor B	LFQ, HAPS/LFQ
53	CFAI_HUMAN	Complement factor I	HAPS/LFQ
54	CLUS_HUMAN	Clusterin	LFQ, HAPS/LFQ
55	CO1A1_HUMAN	Collagen alpha-1 (I) chain	LFQ/Individual
56	CO2_HUMAN	Complement C2	HAPS/LFQ
57	CO3_HUMAN	Complement C3	Lit
58	CO5_HUMAN	Complement C5	Lit
59	CO6_HUMAN	Complement component C6	Lit
60	CO6A3_HUMAN	Collagen alpha-3(VI) chain	iTRAQ, LFQ, HAPS/LFQ, LFQ/Individual
61	CO9_HUMAN	Complement component C9	HAPS/LFQ
62	COFA1_HUMAN	Collagen alpha-1 (XV) chain	Lit
63	COR1A_HUMAN	Coronin-1A	LFQ/Individual
64	COTL1_HUMAN	Coactosin-like protein	iTRAQ, HAPS/LFQ
65	CPN2_HUMAN	Carboxypeptidase N subunit 2	Lit
66	CSF1_HUMAN	Macrophage colony-stimulating factor 1	iTRAQ, LFQ/Individual
67	CUBN_HUMAN	Cubilin	Lit
68	CYTB_HUMAN	Cystatin-B	Lit
69	CYTC_HUMAN	Cystatin-C	Lit
70	CYTM_HUMAN	Cystatin-M	iTRAQ, LFQ, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
71	DAF_HUMAN	Complement decay-accelerating factor	HAPS/LFQ
72	DAG1_HUMAN	Dystroglycan	Lit
73	DERM_HUMAN	Dermatopontin	HAPS/LFQ
74	DIAC_HUMAN	Di-N-acetylchitinase	Lit
75	DMKN_HUMAN	Dermokine	LFQ, HAPS/LFQ, LFQ/Individual
76	DNAS1_HUMAN	Deoxyribonuclease-1	Lit
77	DPP4_HUMAN	Dipeptidyl peptidase 4	Lit
78	DSC2_HUMAN	Desmocollin-2	iTRAQ, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
79	EGF_HUMAN	Pro-epidermal growth factor	Lit

#	UniProtKB Entry Name	Recommended Name	Basis for Inclusion in TUPA ¹
80	ELNE_HUMAN	Neutrophil elastase	Lit
81	ENDD1_HUMAN	Endonuclease domain-containing 1 protein	HAPS/LFQ
82	ENOA_HUMAN	Alpha-enolase	HAPS/LFQ
83	EPCR_HUMAN	Endothelial protein C receptor	Lit
84	FABPL_HUMAN	Fatty acid-binding protein, liver	LFQ, LFQ/Individual
85	FAM3C_HUMAN	Protein FAM3C	DIGE, LFQ, HAPS/LFQ, HAPS/MAPS/LFQ
86	FBLN1_HUMAN	Fibulin-1	Lit
87	FBLN3_HUMAN	EGF-containing fibulin-like extracellular matrix protein 1	HAPS/LFQ
88	FETUA_HUMAN	Alpha-2-HS-glycoprotein	Lit
89	FIBB_HUMAN	Fibrinogen beta chain	LFQ
90	FIBG_HUMAN	Fibrinogen gamma chain	DIGE, iTRAQ
91	FINC_HUMAN	Fibronectin	iTRAQ, LFQ, HAPS/LFQ
92	FOLR1_HUMAN	Folate receptor alpha	Lit
93	G3P_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase	HAPS/MAPS/LFQ, LFQ/Individual
94	G6PI_HUMAN	Glucose-6-phosphate isomerase	HAPS/LFQ
95	GELS_HUMAN	Gelsolin	Lit
96	GGH_HUMAN	Gamma-glutamyl hydrolase	Lit
97	GNS_HUMAN	N-acetylglucosamine-6-sulfatase	Lit
98	GPX3_HUMAN	Glutathione peroxidase 3	HAPS/LFQ
99	GSTP1_HUMAN	Glutathione S-transferase P	Lit
100	GUC2A_HUMAN	Guanylin	LFQ, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
101	H4_HUMAN	Histone H4	Lit
102	HBD_HUMAN	Hemoglobin subunit delta	iTRAQ
103	HEMO_HUMAN	Hemopexin	DIGE, HAPS/LFQ
104	HEP2_HUMAN	Heparin cofactor 2	HAPS/LFQ, LFQ/Individual
105	HPT_HUMAN	Haptoglobin	LFQ
106	HRG_HUMAN	Histidine-rich glycoprotein	Lit
