

Proteomic profile changes associated with diminished expression of T-cell intracellular antigens reveal a hormesis response

José Alcalde¹ and José M. Izquierdo^{1,2}

¹Centro de Biología Molecular 'Severo Ochoa'.
Consejo Superior de Investigaciones Científicas.
Universidad Autónoma de Madrid (CSIC/UAM).
C/ Nicolás Cabrera 1. Cantoblanco.
28049 Madrid, Spain

²Address correspondence and proofs to:
José M. Izquierdo
Phone# +34-911964530
Fax# +34-911964520

Abstract

T-cell intracellular antigen (TIA) proteins function as regulators of cell homeostasis by controlling global gene expression in response to dynamic regulatory changes and environmental stress. Here, we used two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry (MALDI-TOF/TOF) to identify protein changes associated with the down-regulated expression of TIA proteins. We detected 30 differentially expressed proteins (DEPs), 24 of which were identified, and some of these DEPs were validated by western blotting. *In silico* analysis showed that DEPs were associated with metabolic processes, detoxification and proteostasis. We mapped the DEPs to the available biological pathways and networks, which included the metabolism of small molecules such as sugars, lipids, amino acids, and nucleotides. Our findings support previous studies and suggest that low expression of TIA proteins might act as a potential adaptive switch to link gene expression reprogramming to a proliferative phenotype mediated by a hormesis phenomenon.

Keywords: TIA1, TIAR, proteome, adaptive metabolism, gene regulatory networks, TIA-hormesis.

Introduction

T-cell intracellular antigen 1 (TIA1) and the closely related TIA1 related/like (TIAR/TIAL1) are multifunctional RNA-binding proteins involved in the fine-tuning of gene expression in the nucleus and cytoplasm through regulation of many aspects of RNA metabolism, including transcription, splicing/processing, subcellular localization, stability and translation [1-6]. Human TIA proteins bind to specific sequence motifs containing U-, CU-, and AU-rich RNA stretches on RNAs [1-4], and can regulate many properties of the human transcriptome [5,6].

While the roles of TIA-proteins in key cellular processes such as inflammatory and stress response are well recognized, less information is available for their involvement in proliferation/differentiation responses in pathophysiological conditions [1-6]. Nevertheless, it is known that knockdown of TIA1 and/or TIAR in various cell lines triggers proliferative phenotypes [5-11], suggesting that they may function as growth suppressors [5,6,9,12-18].

Two-dimensional polyacrylamide gel electrophoresis (2D-DIGE) is a core technology for protein separation and expression profiling [19,20,21], and is a useful tool for investigating the regulation of protein expression during pathophysiological situations [22]. Accordingly, proteome analysis coupled with systems biology approaches might predict new targets of TIA proteins and provide information on their role in different regulatory networks and homeostasis.

Herein, we used 2D-DIGE to investigate the consequences of reduced TIA expression in HeLa cells. We quantified differences and identified some of the proteins that were differentially expressed. The protein patterns found are consistent with adaptations of carbohydrate, amino acid, lipid, and nucleotide metabolism, free radical detoxification, and proteostasis.

Material and Methods

Sample preparation

HeLa cells were silenced for TIA1/TIAR (TIA) expression as described [9]. Four independent samples from control and TIA-knock down (kd) cells were prepared by differential centrifugation and stored at -80°C. Proteins were precipitated using the 2D Clean-up Kit (GE Healthcare) and resuspended in 30 mM Tris-HCl, 8 M urea, 2 M thiourea, 4% CHAPS (pH 8.5). Protein concentration was determined using the Bradford assay (Bio-Rad).

Protein labeling

Four biological replicates were used from control and TIA-kd cells, generating eight individual samples. The 2D-DIGE was carried out at the Proteomics Facility, Universidad Complutense de Madrid-Parque Científico de Madrid. The samples were distributed across four DIGE gels, with the internal standard pooled sample also present, according to the experimental design shown in Figure 1. Proteins were fluorescently labeled following the protocol of the manufacturer (Sigma). Briefly, 400 pmol of Cy dye in 1 µl anhydrous *N,N*-dimethylformamide (Sigma) was used per 50 µg of protein. After 30 min of incubation on ice in the dark, the reaction was quenched with 1 µl of 10 mM lysine for 10 min.

Electrophoresis and image acquisition

Labeled samples were combined according to the experimental design, processed and visualized with a Typhoon 9400 scanner (GE Healthcare) with CyDye filters as described previously [23,24]. For the Cy3, Cy5, and Cy2 image acquisition, 532/580, 633/670, and 488/520 nm excitation/emission wavelengths were used, respectively, and were acquired with a 100 µm pixel size.

DIGE data analysis

Image analysis was carried out with DeCyder differential analysis software, version 6.5 (GE Healthcare) [23]. The differential in-gel analysis module was used to assign spot boundaries and to calculate parameters such as normalized spot volumes. The integral variability was corrected by normalization of the runs with the internal standard spot maps in the biological variation analysis module. The average ratio was calculated. The false discovery rate was applied to reduce the false positives in the *p*-value calculation [24]. Those protein spots with 1.5-fold as a threshold in the average ratio and with *P* values < 0.05 (Student's *t* test) were considered differentially expressed.

Protein identification by mass spectrometry

The changes observed by 2D-DIGE analysis were aligned with Colloidal Coomassie Blue (CCB) profiling, and spots considered to be of interest were excised and in-gel digested [25]. After digestion, the supernatants were collected and 1 ml was spotted onto a matrix-assisted laser desorption ionization (MALDI) target plate and allowed to air-dry at room temperature. Then, 0.5 μ l of a 3 mg/ml α -cyano-4-hydroxycinnamic acid matrix (Sigma) in 50% (vol/vol) acetonitrile and 0.1% trifluoroacetic acid was added to the dried peptide-digested spots and allowed to air dry.

Mass spectrometry (MS) analysis was performed in a MALDI-TOF/TOF spectrometer 4700 proteomics analyzer (PerSeptives Biosystems, Framingham, MA). The instrument was operated in reflector mode, with an accelerating voltage of 20,000 V. All mass spectra were internally calibrated using autodigested trypsin peptides. The mass fingerprint spectra with a signal-to-noise ratio greater than 20 were collated and represented as a list of monoisotopic molecular weights. Some proteins were subjected to MS/MS sequencing analysis to be more confident of the identification

For protein identification, the nonredundant NCBI database was searched using Mascot 2.1 (www.matrixscience.com) through the Global Protein Server, version 3.6 (Applied Biosystems). The Mascot search parameters were: (i) species: all; (ii) allowed number of missed cleavages: 1; (iii) carbamidomethyl cysteine as fixed modification; (iv) variable modification: methionine oxidation; (v) peptide tolerance: \pm 50–80 ppm; (vi) MS/MS tolerance: \pm 0.3 Da and (vii): peptide charge: +1. In all identified proteins, the probability score was greater than that fixed by Mascot as being significant ($P < 0.05$). The parameters for the combined search (peptide mass fingerprint and MS/MS spectra) were the same as described above.

Western blotting

Protein samples (10 μ g) were separated by 10% SDS-PAGE and transferred to a nylon membrane at 4°C and 50 V for 2 h. The membrane was then blocked with 5% powdered milk in phosphate-buffer saline solution (pH 7.5) containing 0.1% Tween-20 (Sigma) (PBS/T), then probed with specific antibodies against TAR DNA-binding protein 43 (TARDBP), aminoacylase-1 (ACY1), transketolase (TKT), transaldolase (TALDO1), glyceraldehyde-3-P dehydrogenase (GAPDH), proliferating cell nuclear antigen (PCNA), beta subunit of actin (ACTB), citrate synthase (CS), T-cell intracellular antigen 1 (TIA1), TIA1 related/like protein (TIAR), and alpha subunit of tubulin (TUBA). Membranes were incubated with primary antibodies in PBS/T containing 3% BSA (Sigma) overnight at 4°C, washed, and then incubated with appropriate HRP-conjugated secondary antibodies at room temperature for 1 h. The ECL reagent (GE Healthcare) for HRP was used as a developer and band intensity was calculated by densitometry.