107	HV320_HUMAN	Ig heavy chain V-III region GAL	LFQ
108	IBP7_HUMAN	Insulin-like growth factor-binding protein 7	Lit
109	IC1_HUMAN	Plasma protease C1 inhibitor	HAPS/LFQ
110	IGHA1_HUMAN	Ig alpha-1 chain C region	LFQ, LFQ/Individual
111	IGHG1_HUMAN	Ig gamma-1 chain C region	LFQ, LFQ/Individual
112	IGHG2_HUMAN	Ig gamma-2 chain C region	HAPS/MAPS/LFQ
113	IGHG4_HUMAN	Ig gamma-4 chain C region	Lit
114	IGHM_HUMAN	Ig mu chain C region	iTRAQ
115	IGJ_HUMAN	Immunoglobulin J chain	Lit
116	IGKC_HUMAN	Ig kappa chain C region	HAPS/LFQ, HAPS/MAPS/LFQ
117	IGSF8_HUMAN	Immunoglobulin superfamily member 8	Lit
118	IPSP_HUMAN	Plasma serine protease inhibitor	Lit
119	ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1	LFQ, HAPS/LFQ
120	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2	LFQ, HAPS/LFQ

#	UniProtKB Entry Name	Recommended Name	Basis for Inclusion in TUPA ¹
121	ITIH3_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H3	Lit
122	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	LFQ, HAPS/LFQ
123	JAM1_HUMAN	Junctional adhesion molecule A	Lit
124	KLK1_HUMAN	Kallikrein-1	Lit
125	KLK3_HUMAN	Prostate-specific antigen	iTRAQ, HAPS/LFQ
126	KNG1_HUMAN	Kininogen-1	HAPS/LFQ
127	KPYM_HUMAN	Pyruvate kinase PKM	Lit
128	LBP_HUMAN	Lipopolysaccharide-binding protein	Lit
129	LDHA_HUMAN	L-lactate dehydrogenase A chain	HAPS/LFQ
130	LDHB_HUMAN	L-lactate dehydrogenase B chain	HAPS/LFQ
131	LMAN2_HUMAN	Vesicular integral-membrane protein VIP36	HAPS/LFQ
132	LYAG_HUMAN	Lysosomal alpha-glucosidase	Lit
133	LYSC_HUMAN	Lysozyme C	iTRAQ, LFQ
134	LYVE1_HUMAN	Lymphatic vessel endothelial hyaluronic acid receptor 1	HAPS/LFQ
135	MASP2_HUMAN	Mannan-binding lectin serine protease 2	Lit
136	MGA_HUMAN	Maltase-glucoamylase, intestinal	Lit
137	MIME_HUMAN	Mimecan	Lit
138	MYG_HUMAN	Myoglobin	Lit
139	NAPSA_HUMAN	Napsin-A	Lit
140	NGAL_HUMAN	Neutrophil gelatinase-associated lipocalin	LFQ, HAPS/MAPS/LFQ
141	NID1_HUMAN	Nidogen-1	iTRAQ
142	NPC2_HUMAN	Epididymal secretory protein E1	Lit
143	NTF2_HUMAN	Nuclear transport factor 2	Lit
144	OLFM4_HUMAN	Olfactomedin-4	Lit
145	OSTP_HUMAN	Osteopontin	LFQ, HAPS/LFQ, LFQ/Individual
146	P3IP1_HUMAN	Phosphoinositide-3-kinase-interacting protein 1	LFQ, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
147	PCOC1_HUMAN	Procollagen C-endopeptidase enhancer 1	HAPS/LFQ
148	PEBP1_HUMAN	Phosphatidylethanolamine-binding protein	HAPS/LFQ
149	PEBP4_HUMAN	Phosphatidylethanolamine-binding protein	LFQ, HAPS/MAPS/LFQ
150	PERM_HUMAN	Myeloperoxidase	Lit
151	PGBM_HUMAN	Basement membrane-specific heparan sulfate proteoglycan core protein	DIGE, iTRAQ, LFQ, HAPS/MAPS/DIGE, LFQ/Individual
152	PGCP_HUMAN	Carboxypeptidase Q	Lit
153	PGK1_HUMAN	Phosphoglycerate kinase 1	HAPS/LFQ
154	PGRP1_HUMAN	Peptidoglycan recognition protein 1	HAPS/LFQ, LFQ/Individual
155	PGRP2_HUMAN	N-acetylmuramoyl-L-alanine amidase	HAPS/LFQ, HAPS/MAPS/LFQ
156	PI16_HUMAN	Peptidase inhibitor 16	Lit
157	PIGR_HUMAN	Polymeric immunoglobulin receptor	iTRAQ, HAPS/LFQ
158	PLSL_HUMAN	Plastin-2	HAPS/LFQ
159	PMGE_HUMAN	Bisphosphoglycerate mutase	Lit
160	PON1_HUMAN	Serum paraoxonase/arylesterase 1	Lit
161	PPAP_HUMAN	Prostatic acid phosphatase	Lit

#	UniProtKB Entry Name	Recommended Name	Basis for Inclusion in TUPA ¹
162	PRG2_HUMAN	Bone marrow proteoglycan	Lit
163	PRTN3_HUMAN	Myeloblastin	iTRAQ
164	PTGDS_HUMAN	Prostaglandin-H2 D-isomerase	DIGE, iTRAQ, LFQ, HAPS/MAPS/DIGE, HAPS/LFQ, LFQ/Individual
165	QPCT_HUMAN	Glutaminyl-peptide cyclotransferase	Lit
166	QSOX1_HUMAN	Sulfhydryl oxidase 1	Lit
167	RET4_HUMAN	Retinol-binding protein 4	DIGE, iTRAQ, LFQ, HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
168	RNAS1_HUMAN	Ribonuclease pancreatic	iTRAQ, LFQ, HAPS/LFQ, LFQ/Individual
169	S10A6_HUMAN	Protein S100-A6	HAPS/LFQ, LFQ/Individual
170	S10A8_HUMAN	Protein S100-A8	HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
171	S10A9_HUMAN	Protein S100-A9	iTRAQ, HAPS/LFQ, HAPS/MAPS/LFQ
172	SAMP_HUMAN	Serum amyloid P-component	HAPS/LFQ
173	SAP_HUMAN	Prosaposin	HAPS/LFQ
174	SAP3_HUMAN	Ganglioside GM2 activator	HAPS/LFQ, HAPS/MAPS/LFQ, LFQ/Individual
175	SH3L3_HUMAN	SH3 domain-binding glutamic acid-rich-like protein 3	LFQ, HAPS/LFQ
176	SODC_HUMAN	Superoxide dismutase [Cu-Zn]	LFQ, HAPS/LFQ
177	SPRL1_HUMAN	SPARC-like protein 1	iTRAQ, HAPS/LFQ, LFQ/Individual
178	TETN_HUMAN	Tetranectin	HAPS/MAPS/LFQ
179	THBG_HUMAN	Thyroxine-binding globulin	HAPS/LFQ, HAPS/MAPS/LFQ
180	THIO_HUMAN	Thioredoxin	HAPS/MAPS/LFQ
181	THRB_HUMAN	Prothrombin	LFQ/Individual
182	THTM_HUMAN	3-mercaptopyruvate sulfurtransferase	Lit
183	THY1_HUMAN	Thy-1 membrane glycoprotein	Lit
184	TKT_HUMAN	Transketolase	Lit
185	TPIS_HUMAN	Triosephosphate isomerase	HAPS/MAPS/LFQ
186	TPP1_HUMAN	Tripeptidyl-peptidase 1	Lit
187	TRFE_HUMAN	Serotransferrin	DIGE, HAPS/MAPS/LFQ
188	TRFL_HUMAN	Lactotransferrin	iTRAQ
189	TRY2_HUMAN	Trypsin-2	HAPS/LFQ
190	TTHY_HUMAN	Transthyretin	iTRAQ, HAPS/MAPS/LFQ
191	TXD17_HUMAN	Thioredoxin domain-containing protein 17	Lit
192	UFO_HUMAN	Tyrosine-protein kinase receptor UFO	Lit
193	UROM_HUMAN	Uromodulin	Lit
194	VASN_HUMAN	Vasorin	Lit
195	VCAM1_HUMAN	Vascular cell adhesion protein 1	Lit
196	VSIG4_HUMAN	V-set and immunoglobulin domain-containing protein 4	HAPS/LFQ
197	VTDB_HUMAN	Vitamin D-binding protein	LFQ/Individual
198	VTNC_HUMAN	Vitronectin	Lit
199	WFDC2_HUMAN	WAP four-disulfide core domain protein 2	LFQ, HAPS/LFQ
200	ZA2G_HUMAN	Zinc-alpha-2-glycoprotein	LFQ, LFQ/Individual