Bioinformatic analysis

Ingenuity Pathway Analysis (IPA) (<http://apps.ingenuity.com>) was used to identify the pathways and networks of the identified proteins and to constitute molecular partners. Two master global networks of all differentially expressed proteins (DEPs) (input objects) were suggested according to published literature-based annotations, and further sub-networks were built from the master network to focus on activated targets and/or pathways. Biological functions, in terms of gene ontology (GO) and interaction networks, were also analyzed using IPA. Based on the local networks created by computational algorithms, identified proteins were connected with hub proteins,

forming a functional protein cluster. The heterogeneous set of DEPs was also analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Analyses were performed using software programs provided by GenCodis3 (<http://genecodis.cnb.csic.es>) [26,27], Bioprofiling (<http://bioprofiling.de>) [28] and GeneAnalytics (www.geneanalytics.com). Data from individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) analysis of TIA1 and TIAR from HeLa cells were also used [4].

Results and Discussion

Identification of differentially expressed proteins in TIA-knockdown HeLa cells

To gain more insight into the role of TIA proteins in human cells, we compared the proteome of TIA-kd and control HeLa cells [9] using 2D-DIGE technology. The design involved mixing aliquots of every individual sample, which were included in equal amounts in the experiment. One-half of the samples of each condition were labeled with Cy3 dye, and the other one-half were labeled with Cy5 dye, to avoid any possible bias derived from the labeling efficiency. An internal standard sample was labeled with Cy2 dye and run on each gel. Thus, two samples from different replicates (Cy3 and Cy5) and an aliquot of the internal standard pool (Cy2) were separated by 2D electrophoresis in each one of the four gels. A scheme of the experimental design and the distribution of the samples is shown in Figure 1.

The four proteome samples were individually analyzed by 2D-DIGE, which resulted in the identification of ~1780 protein spots that tallied in the four gels. Among these were 30 protein spots that showed differential ratios with 1.5-fold change in abundance and consistent differences between the two backgrounds analyzed ($P < 0.05$) (Figure S1). Twenty-one spots increased in intensity in TIA-kd cells, whereas nine decreased (Figure S1). Twenty-five of the 30 protein spots were excised and digested and the peptides were analyzed by MS. Two spots contained more than one protein; in these cases, the individual proteins were identified by MS/MS sequencing analysis (Figure S2). Table I summarizes the identified peptides including the average ratio and the statistical data from each comparison, as well as the data on Mascot identifications (Figure S2).

Validation of predicted changes in proteomic analysis

We validated some of the 2D-DIGE data using western blotting to measure the expression of the following proteins linked to relevant biological processes or pathways: TARDBP, ACY1, TKT, TALDO1, GAPDH, PCNA, ACTB, CS, TIA1, TIAR, and TUBA. The results obtained were in part consistent with those of the proteomic analysis, suggesting that several changes observed in our proteomic analysis could represent putative novel targets of TIA proteins (Figure S3).

To gain a more complete view on the role of TIA proteins in gene expression control, the relative mRNA abundance of genes encoding for 23 of the proteins identified in the 2D-DIGE analysis were estimated from transcriptome profiles of control and TIA-kd cells using a previous genome-wide oligonucleotide-based DNA microarray analysis [29]. Six out of 23 DEPs (TKT, G6PD, IDH1, AKR1C2, AKR1C3 and CS) corresponded to genes that were differentially decreased in the transcriptomic assay [29], suggesting that their regulation was exerted *via* controlling their rates of transcription, alternative pre-mRNA splicing, and/or mRNA stability/turnover. The remainder of the genes showed unaltered mRNA levels, suggesting that their differential expression could reflect a post-transcriptional control *via* modulating the rates of mRNA translation and/or protein stability.

In the light of the above data, a question emerges as to how the under-expression of TIA proteins in HeLa cells can regulate the expression of so many functionally related genes. One possibility is that these mRNAs or their precursors (pre-mRNAs) could be targeted by TIA proteins at different levels to control their gene expression. In this regard, the experimental profiles of TIA1 and TIAR proteins (Figure 2 and S4) were examined using the iCLIP database of TIA proteins, kindly provided by the Ule lab [4]. The results showed that many of the gene targets identified contain multiple TIA-binding sites located across the full-length pre-mRNAs, both exons and introns, with a high frequency in the last exons and particularly at the 3'-untranslated regions of the mature mRNAs. Therefore, many of these mRNAs might be TIA-target genes that are subject to post-transcriptional and/or translational regulation. Pre-mRNAs and/or mRNAs are tethered to regulatory factors, and the combinatorial binding of these RNA-binding proteins and/or microRNAs to mRNA targets coordinates different steps of RNA metabolism [6]. It will be important to determine whether ectopic and/or endogenous TIA protein expression, as well as potential post-translational modifications of these factors, can also regulate the gene expression patterns of these target mRNAs at transcriptional and/or post-transcriptional levels. Our observations suggest a direct interaction between some mRNAs and TIA proteins, which could contribute to the post-transcriptional regulation of these targets, indicating that these multifunctional regulators are able to function as global and/or specific translational repressors, possibly by temporarily contributing to plasticity in growth and metabolism that enables cells/tissues to adapt/survive in a dynamic environment.

Classification of differentially expressed genes

Proteins were classified according to biological processes as described in the protein knowledge base (UniProtKB) (www.uniprot.org) (Table I). We used IPA to map the biological functions, pathways and networks for the 30 DEPs. The analysis highlighted several metabolic pathways including pentose phosphate pathway (non-oxidative and oxidative branch), TCA cycle, amino acid turnover and/or biosynthesis, and NADH repair (Figure 3A). Canonical pathways identified at statistically significant levels ($P < 0.05$) are shown in Figure 3A. The scores ($-\log [p \text{ values}]$) reflect the probabilities of such associations occurring by chance, with the threshold value for significance set at 1.25. Cellular compartments as designated by IPA (GO-based) indicated that the majority of identified proteins were cytoplasmic and mitochondrial in origin (data not shown).

GO analysis identified the main categories of biological processes and molecular functions of DEPs controlled by TIA proteins (Figures S5 and S6). GO categories related to small molecule and carbohydrate metabolic processes, citric acid cycle (TCA cycle) and pentose-phosphate, among others, were especially prevalent among the enriched categories of "Biological Process and Molecular Function" (Figures S5 and S6). KEGG/pathways database analysis was used to identify the enrichment of specific pathways in functionally regulated gene groups. Several KEGG pathways were significantly enriched in regulated/modulated proteins, including those involved in carbon, glucose and energy metabolism, pentose-phosphate pathway, MPS VI-Maroteaux-Lamy syndrome, (TCA cycle) and glyoxylate and dicarboxylate metabolism. In addition, several human diseases clustered as global and anatomical categories were related with the differentially expressed proteins: global categories were associated with rare, genetic, infectious, cancer and fetal diseases; and anatomical categories were

linked to neuronal, bone, blood, skin, eye, nephrological, liver, mental, muscle, reproductive, respiratory, ear, gastrointestinal, endocrine, cardiovascular and immune diseases. Furthermore, several pathophysiological aspects were also associated with the genes identified, such as liver disease, asthma, allergy, arthritis, congenital dyserythropoietic anemia, distant metastasis, carcinoma (breast, renal and colon), leukemia, coronary heart disease, insulin resistance (diabetes) and obesity (data not shown). Bioprofiling tool analysis showed that most of DEPs are implicated in carbohydrate and small molecule metabolic processes in cytoplasm related to the metabolism of amino acids, sugars and nucleotides. Taken together, these observations suggest that most of the genes are implicated in the metabolism of sugars, nucleotides, and amino acids as well as in metabolism-associated diseases. These *in silico* analysis were completed with integration analysis using IPA to build gene networks. The results obtained showed a top-scoring network connecting 18 DEPs (11 up- and 7-down-regulated genes) strongly linked to metabolic and proteostasis features (Figure 3B). A secondary subnetwork involving only 7 up-regulated DEPs was associated with detoxification and metabolism (Figure S7). Thus, metabolism, proteostasis, and detoxification dynamics appear as hallmarks in all the analyses performed (Figure 3 and S5-S7).

Cellular metabolism is generally a primary target to favor the development and establishment of new cellular phenotypes. For example, cells with higher proliferative capacity reprogram basal metabolism to adapt to the new requirements associated with phenotypic evolution. Against this background, at least two metabolic aspects are fundamental: how cellular energy is derived and what are the sources. Thus, depending on whether oxygen availability is sufficient, glycolysis provides energy in the form of ATP, precursors for the synthesis of amino acids (for protein biosynthesis), nucleotides (for the processes of synthesis and replication of cellular DNA and DNA-dependent transcription), lipids (for the synthesis of biological membranes), and reducing power (to achieve the synthesis of the above precursors) [30]. Furthermore, mitochondria also play an essential role in metabolic renewal and the processes associated with this organelle are readapted, favoring the production of anabolic precursors to the detriment of the production of energy [31]. Mitochondria also contribute to intracellular signaling, retrograde communication and detoxification at this regulatory crossroads, to make possible the acquisition of a stronger proliferative phenotype [30,31].

Mice lacking both TIA1 and TIAR are nonviable and die before embryonic day 7, implying that at least one of these proteins is necessary for normal embryonic development [32,33]. HEK293 cells with CRISPR-mediated deletion of TIA1 present a chronic stress response mediated by the EIF2AK2 kinase, triggering cell-cycle arrest at G2/M phase, and a progressive DNA damage response that enhances cell death after 7 days [34]. By contrast, several cell models with reduced expression of TIA1 and/or TIAR by RNA interference mainly show an adaptive response accompanied by a proliferative phenotype [5-13]. This is reminiscent of that observed when a low dose of a stressful stimulus activates an adaptive response that increases the resistance of the cell or organism to higher stress levels [35]. Therefore, we propose that in cases of low TIA expression, there is an adaptive response known as hormesis. Accordingly, TIA-associated hormetic response involves the expression of genes that encode cytoprotective proteins such as metabolic and antioxidant enzymes, chaperones, and survival factors.

Crosstalk of gene expression regulation associated with TIA proteins may involve complex autoregulatory loops with key factors such as other RNA-binding proteins [36]. For example, TIA proteins are recognized transcriptional and post-transcriptional regulators [6], yet, both their overexpression and knockdown increase stress granule formation [37,38]. Such positive and negative assembly roles for TIA proteins could be determined by cellular conditions, by their overall concentration, or be a consequence of altered regulation of other stress granule assembly factors, such as TIA1, whose translation and alternative splicing is regulated by TIAR [39,40]. By contrast, the RNA binding protein HuR, which is operatively close to TIA proteins, enhances TIA1 expression [39]. Given these types of regulatory hubs, care must be exercised in interpreting loss- and gain-of-function phenotypes without a clear understanding of the underlying mechanism [36]. In addition, distinct size changes to 3'-untranslated regions (UTR) have been recently described under stress conditions in proliferating and differentiated cells, correlating with stress granule association and mRNA decay of long 3'-UTR isoforms in a TIA1-dependent manner. This observation underlies a context-specific impact on the 3'UTR landscape associated with TIA1 expression [41].

Acknowledgements

We are indebted to the generosity of the following researchers and facilities: T Alberio, FE Baralle, M Martínez-Gomariz, B Peral, JM Sierra, N Zambrano, and Proteomics Facility at Universidad Complutense de Madrid-Parque Científico de Madrid (UCM-PCM). This work was supported by grants from Ministry of Economic Affairs and Competitiveness (BFU2011-29653 and BFU2014-57735-R). The CBMSO receives an institutional grant from Fundación Ramón Areces and Banco Santander, Spain.

Competing interests statements

The authors declare that they have no competing interests.

References

- [1] I. López de Silanes, S. Galbán, J.L. Martindale, et al, Identification and functional outcome of mRNAs associated with RNA-binding protein TIA-1, *Mol. Cell. Biol.* 25 (2005) 9520-9531.
- [2] K. Mazan-Mamczarz, A. Lal, J.L. Martindale, et al, Translational repression by RNA-binding protein TIAR, *Mol. Cell. Biol.* 26 (2006) 2716-2727.
- [3] H.S. Kim, Y. Kuwano, M. Zhan, et al, Elucidation of a C-rich signature motif in target mRNAs of RNA-binding protein TIAR, *Mol. Cell. Biol.* 27 (2007) 6806-6817.
- [4] Z. Wang, M. Kayikci, M. Briese, et al, iCLIP predicts the dual splicing effects of TIA-RNA interactions, *PLoS Biol.* 8 (2010) e1000530.
- [5] R. Reyes, J. Alcalde, J.M. Izquierdo, Depletion of T-cell intracellular antigen proteins promotes cell proliferation, *Genome Biol.* 10 (2009) R87.
- [6] C. Sánchez-Jiménez, J.M. Izquierdo, T-cell intracellular antigens in health and disease, *Cell Cycle.* 14 (2015) 2033-2043.
- [7] T. Kawai T, A. Lal, X. Yang, et al, Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR, *Mol. Cell. Biol.* 26 (2006) 3295-3307.
- [8] B. Liao, Y. Hu, G. Brewer, Competitive binding of AUF1 and TIAR to MYC mRNA controls its translation, *Nat. Struct. Mol. Biol.* 14 (2007) 511-518.
- [9] J.M. Izquierdo, J. Alcalde, I. Carrascoso, et al, Knockdown of T-cell intracellular antigens triggers cell proliferation, invasion and tumour growth. *Biochem J.* 435 (2011) 337-344.

- [10] Y. Liu, R. Liu, F. Yang F, et al, miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1, *Mol. Cancer*. 16 (2017) 53.
- [11] C.P. Wigington, J. Jung, E.A. Rye, et al, Post-transcriptional regulation of programmed cell death 4 (PDCD4) mRNA by the RNA-binding proteins human antigen R (HuR) and T-cell intracellular antigen 1 (TIA1), *J. Biol. Chem.* 290 (2015) 3468-3487.
- [12] M.A. Hamdollah Zadeh, E.M. Amin, C. Hoareau-Aveilla, C, et al, Alternative splicing of TIA-1 in human colon cancer regulates VEGF isoform expression, angiogenesis, tumour growth and bevacizumab resistance, *Mol. Oncol.* 9 (2015) 167-178.
- [13] J. Hamada, K. Shoda, K. Masuda, et al, Tumor-promoting function and prognostic significance of the RNA-binding protein T-cell intracellular antigen-1 in esophageal squamous cell carcinoma, *Oncotarget*. 7 (2016) 17111-17128.
- [14] C. Sánchez-Jiménez, M.D. Ludeña, J.M. Izquierdo, *Cell Death Dis.* 6 (2015) e1669.
- [15] I. Carrascoso, J. Alcalde, C. Sánchez-Jiménez C, et al, T-cell intracellular antigens and Hu antigen R antagonistically modulate mitochondrial activity and dynamics by regulating optic atrophy 1 gene expression. *Mol. Cell. Biol.* 37 (2017) e00174-17.
- [16] K. Arimoto-Matsuzaki, H. Saito, M. Takekawa, TIA1 oxidation inhibits stress granule assembly and sensitizes cells to stress-induced apoptosis, *Nat Commun.* 7 (2016) 10252.
- [17] L. Liu, H. Yue, Q. Liu, et al, LncRNA MT1JP functions as a tumor suppressor by interacting with TIAR to modulate the p53 pathway, *Oncotarget*. 7 (2016) 15787-15800.
- [18] M. Seiler, S. Peng, A.A. Agrawal, et al, Somatic mutational landscape of splicing factor genes and their functional consequences across 33 cancer types. *Cell Rep.* 23 (2018) 282-296.
- [19] R. Marouga, S. David, E. Hawkins, The development of the DIGE system: 2D fluorescence difference gel analysis technology, *Anal. Bioanal. Chem.* 382 (2005) 669-678.
- [20] B. Bjellquist, K. Ek, P.G. Righetti, et al, Isoelectric focusing in immobilized pH gradients: principle, methodology and some applications, *J. Biochem. Biophys. Methods* 6 (1982) 317-339.
- [21] P.H. O'Farrell, High resolution two-dimensional electrophoresis of proteins, *J. Biol. Chem.* 250 (1975) 4007-4021.
- [22] R. Alessandro, C. Belluco, E.C. Kohn, Proteomic approaches in color cancer: promising tools for new cancer markers and drug target discovery, *Clin. Colorectal Cancer* 4 (2005) 396-402.
- [23] C. Ruiz-Romero, V. Calamia, J. Mateos, et al, Mitochondrial dysregulation of osteoarthritic human articular chondrocytes analyzed by proteomics: a decrease in mitochondrial superoxide dismutase points to a redox imbalance. *Mol. Cell. Proteomics.* 8 (2009) 172-189.
- [24] N.A. Karp, K.S. Lilley, Design and analysis issues in quantitative proteomics studies, *Proteomics.* 7 (2007) 42-50.
- [25] S. Sechi, B.T. Chait, Modification of cysteine residues by alkylation. A tool in peptide mapping and protein identification. *Anal Chem.* 70 (1998) 5150-5158.