¹As indicated in this column proteins were chosen for inclusion in TUPA that had 2-fold or greater change in expression in IGF versus DGF patient urine samples based on DIGE, iTRAQ, or LFQ analyses of undepleted, pooled samples; DIGE analysis of HAPS/MAPS depleted, pooled samples; and LFQ analyses of HAPS and HAPS/MAPS depleted samples; and also, LFQ analyses of individual, undepleted samples.

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Table 2
Results from Interference Analysis of 167 TUPA Proteins with Qualified “Heavy” SIS Peptides

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
1	1433E_HUMAN	1	1433E_HUMAN.EAAENSLVAYK	2y3/2y7	Yes	
	1433E_HUMAN	2	1433E_HUMAN.HLIPANTGESK	2y10/2y9	Yes	Yes
2	6PGD_HUMAN	3	6PGD_HUMAN.GILFVGSGVSGGEEGAR	2y8/2y12	Yes	Yes
	6PGD_HUMAN	4	6PGD_HUMAN.NPELQNLLDDDFEK	2b8*/2b7*	Yes	
3	6PGL_HUMAN	5	6PGL_HUMAN.VTLTLPVLNAAR	2b5o/2y7	Yes	
4	A1AG1_HUMAN	6	A1AG1_HUMAN.SDVVYTDWK	2y6/2y5	Yes	
5	A1AT_HUMAN	7	A1AT_HUMAN.LSITGTYDLK	2y8/2y7	Yes	Yes
	A1AT_HUMAN	8	A1 AT_HUMAN.SVLGQLGITK	2y8/2y7	Yes	Yes
6	A1BG_HUMAN	9	A1BG_HUMAN.ATWSGAVLAGR	2y7/2y8	Yes	Yes
7	A2AP_HUMAN	10	A2AP_HUMAN.LCQDLPGAFR	2y6/2y9	Yes	
8	A2GL_HUMAN	11	A2GL_HUMAN.DLLLPPQDLR	2y7/2y6	Yes	Yes
9	A2MG_HUMAN	12	A2MG_HUMAN.LLLQQVSLPELPGEYSMK	3y7++/3y7	Yes	Yes
10	A4_HUMAN	13	A4_HUMAN.LVFFAEDVGSNK	2y9/2y8	Yes	Yes
11	ALBU_HUMAN	14	ALBU_HUMAN.VPQVSTPTLVEVSR	2y11/2y10	Yes	Yes
12	ALDOA_HUMAN	15	ALDOA_HUMAN.ALANSLACQK	2y7/2y9	Yes	
13	ALDOB_HUMAN	16	ALDOB_HUMAN.LDQGAPLAGTNK	2y10/2y7	Yes	Yes
14	AMBP_HUMAN	17	AMBP_HUMAN.AFIQLWAFDAVK	2y8/2y7	Yes	Yes
15	AMPN_HUMAN	18	AMPN_HUMAN.ENSLFFDPLSSSSSNK	2b9/2y9	Yes	
16	ANAG_HUMAN	19	ANAG_HUMAN.FLLGSWLEQAR	2y9/2y8	Yes	
	ANAG_HUMAN	20	ANAG_HUMAN.LLTSAPSLATSPAFR	3y6/3y4	Yes	
17	ANGT_HUMAN	21	ANGT_HUMAN.DPTFIPAPIQAK	2b4/2y7	Yes	Yes
18	ANT3_HUMAN	22	ANT3_HUMAN.VAEGTQVLELPFK	2y6/2y3	Yes	Yes
19	APOA1_HUMAN	23	APOA1_HUMAN.DYVVSQFEGSALGK	2y8/2y10	Yes	Yes
	APOA1_HUMAN	24	APOA1_HUMAN.VSFLSALBEYTK	2y9/2y8	Yes	Yes
20	APOA2_HUMAN	25	APOA2_HUMAN.EQLTPLIK	2y6/2y4	Yes	Yes
	APOA2_HUMAN	26	APOA2_HUMAN.SPELQAEAK	2y7/2y6	Yes	Yes
21	APOA4_HUMAN	27	APOA4_HUMAN.ISASAEELR	2y8/2y7	Yes	Yes
22	APOC2_HUMAN	28	APOC2_HUMAN.ESLSYWESAK	2y7/2b8*	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
23	APOD_HUMAN	29	APOD_HUMAN.VLNQELR	2y4/2y5	Yes	Yes
24	APOE_HUMAN	30	APOE_HUMAN.LAVYQAGAR	2b8/2y6	Yes	
	APOE_HUMAN	31	APOE_HUMAN.LGPLVEQGR	2y4/2y5	Yes	
25	APOH_HUMAN	32	APOH_HUMAN.ATVVYQGER	2b6/2y6	Yes	
26	APOLI_HUMAN	33	APOLI_HUMAN.VAQELEEK	2y7/2y6	Yes	
27	B3AT_HUMAN	34	B3AT_HUMAN.ADFLEQPVLGFR	2y8/2y7	Yes	Yes
	B3AT_HUMAN	35	B3AT_HUMAN.LQEAEELEAVELPPIR	2y7/2y5	Yes	
28	BGAL_HUMAN	36	BGAL_HUMAN.AYVAVDGIPIQGVLER	2b8/2y7	Yes	
	BGAL_HUMAN	37	BGAL_HUMAN.TEAVASSLYDILAR	2y9/2y10	Yes	
29	BINI_HUMAN	38	BINI_HUMAN.GVFPENFIER	2y7+/+2y7	Yes	Yes
30	BLVRB_HUMAN	39	BLVRB_HUMAN.NDLSPTTVMSEGAR	2y11/2y10	Yes	Yes