- [26] P. Carmona-Sáez, M. Chagoyen, F. Tirado, et al, GENECODIS: a web-based tool for finding significant concurrent annotations in gene lists, *Genome Biol.* 8 (2007) R3.
- [27] R. Nogales-Cadenas, P. Carmona-Saez, M. Vazquez, et al, (2009) GeneCodis: interpreting gene lists through enrichment analysis and integration of diverse biological information, *Nucleic Acids Res.* 37 (2009) W317-322.
- [28] A.V. Antonov, BioProfiling.de: analytical web portal for high-throughput cell biology, *Nucleic Acids Res.* 39 (2011) W323-327.
- [29] M. Núñez, C. Sánchez-Jiménez, J. Alcalde, et al, Long-term reduction of T-cell intracellular antigens reveals a transcriptome associated with extracellular matrix and cell adhesion components. *PLoS One* 9 (2014) e113141.
- [30] P.S. Ward, C.B. Thompson, Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell.* 21 (2012) 297-308.
- [31] P.B. Esparza-Moltó, J.M. Cuezva, The role of mitochondrial H⁺-ATP synthase in cancer. *Front Oncol.* 8 (2018) 53.
- [32] M. Piecyk, S. Wax, A.R. Beck, et al, TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha, *EMBO J.* 19 (2000) 4154-4163.
- [33] A.R. Beck, I.J. Miller, P. Anderson P, M. Streuli, RNA-binding protein TIAR is essential for primordial germ cell development, *Proc. Natl. Acad .Sci.* 95 (1998) 2331-2336.
- [34] C. Meyer, A. Garzia, M. Mazzola M, et al, The TIA1 RNA-binding protein family regulates EIF2AK2-mediated stress response and cell cycle progression, *Mol. Cell.* 69 (2018) 622-635.
- [35] Yun J, T. Finkel T. Mitohormesis. *Cell Metab.* 2014; 19: 757-766.
- [36] J.R. Buchan, R. Parker. Eukaryotic stress granules: the ins and outs of translation. *Mol. Cell.* 36 (2009) 932-941.
- [37] N. Gilks, N. Kedersha, M. Ayodele, et al, Stress granule assembly is mediated by prion-like aggregation of TIA-1, *Mol. Biol. Cell.* 15 (2004) 5383-5398.
- [38] F. De Leeuw, T Zhang, C. Wauquier, et al, The cold-inducible RNA-binding protein migrates from the nucleus to cytoplasmic stress granules by a methylation-dependent mechanism and acts as a translational repressor. *Exp. Cell. Res.* 313 (2007) 4130-4144.
- [39] R. Jr. Pullmann, H.H. Kim, K. Abdelmohsen et al, Analysis of turnover and translation regulatory RNA-binding protein expression through binding to cognate mRNAs, *Mol. Cell. Biol.* 27 (2007) 6265-6278.
- [40] J.M. Izquierdo, J. Valcárcel, Two isoforms of the T-cell intracellular antigen 1 (TIA-1) splicing factor display distinct splicing regulation activities. Control of TIA-1 isoform ratio by TIA-1-related protein. *J. Biol. Chem.* 282 (2007) 19410-19417.
- [41] D. Zheng, R. Wang, Q. Ding, et al, Cellular stress alters 3'UTR landscape through alternative polyadenylation and isoform-specific degradation, *Nat. Commun.* 9 (2018) 2268.

Figure / Table legends

Figure 1. Schematic representation of the 2D-DIGE proteomic strategy. Workflow followed to evaluate and analyze the 2D-DIGE data.

Figure 2. *In vivo* crosslinking sites (iCLIP) of TIA1 and TIAR proteins in 2D-DIGE-identified genes. The RNA map, corresponding to TIA proteins on 2D-DIGE-predicted genes in HeLa cells, was adapted using the TIA-iCLIP analysis provided by the Ule laboratory [4]. The bar graphs show the number of cDNAs that identified each

crosslinking site. The localization of target genes on human chromosomes and the exon and intron positions of the human pre-mRNAs are shown. The following gene names are used: LMNB1, lamin-B1; TARDBP, TAR DNA-binding protein 43; EIF2B2, eIF-2B subunit beta; PSMB8, proteasome subunit β type-8; SELENBP1, selenium-binding protein 1; PRDX2, peroxiredoxin-2; TKT, transketolase; G6PD, glucose-6-phosphate 1-dehydrogenase; TALDO1, transaldolase; GAPDH, glyceraldehyde-3-P; CS, citrate synthase; GOT1, aspartate aminotransferase.

Figure 3. The top-scoring regulatory networks identified by Ingenuity Pathway Analysis. (A) IPA of biological processes associated with differentially expressed proteins (DEPs) in TIA-kd HeLa cells. The classical IPA bar chart displays biological functions along the x-axis. The y-axis displays the $-\log$ significance ($-\log P$ values) calculated by Fisher's exact test ($P < 0.05$). Functions are listed from most (higher bars) to least (lower bars) significant and the orange horizontal line denotes the threshold for significance ($P = 0.05$). (B) DEPs that are upregulated and downregulated in TIA-kd HeLa cells are displayed within red and green nodes, respectively. The intensity of the shading shows to what degree each gene was up or downregulated. Solid and dashed lines between genes represent known direct and indirect gene interactions, respectively. The shapes of the nodes reflect the functional class of each gene product: transcriptional regulator (horizontal ellipse), transmembrane receptor (vertical ellipse), enzyme (vertical rhombus), cytokine/growth factor (square), kinase (inverted triangle) and complex/group/other (circle). The following colored gene names are shown: AKR1C1, aldo-keto reductase 1 C1; AKR1C2, aldo-keto reductase 1 C2; AKR1C3, aldo-keto reductase 1 C3; CAT, catalase; CS, citrate synthase; CRKL, Crk-like protein; EIF2B2, eIF-2B subunit beta; G6PD, glucose-6-phosphate 1-dehydrogenase; GAPDH, glyceraldehyde-3-P dehydrogenase; GOT1, aspartate aminotransferase; HSPA8, heat shock cognate 71 kDa; IDH1, isocitrate DH [NADP], IDH3A, isocitrate DH [NAD] alpha; PRDX2, peroxiredoxin-2; PSMB8, proteasome subunit β type-8; TALDO1, transaldolase; TARDBP, TAR DNA-binding protein 43; BLVRA, biliverdin reductase A; MDH1, malate dehydrogenase.

Table I. Summary of the identified proteins classified according to biological function, with the average ratio, statistical data from the comparison and the data from Mascot identifications. ^(a)Average volume ratio TIA1 + TIAR/control and t test P value, quantified by DeCyder BVA module. ^(b)Score: Mascot MS protein score or MS/MS, obtained from MALDI-TOF/TOF spectra. In all of the cases, a probability score of < 0.05 was obtained. ^(c)Number of peptide mass value searched/mapped. ^(d)Amino acid sequence coverage for the identified proteins or sequence identified by MS/MS.

Figure 1

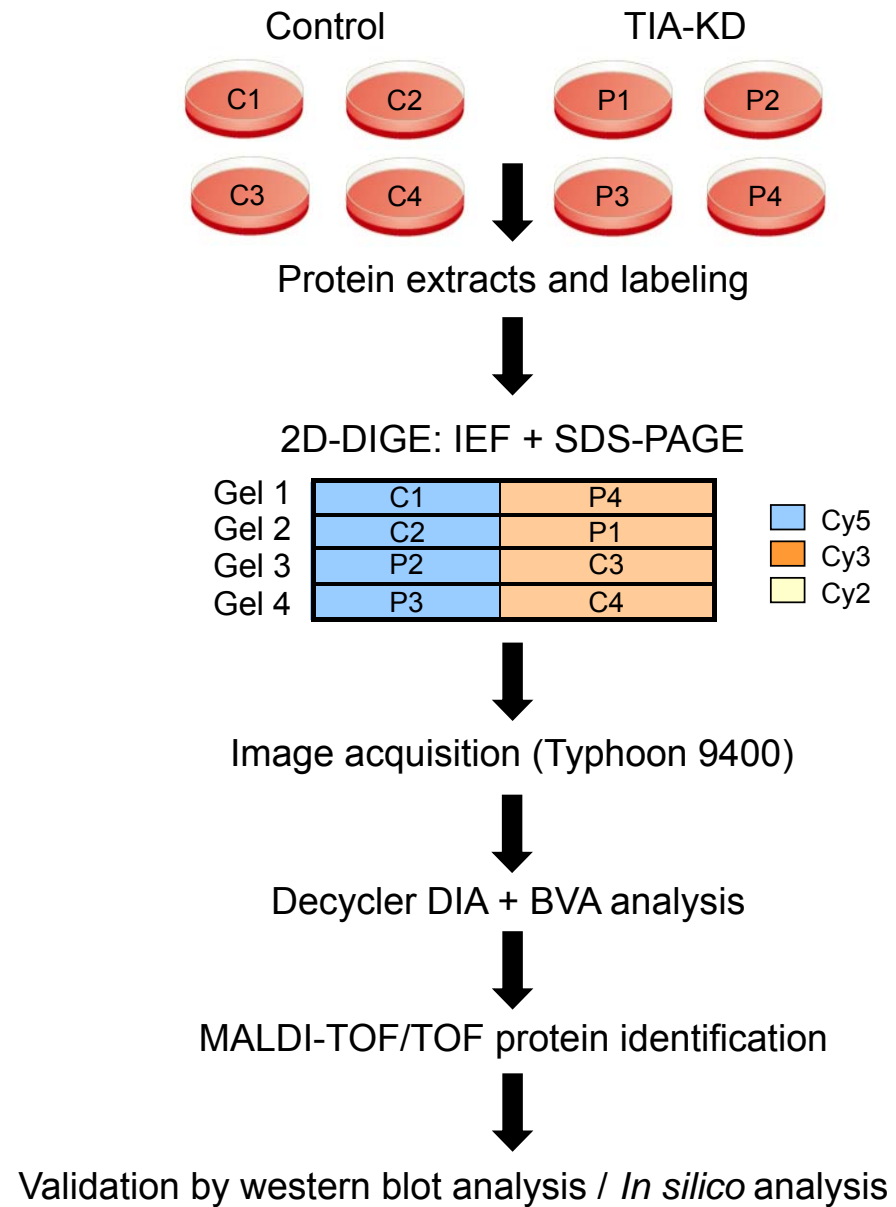
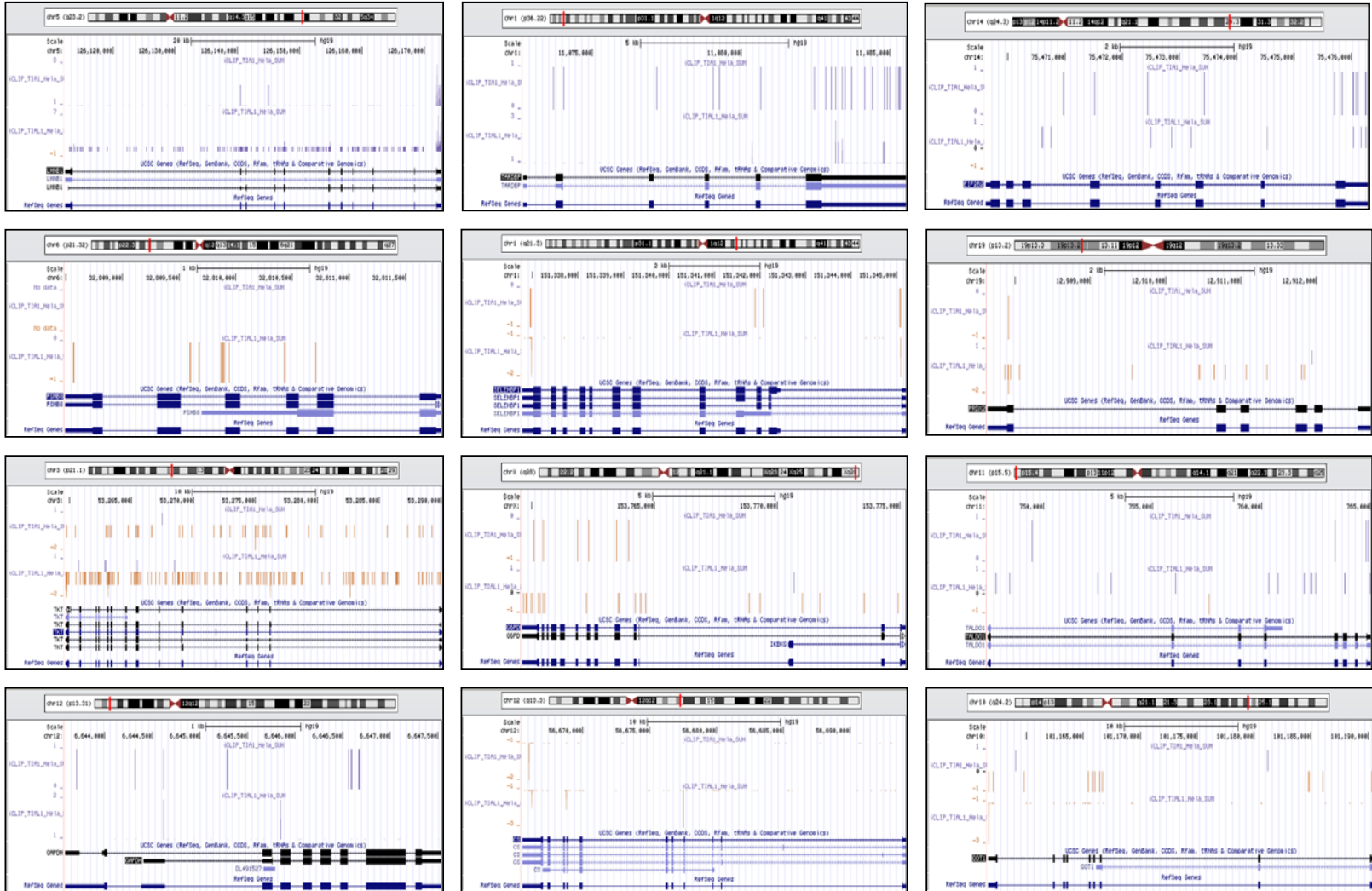
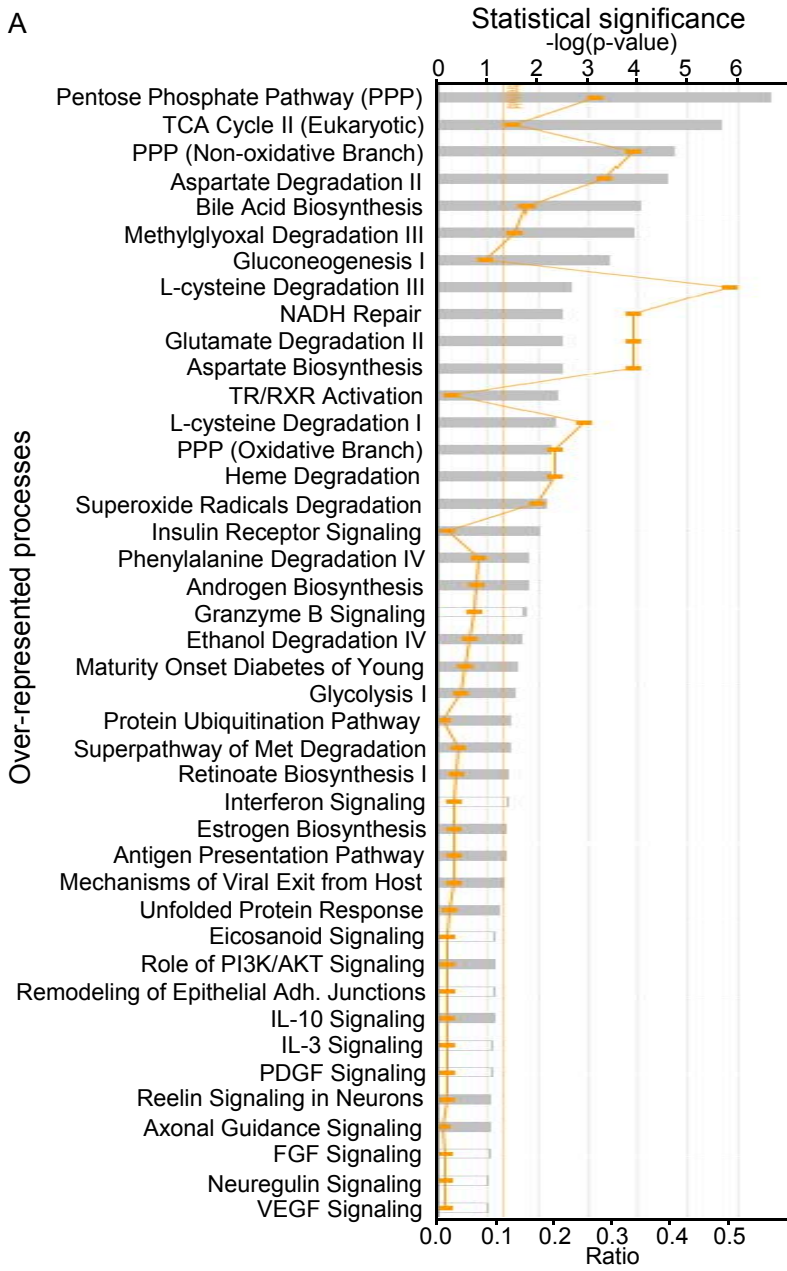