32	C1RL_HUMAN	40	C1RL_HUMAN.GSEAINAPGDNPAK	2y9/2y7	Yes	Yes
32	C4BPA_HUMAN	41	C4BPA_HUMAN.EDVYWGTVLR	2y8/2y6	Yes	Yes
33	CADI3_HUMAN	42	CADI3_HUMAN.DIQGSLQDIFK	2y9/2y8	Yes	Yes
	CADI3_HUMAN	43	CADI3_HUMAN.VNSDGGGLVALR	2y7/2y9	Yes	Yes
34	CADH1_HUMAN	44	CADH1_HUMAN.NTGVISVVTGLDR	2y10/2y9	Yes	
	CADH1_HUMAN	45	CADH1_HUMAN.VTEPLDR	2y5/2y4	Yes	Yes
35	CADM4_HUMAN	46	CADM4_HUMAN.VW/SVASTVR	2b5/2y7	Yes	
36	CAH2_HUMAN	47	CAH2_HUMAN.YAAELHLVHWNTK	3y7/3y4	Yes	Yes
37	CAH3_HUMAN	48	CAH3_HUMAN.FDPSCLFPACR	2y5/2y4	Yes	Yes
	CAH3_HUMAN	49	CAH3_HUMAN.VVFDDTYDR	2y6/2y7	Yes	
38	CATA_HUMAN	50	CATA_HUMAN.LEFAYPDTHR	2y7/2y5	Yes	
39	CATC_HUMAN	51	CATC_HUMAN.GTDECAIESIAVAATPIPK	2y7/2y12	Yes	
40	CATD_HUMAN	52	CATD_HUMAN.LSPEDYTLK	2y7/2y7++	Yes	
	CATD_HUMAN	53	CATD_HUMAN.VSTLPATLTK	2y8/2y6	Yes	Yes
41	CD14_HUMAN	54	CD14_HUMAN.AFPALTSLDLSDNPGLGER	2y11/2y10	Yes	
	CD14_HUMAN	55	CD14_HUMAN.VLDLSCNR	2y4/2y6	Yes	
42	CD44_HUMAN	56	CD44_HUMAN.ALSIGFETCR	2b9/2y6	Yes	
	CD44_HUMAN	57	CD44_HUMAN.TEAADLCK	2b2/2y6	Yes	Yes
43	CD59_HUMAN	58	CD59_HUMAN.AGLQVYNK	2y5/2y4	Yes	Yes
	CD59_HUMAN	59	CD59_HUMAN.ENELTYCYCK	2y3/2y6	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
44	CD5L_HUMAN	60	CD5L_HUMAN.LVGGDNLCSGR	2y4/2y9	Yes	
45	CERU_HUMAN	61	CERU_HUMAN.GAYPLSIEPIGVR	2y8/2y10++	Yes	
46	CFAB_HUMAN	62	CFAB_HUMAN.DFHINLFQVLPWLK	3b6/3y4	Yes	Yes
47	CFAL_HUMAN	63	CFAL_HUMAN.AQLGDLPWQVAIK	2y10/2y7	Yes	Yes
	CFAL_HUMAN	64	CFAL_HUMAN.GLETSLAECTFTK	2y6/2y7	Yes	
48	CLUS_HUMAN	65	CLUS_HUMAN.EILSVDCSTNNPSQAK	2y5/2y11	Yes	Yes
49	CO2_HUMAN	66	CO2_HUMAN.AVISPGEDVFAK	2y9/2y8	Yes	
	CO2_HUMAN	67	CO2_HUMAN.VLMSVLNDSNR	2y9/2y6	Yes	
50	CO3_HUMAN	68	CO3_HUMAN.NEQVEIR	2y4/2y5	Yes	
	CO3_HUMAN	69	CO3_HUMAN.SGIPVTSPYQIHFTK	3y13+++/3y8	Yes	Yes
51	CO5_HUMAN	70	CO5_HUMAN.IPLDLVPK	2b6++/2y6	Yes	
52	CO6_HUMAN	71	CO6_HUMAN.ENPAVIDFELAPIVDLVR	2y10/2y7	Yes	Yes
	CO6_HUMAN	72	CO6_HUMAN.IGESIELTCPK	2y6/2y3	Yes	
53	CO6A3_HUMAN	73	CO6A3_HUMAN.EVQVEITENSAK	2y9/2y6	Yes	Yes
	CO6A3_HUMAN	74	CO6A3_HUMAN.FWYGGCGGNEK	2b5+++/2y10	Yes	
54	CO9_HUMAN	75	CO9_HUMAN.AIEDYINEFSVR	2y7/2y6	Yes	Yes
	CO9_HUMAN	76	CO9_HUMAN.LSPIYNLVPVK	2y3/2y9	Yes	Yes
55	COFA1_HUMAN	77	COFA1_HUMAN.TLIPSTFR	2y7/2y6	Yes	Yes
56	CORIA_HUMAN	78	CORIA_HUMAN.HVFGQPAK	2b2/2y6	Yes	Yes
	CORIA_HUMAN	79	CORIA_HUMAN.ILTTFGR	2y3o/2y6	Yes	
57	COTL1_HUMAN	80	COTL1_HUMAN.FALITWIGENVSLQR	2y10/2y9	Yes	Yes
58	CPN2_HUMAN	81	CPN2_HUMAN.GQVVPALNEK	2y8/2y6	Yes	Yes
	CPN2_HUMAN	82	CPN2_HUMAN.LSNNALSGLPQGVFGK	2y10/2b8*	Yes	
59	CSF1_HUMAN	83	CSF1_HUMAN.FNSVPLTDTGHER	3y7/3y9	Yes	
	CSF1_HUMAN	84	CSF1_HUMAN.SHSSGSLPLGLEGR	3y6/3y8	Yes	
60	CUBN_HUMAN	85	CUBN_HUMAN.DGVDSADPILSK	2y5/2y9	Yes	
	CUBN_HUMAN	86	CUBN_HUMAN.FVTDGSGVTASGR	2y6/2y11	Yes	
61	CYTB_HUMAN	87	CYTB_HUMAN.SQVVAGTNYFIK	2y10/2y8	Yes	Yes
62	CYTC_HUMAN	88	CYTC_HUMAN.ALDFAVGEYNK	2y6/2y9	Yes	Yes
	CYTC_HUMAN	89	CYTC_HUMAN.TQPNLDCPFHDQPHLK	4y9++/4b9*++	Yes	
63	CYTM_HUMAN	90	CYTM_HUMAN.DLSPDDPQVQK	2y8++/2y8	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
64	DAF_HUMAN	91	DAF_HUMAN.TSFPEDTVITYK	2y10/2b10o	Yes	
65	DIAC_HUMAN	92	DIAC_HUMAN.SYDWSQITTVATFGK	2y7/2y11	Yes	
66	DMKN_HUMAN	93	DMKN_HUMAN.VSEALGQGTR	2y7/2y5	Yes	Yes
67	DNAS1_HUMAN	94	DNAS1_HUMAN.IAAFNQITFGETK	2b10o/2y7	Yes	
68	DPP4_HUMAN	95	DPP4_HUMAN.VLEDNSALDK	2y7/2y8	Yes	
69	EGF_HUMAN	96	EGF_HUMAN.IESSSLQGLGR	2y8/2y9	Yes	Yes
	EGF_HUMAN	97	EGF_HUMAN.ITAVSLDVLDK	2y9/2y7	Yes	
70	ENDD1_HUMAN	98	ENDD1_HUMAN.ILEVNVNQIODEER	2y8/2y9	Yes	
71	FABPL_HUMAN	99	FABPL_HUMAN.AIGLPEELIQK	2y9/2y7	Yes	Yes
72	FAM3C_HUMAN	100	FAM3C_HUMAN.SALDTAAR	2y4/2y5	Yes	
73	FBLN1_HUMAN	101	FBLN1_HUMAN.MCVDVNECQR	2y7/2y8	Yes	
74	FBLN3_HUMAN	102	FBLN3_HUMAN.ADQVCINLR	2b7*/2y5	Yes	
	FBLN3_HUMAN	103	FBLN3_HUMAN.DIDECDIVPDACK	2y6/2y5	Yes	Yes
75	FIBB_HUMAN	104	FIBB_HUMAN.TPCTVSCNIPVVS GK	2y10/2y6	Yes	Yes
76	FIBG_HUMAN	105	FIBG_HUMAN.DNCCILDER	2y4/2y3	Yes	Yes
	FIBG_HUMAN	106	FIBG_HUMAN.EGFGHLSPGTGTFEVLGNEK	3y7/3b9*++	Yes	
77	FINC_HUMAN	107	FINC_HUMAN.VTWAPPSIDLTLNVLVR	3y5/3b4	Yes	Yes
78	FOLR1_HUMAN	108	FOLR1_HUMAN.EDCEQWWEDCR	2y6/2y5	Yes	
	FOLR1_HUMAN	109	FOLR1_HUMAN.VLNVPLCK	2y6/2y4	Yes	Yes
79	G3P_HUMAN	110	G3P_HUMAN.GALQNIIPASTGA AK	2y10/2y9	Yes	Yes
	G3P_HUMAN	111	G3P_HUMAN.LVINGNPITIFQER	2b8o/2y8	Yes	
80	G6P1_HUMAN	112	G6P1_HUMAN.TLAQLNPESLFIASK	3y11/3y6	Yes	Yes
81	GGH_HUMAN	113	GGH_HUMAN.FFNVLTNTIDGK	2y10/2y8	Yes	Yes
	GGH_HUMAN	114	GGH_HUMAN.SINGILFPGGSVDLR	2y9/2y8	Yes	
82	GNS_HUMAN	115	GNS_HUMAN.IQEPNTFFAILR	2y5/2y9	Yes	
83	GPX3_HUMAN	116	GPX3_HUMAN.FLYGPDGPIIMR	2y4/2y9	Yes	Yes
84	GSTP1_HUMAN	117	GSTP1_HUMAN.FQDGDLTLYQSNITLR	2y10/2y8	Yes	
85	GUC2A_HUMAN	118	GUC2A_HUMAN.DLQEPQEP R	2y6/2y5	Yes	Yes
86	H4_HUMAN	119	H4_HUMAN.ISGLIYEETR	2y8/2y5	Yes	Yes
	H4_HUMAN	120	H4_HUMAN.VFLENVIR	2y5/2y6	Yes	
87	HBD_HUMAN	121	HBD_HUMAN.VNVDAVGG EALGR	2y10/2y7	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
88	HEP2_HUMAN	122	HEP2_HUMAN.TLEAQLTPR	2y4/2y7	Yes	Yes
89	HPT_HUMAN	123	HPT_HUMAN.VGYVSGWGR	2y8/2y5	Yes	Yes
	HPT_HUMAN	124	HPT_HUMAN.VTSIQDVWQK	2y6/2y8	Yes	Yes
90	HV320_HUMAN	125	HV320_HUMAN.GLEVWANI	2y6/2y4	Yes	Yes
	HV320_HUMAN	126	HV320_HUMAN.VEDTALYYCAR	2y4/2y5	Yes	
91	IBP7_HUMAN	127	IBP7_HUMAN.GTCEQGPSIVTPPK	2y9/2y3	Yes	Yes
	IBP7_HUMAN	128	IBP7_HUMAN.HEVTGWVLSPLSK	3y5/3y6	Yes	
92	ICI_HUMAN	129	ICI_HUMAN.TNLESILSYPK	2y9/2y7	Yes	Yes
93	IGHA1_HUMAN	130	IGHA1_HUMAN.DASGVTFITWTPSSGK	2y10/2b9++	Yes	
	IGHA1_HUMAN	131	IGHA1_HUMAN.TFTCTAAYPESK	2y10/2y4	Yes	Yes
94	IGHG1_HUMAN	132	IGHG1_HUMAN.TTPPVLDSDGSFFLYSK	2y3/2y15++	Yes	
95	IGHG4_HUMAN	133	IGHG4_HUMAN.TTPPVLDSDGSFFLYSR	2y10/2b16o++	Yes	
96	IGHM_HUMAN	134	IGHM_HUMAN.YAATSQVLLPSK	2y10/2y3	Yes	Yes