Figure 2



A



B

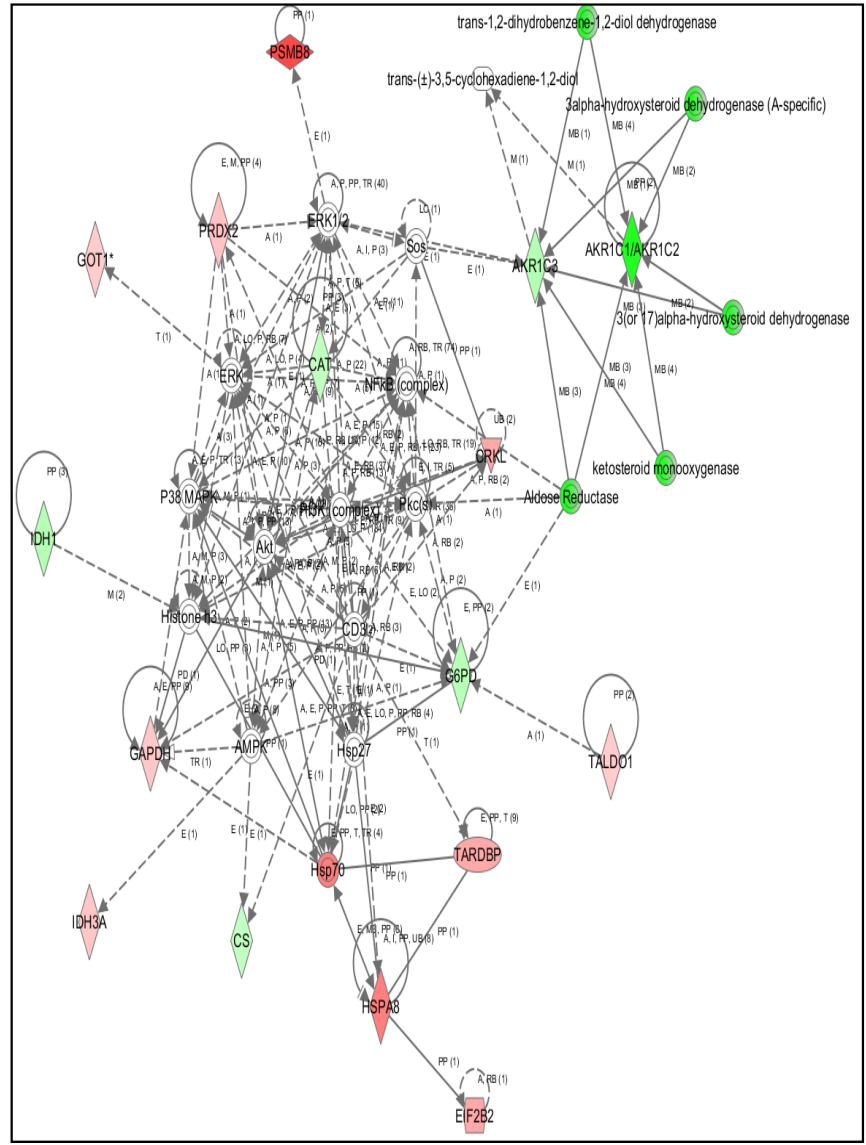
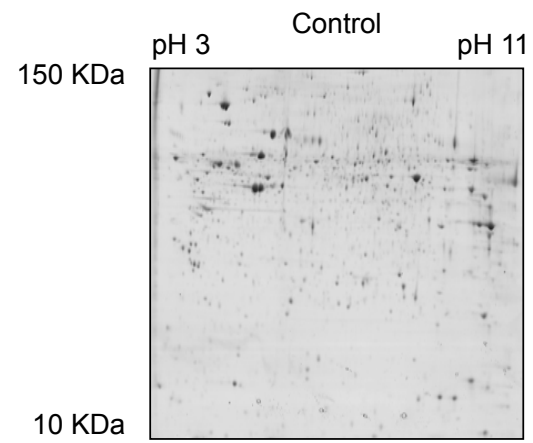