97	IGKC_HUMAN	135	IGKC_HUMAN.DSTYSLSTLTLSK	2y10/2y8	Yes	Yes
98	IGSF8_HUMAN	136	IGSF8_HUMAN.VVAGEVQVQR	2y7/2y8	Yes	Yes
99	IPSP_HUMAN	137	IPSP_HUMAN.TLYLADTFPTNFR	2y8/2y9	Yes	
100	ITIH1_HUMAN	138	ITIH1_HUMAN.AAISGENAGLVR	2y8/2y9	Yes	Yes
101	ITIH2_HUMAN	139	ITIH2_HUMAN.FYNQVSPTLLR	2y7/2y6	Yes	Yes
102	ITIH3_HUMAN	140	ITIH3_HUMAN.EVSFDVELPK	2y8/2y6	Yes	Yes
103	ITIH4_HUMAN	141	ITIH4_HUMAN.TGLLLSDPDK	2b8o/2b4o	Yes	
104	KLK1_HUMAN	142	KLK1_HUMAN.LTEPADTITDAVK	2y9/2y10	Yes	
105	KNK1_HUMAN	143	KNK1_HUMAN.DFVQPPTK	2y6/2y4	Yes	Yes
106	KPYM_HUMAN	144	KPYM_HUMAN.GVNLPGAADVLPVSEK	2b4/2y6	Yes	
107	LBP_HUMAN	145	LBP_HUMAN.LAEGFPLPK	2y4/2y6	Yes	Yes
108	LDHA_HUMAN	146	LDHA_HUMAN.SADTLWGQK	2y8/2y5	Yes	Yes
109	LDHB_HUMAN	147	LDHB_HUMAN.SLADELALVDVLEDK	2b10o/2y6	Yes	
110	LYAG_HUMAN	148	LYAG_HUMAN.GAYTQVIFLAR	2y5/2y4	Yes	
111	LYSC_HUMAN	149	LYSC_HUMAN.STDYGIQFINSR	2y8/2y6	Yes	Yes
112	LYVE1_HUMAN	150	LYVE1_HUMAN.NGVGLIWK	2b8*/2y6	Yes	
113	MASP2_HUMAN	151	MASP2_HUMAN.LASGPFGEYANDQER	2y13+/2y10	Yes	Yes
114	MGA_HUMAN	152	MGA_HUMAN.FAGFPALINR	2y3*/2y6	Yes	

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
115	MGA_HUMAN	153	MGA_HUMAN.SSVYANAFPSTPVNPLR	2y10/2y9	Yes	Yes
	MIME_HUMAN	154	MIME_HUMAN.ETVIIPNEK	2y5/2y4	Yes	Yes
	MIME_HUMAN	155	MIME_HUMAN.LEGNPIVLGK	2y6/2y8	Yes	
116	NAPSA_HUMAN	156	NAPSA_HUMAN.FAIQYGTGR	2y5/2y6	Yes	
	NAPSA_HUMAN	157	NAPSA_HUMAN.VGPGITLCAK	2y5/2y8	Yes	
117	NID1_HUMAN	158	NID1_HUMAN.EDLSPSITQR	2y7/2y6	Yes	Yes
	NID1_HUMAN	159	NID1_HUMAN.ESVENLAK	2y5o/2y5	Yes	
118	NPC2_HUMAN	160	NPC2_HUMAN.SGINCPQIK	2y4/2y6	Yes	
	OLFM4_HUMAN	161	OLFM4_HUMAN.VQSINYNPEDQK	2y5/2y10	Yes	
119	OSTP_HUMAN	162	OSTP_HUMAN.AIPVAQDLNAPSDWDSR	2y7/2b16++	Yes	Yes
120	OSTP_HUMAN	163	OSTP_HUMAN.YPDAAVATWLNPDPSQK	2y4/2y6	Yes	Yes
121	P3IP1_HUMAN	164	P3IP1_HUMAN.GPWCYVSGEAGVPEK	2y10/2y3	Yes	Yes
	P3IP1_HUMAN	165	P3IP1_HUMAN.SEAAAVQPVIGISQR	2y9/2y8	Yes	Yes
122	PCOC1_HUMAN	166	PCOC1_HUMAN.YDALEVFAGSGTSGQR	2y9/2y10	Yes	
123	PEBP1_HUMAN	167	PEBP1_HUMAN.LYEQLSGK	2y7/2y6	Yes	Yes
124	PEBP4_HUMAN	168	PEBP4_HUMAN.VVPDCNNYR	2y6/2y7	Yes	Yes
125	PGBM_HUMAN	169	PGBM_HUMAN.DFISLGLQDGHVFR	3y8/3y6	Yes	
126	PGCP_HUMAN	170	PGCP_HUMAN.LALLVDVTGPR	2y6/2y7	Yes	
127	PGK1_HUMAN	171	PGK1_HUMAN.ITLPVDFVTADK	2y10/2y9	Yes	Yes
128	PGRP1_HUMAN	172	PGRP1_HUMAN.AAQGLLACGVAQ.GALR	2b10/2b11	Yes	Yes
129	PGRP2_HUMAN	173	PGRP2_HUMAN.DTLPSCAVR	2y5/2y6	Yes	Yes
130	PGRP2_HUMAN	174	PGRP2_HUMAN.EFTEAFLGCPAIIHPR	3y3/3y6	Yes	Yes
	PI16_HUMAN	175	PI16_HUMAN.WDEELAAFAK	2y4/2y9	Yes	
131	PIGR_HUMAN	176	PIGR_HUMAN.IILLNPQDK	2y4/2y6	Yes	Yes
132	PIGR_HUMAN	177	PIGR_HUMAN.TVTINCPFK	2y3/2y7	Yes	Yes
	PLSL_HUMAN	178	PLSL_HUMAN.NEALIALLR	2y7/2b7*	Yes	
133	PMGE_HUMAN	179	PMGE_HUMAN.VCDVPLDQLPR	2y9/2y7	Yes	
134	PONI_HUMAN	180	PONI_HUMAN.IFFYDSENPPASEVLR	2y9/2y8	Yes	Yes