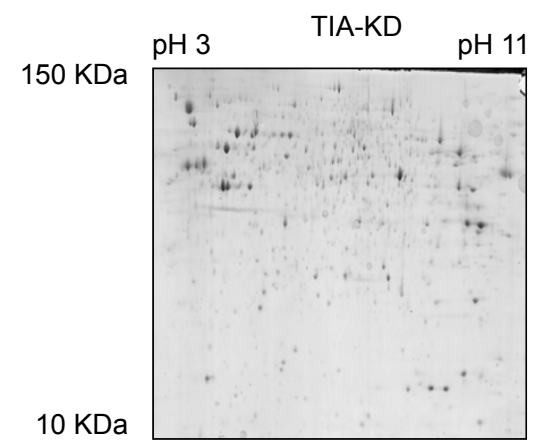
Figure 3

Figure S1

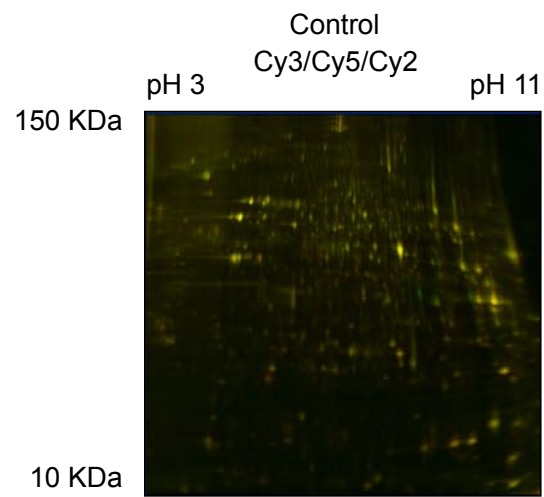
A



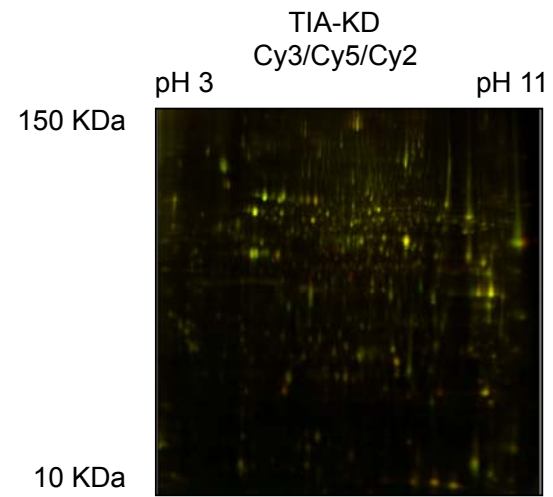
B



C



D



Mascot Search Results

Protein View

Match to: [sp|Q9BQE3|TBA1C_HUMAN](#) Score: 51
 Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1

Nominal mass (M_r): 50548; Calculated pI value: 4.96
 NCBI BLAST search of [sp|Q9BQE3|TBA1C_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Sequence Coverage: 3%

Matched peptides shown in **Bold Red**

```

1 MRECISIHVG QAGVQIGNAC WELYCLEHGI QPDGQMPSDK TIGGGDDSFN
51 TFFSETGAGK HVPRAVFVDL EPTVIDEVRT GTYRQLFHPE QLITGKEDAA
101 NNYARGHYTI GKEIIDLVLD RIRKLADQCT GLQGFLVFHS FGGGTGSGFT
151 SLLMERLSVD YGKSKLEFS IYPAPQVSTA VVEPYNSILT THTTLEHSDC
201 AFMVDNEAIY DICRRNLDIE RPTYTNLNLRL ISQIVSSITA SLRFDGALNV
251 DLTEFQTNLV PYPRIHFPLA TYAPVISAEK AYHEQLTVAE ITNACFEPAN
301 QMVKCDPRHG KYMACCLLYR GDVVPKDVNA AIATIKTKRT IQFVDWCPTG
351 FKVGINYQPP TVVPGGDLAK VQRAVCMLSN TTAVAEAWAR LDHKFDLMYA
401 KRAFVHWYVG EGMEEGEFSE AREDMAALEK DYEEVGADSA DGEDEGEEY
  
```

Show predicted peptides also

Sort Peptides By



Residue Number



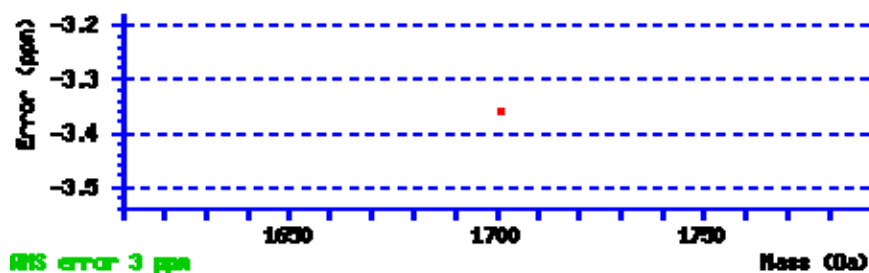
Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	M_r (expt)	M_r (calc)	Delta	Miss
65 - 79 R.AVFVDLEPTVIDEVR.T	1701.9000	1700.8927	1700.8984	-0.0057	0

([Ions score 51](#))



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P20700|LMNB1_HUMAN** Score: **75** Expect: **0.00063**
Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2

Nominal mass (M_r): **66653**; Calculated pI value: **5.11**
 NCBI BLAST search of [sp|P20700|LMNB1_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **71**
 Number of mass values matched: **15**
 Sequence Coverage: **28%**

Matched peptides shown in **Bold Red**

1 **MATATPVPPR MGSR**AGGPTT PLSPTRLSRL QEKEELRELN DRLAVYIDKV
 51 **R****SLETENSAL QLQVTER**EEV RGRELTGLKA **LYETELADAR RALDDTARER**
 101 AKLQIELGKC KAEHDQLLLN YAKKESDLNG AQIKLREYEA ALNSKDAALA
 151 TALGDKKSLE GDLEDLKDQI AQLEASLAAA **KKQLADETLL KVDLENRCQS**
 201 LTEDLEFRKS **MYEEEEINETR RKHETRLVEV DSGRQIEYFY KLAQALHEMR**
 251 EQHDAQVRLY **KEELEQTYHA KLENARLSSE MNTSTVNSAR EELMESRMRI**
 301 ESLSSQLSNL QKESRACLER IQELEDLLAK EKDNSRRMLT DKEREMAEIR
 351 DQMQQQLNDY EQLLDVKLAL DMEISAYRKL LEGEEERLKL SPSRSSRVTV
 401 SRASSRSRVR TTRGKRKRVD VEESEASSV SIHSASATG NVCIEEIDVD
 451 GKFIRLKN**TS EQDQPMGGWE MIR**KIGDTSV SYKYTSRYVL KAGQTVTIWA
 501 ANAGVTASPP TDLIWK**NQNS WGTGEDVKVI LKNSQGEEVA QR**STVFKTTI
 551 PEEEEEEEEEA AGVVVEEELF HQQGTPRASN RSCAIM

Show predicted peptides also

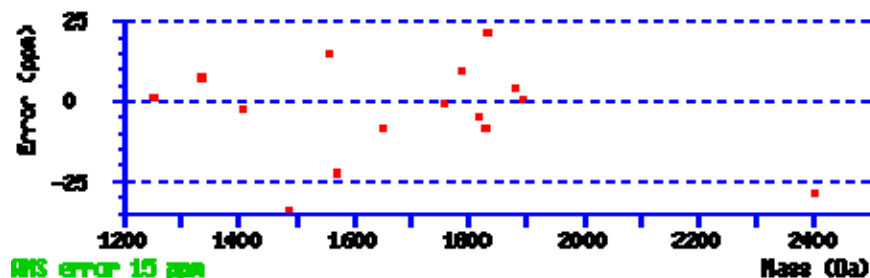
Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
1 - 14 .MATATPVPPRMGSR.A	1487.6952	1486.6879	1486.7384	-0.0505	1 -
52 - 67 R.SLETENSALQLQVTER.E	1817.9147	1816.9074	1816.9166	-0.0092	0
80 - 90 K.ALYETELADAR.R	1251.6227	1250.6154	1250.6142	0.0012	0
80 - 91 K.ALYETELADARR.A	1407.7191	1406.7118	1406.7153	-0.0035	1
183 - 197 K.QLADETLLKVDLENR.C	1756.9430	1755.9357	1755.9366	-0.0009	1
210 - 221 K.SMYEEEEINETR.R.K	1556.7244	1555.7171	1555.6936	0.0235	1