135	PPAP_HUMAN	181	PPAP_HUMAN.SPIDTFPTDPIK	2y3/2y6	Yes	
136	PRG2_HUMAN	182	PRG2_HUMAN.FQWVDGSR	2y3/2y6	Yes	
	PRG2_HUMAN	183	PRG2_HUMAN.VVGIPGCQTCR	2y7++/2y7	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
137	PTGDS_HUMAN	184	PTGDS_HUMAN.AQGTEDTIVFLPQTDK	2y8/2b1lo++	Yes	
138	QPCT_HUMAN	185	QPCT_HUMAN.SFSNIITLNPATAK	2y9/2y8	Yes	
	QPCT_HUMAN	186	QPCT_HUMAN.YFQNYSYGGVIQDDHIFFLR	3y6/3y4	Yes	Yes
139	QSOX1_HUMAN	187	QSOX1_HUMAN.LAGAPSEDPQFPK	2y5/2y9	Yes	
140	RET4_HUMAN	188	RET4_HUMAN.DPNGLPPEAQK	2b5/2y6	Yes	Yes
141	RNAS1_HUMAN	189	RNAS1_HUMAN.YPNCAYR	2y6/2y5	Yes	Yes
142	S10A6_HUMAN	190	S10A6_HUMAN.LM(ox)EDLDR	2y6/2y5	Yes	Yes
	S10A6_HUMAN	191	S10A6_HUMAN.LMEDLDR	2y6/2y5	Yes	Yes
	S10A6_HUMAN	192	S10A6_HUMAN.LQDAELAR	2y7*/2y6	Yes	Yes
143	S10A9_HUMAN	193	S10A9_HUMAN.LGHPDTLNLQGEFK	2y11++/2y10	Yes	
	S10A9_HUMAN	194	S10A9_HUMAN.NIETIITFHQYSVK	3y10/3y9	Yes	Yes
144	SAMP_HUMAN	195	SAMP_HUMAN.IVLGQEQQDSYGGK	2y11/2y10	Yes	Yes
	SAMP_HUMAN	196	SAMP_HUMAN.VGEYSLYIGR	2y9/2y7	Yes	
145	SAP_HUMAN	197	SAP_HUMAN.EIVDSYLPVLDIHK	2y9/2y8	Yes	Yes
	SAP_HUMAN	198	SAP_HUMAN.GCSFLPDYQK	2y4/2y6	Yes	
146	SAP3_HUMAN	199	SAP3_HUMAN.VDLVLEK	2y5/2y6	Yes	
147	SH3L3_HUMAN	200	SH3L3_HUMAN.VYSTSVTGSR	2y7/2y8	Yes	Yes
148	SODC_HUMAN	201	SODC_HUMAN.HVGD LGNTADK	2y4/2y10	Yes	Yes
149	THBG_HUMAN	202	THBG_HUMAN.NALALFVLPK	2y8/2y7	Yes	Yes
150	THIO_HUMAN	203	THIO_HUMAN.TAFQEALDAAAGDK	2y6/2y9	Yes	Yes
151	THRB_HUMAN	204	THRB_HUMAN.NPDSSTTGPWCYTTPDPTVR	3y4/3y7	Yes	Yes
152	THY1_HUMAN	205	THY1_HUMAN.HVLFGTGVGPEHTYR	3b6o/3y6	Yes	Yes
153	TKT_HUMAN	206	TKT_HUMAN.AVELAANTK	2y5/2y7	Yes	
	TKT_HUMAN	207	TKT_HUMAN.IIALDGDGDK	2y8/2y7	Yes	
154	TPIS_HUMAN	208	TPIS_HUMAN.VVLAYPEVWAGTGK	2y10/2y9	Yes	Yes
155	TPPI_HUMAN	209	TPPI_HUMAN.LFGGNFAHQASVAR	2y12++/2y6	Yes	Yes
156	TRFE_HUMAN	210	TRFE_HUMAN.EGYGYTGAFR	2b9o/2y7	Yes	
157	TRFL_HUMAN	211	TRFL_HUMAN.DGAGDVAFIR	2y5/2y7	Yes	
	TRFL_HUMAN	212	TRFL_HUMAN.YLGPQYVAGITNLK	2y6/2y7	Yes	Yes
158	TRY2_HUMAN	213	TRY2_HUMAN.TLDNDILLIK	2y3/2y8	Yes	Yes
159	TTHY_HUMAN	214	TTHY_HUMAN.AADDTWEPFASGK	2y8/2y6	Yes	Yes

Protein #	Protein Name	SIS#	Peptide	Transition Ratio	Qualified SIS Peptide?	Qualified Light Peptide?
160	UFO_HUMAN	215	UFO_HUMAN.APLQGTLLGYR	2y8/2y7	Yes	
	UFO_HUMAN	216	UFO_HUMAN.TAITTVLPQQPR	2y6/2y5	Yes	Yes
161	VASN_HUMAN	217	VASN_HUMAN.LAGLGLQQLDEGLFSR	2y10/2y9	Yes	
	VASN_HUMAN	218	VASN_HUMAN.YLQSSSVQLR	2y8/2y7	Yes	Yes
162	VCAM1_HUMAN	219	VCAM1_HUMAN.SLEVTFITPVIEDIGK	2y11/2y8	Yes	Yes
163	VSIG4_HUMAN	220	VSIG4_HUMAN.GSDPVTIFLR	2y7/2y5	Yes	Yes
164	VTDB_HUMAN	221	VTDB_HUMAN.HLSLLTTLNLR	2y7/2y9	Yes	
165	VTNC_HUMAN	222	VTNC_HUMAN.FEDGVLDPPDYPR	2y7/2y5	Yes	Yes
166	WFDC2_HUMAN	223	WFDC2_HUMAN.EGSCPVNINFPQLGLCR	2y9/2y7	Yes	Yes
167	ZA2G_HUMAN	224	ZA2G_HUMAN.AYLEEECPATLR	2y8/2y5	Yes	Yes
Total					224	124