227 - 241	1827.8980	1826.8907	1826.9050	-0.0143	1
R.LVEVDSGRQIEYKYK.L					
259 - 271	1651.8195	1650.8122	1650.8253	-0.0131	1
R.LYKEELEQTYHAK.L					
262 - 276	1830.9373	1829.9300	1829.8907	0.0393	1
K.EELEQTYHAKLENAR.L					
277 - 297	2403.0073	2402.0000	2402.0689	-0.0689	1
R.LSSEMNTSTVNSAREELMESR.M 2 Oxidation (M)					
458 - 473	1878.8190	1877.8117	1877.8036	0.0081	0
K.NTSEQDQPMGGWEMIR.K					
458 - 473	1894.8064	1893.7991	1893.7985	0.0006	0
K.NTSEQDQPMGGWEMIR.K Oxidation (M)					
517 - 528	1334.6069	1333.5997	1333.5898	0.0098	0
K.NQNSWGTGEDVK.V					
517 - 532	1787.9456	1786.9383	1786.9213	0.0170	1
K.NQNSWGTGEDVKVILK.N					
529 - 542	1570.8195	1569.8122	1569.8474	-0.0352	1
K.VILKNSQGEEVAQR.S					

No match to: 1145.5675, 1358.7142, 1391.7073, 1393.7199, 1417.7304, 1431.7078, 1434.7300, 1465.7087, 1480.7518, 1521.7516, 1529.7046, 1539.7914, 1562.7950, 1564.7987, 1566.7902, 1567.7548, 1590.7831, 1606.7422, 1616.7672, 1628.8868, 1638.8558, 1653.8372, 1656.7396, 1658.7323, 1671.8671, 1674.7290, 1687.8832, 1690.7231, 1691.7117, 1701.8927, 1703.9197, 1718.8568, 1740.8617, 1750.8823, 1771.8404, 1805.8793, 1837.9275, 1851.9396, 1885.9940, 1901.0007, 1918.8909, 1941.9677, 1964.0448, 1982.0103, 1992.9533, 2020.0671, 2049.9717, 2069.0117, 2103.9807, 2119.0481, 2128.0256, 2175.0503, 2566.2751, 2572.2398, 2582.2341, 3412.5830



Mascot Search Results

Protein View

Match to: [sp|Q13148|TADBP_HUMAN](#) Score: 72 Expect: 0.0014
TAR DNA-binding protein 43 OS=Homo sapiens GN=TARDBP PE=1 SV=1

Nominal mass (M_r): 45053; Calculated pI value: 5.85
NCBI BLAST search of [sp|Q13148|TADBP_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 6%

Matched peptides shown in **Bold Red**

```
1 MSEYIRVTED ENDEPIEIPS EDDGTVLLST VTAQFPGACG LRYRNPVSQC
51 MRGVRLEVEGI LHAPDAGWGN LVYVVNYPKD NKRKMDETDA SSAVKVKRAV
101 QKTSDLIVLG LPWKTTEQDL KEYFSTFGEV LMVQVKKDLK TGHSKGFGFV
151 R.FTEYETQVK VMSQRHMIDG RWCDCKLPNS KQSQDEPLRS RKVFGVGRCTE
201 DMTDELREF FSQYGDVMDV FIPKPFRAFA FVTFADDQIA QSLCGEDLII
251 KGISVHISNA EPKHNSNRQL ERSGRFGGNP GGFNQGQGF NSRGGGAGLG
301 NNQGSNMGGG MNFGAFSINP AMMAAAQAAL QSSWGMMGML ASQQNQS GPS
351 GNNQNQGNMQ REPNQAFGSG NNSYSGSNSG AAIGWGSASN AGSGSGFNNG
401 FGSSMDSKSS GWGM
```

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
152 - 160	1144.5558	1143.5485	1143.5448	0.0038	0
R.FTEYETQVK.V	(No match)				
276 - 293	1726.7751	1725.7678	1725.7607	0.0072	0
R.FGGNPGGFNQGQGFNSR.G	(Ions score 65)				
276 - 293	1726.7751	1725.7678	1725.7607	0.0072	0
R.FGGNPGGFNQGQGFNSR.G	(No match)				

MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P49770|EI2BB_HUMAN** Score: **119** Expect: **2.6e-008**
 Translation initiation factor eIF-2B subunit beta OS=Homo sapiens
 GN=EIF2B2 PE=1 SV=3

Nominal mass (M_r): **39193**; Calculated pI value: **5.77**
 NCBI BLAST search of [sp|P49770|EI2BB_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **81**
 Number of mass values matched: **23**
 Sequence Coverage: **49%**

Matched peptides shown in **Bold Red**

1 MPGSAAK**GSE L**SERIESFVE TLKRGGGPRS **SEEMARETLG LLR**QIITDHR
 51 **WSNAGELMEL IRRE**GRRMTA **AQPSETTVGN MVRR**VLKIIR **EEYGR**LHGERS
 101 **DESDQQESLH KLL**TSGGLNE DFSFYAQLQ SNIIEAINEL LVELEGTMEN
 151 IAAQALEHIH SNEVIMTIGF SR**TVEAF**LKE **AAR**KRKFHVI VAECAPFCQG
 201 HEMAVNLSKA **GIET**TVMTDA **AIFAV**MSRVN KVIIGTKTIL ANGALRAVTVG
 251 THTLALAAKH **HSTPL**IVCAP **MFKL**SPQFPN **EEDSF**HKFVA **PEEV**LPFTEG
 301 **DILEK**VSVHC PVFDYVPEEL ITLFISNIGG NAPSYYR**LM SELY**HPDDHV
 351 **L**

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

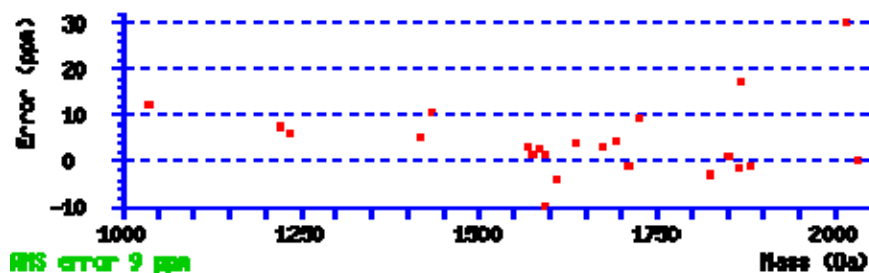


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
8 - 23 K.GSELSERIESFVETLK.R	1823.9330	1822.9257	1822.9312	-0.0055	1
15 - 24 R.IESFVETLKR.G	1221.6931	1220.6858	1220.6764	0.0094	1
30 - 43 R.SSEEMARETLGLLR.Q	1591.8129	1590.8056	1590.8035	0.0021	1
30 - 43 R.SSEEMARETLGLLR.Q Oxidation (M)	1607.7998	1606.7925	1606.7984	-0.0059	1
51 - 62 R.WSNAGELMELIR.R	1418.7170	1417.7098	1417.7023	0.0074	0
51 - 62 R.WSNAGELMELIR.R Oxidation (M)	1434.7196	1433.7123	1433.6972	0.0151	0
51 - 63 R.WSNAGELMELIRR.E	1574.8131	1573.8058	1573.8034	0.0024	1
51 - 63 R.WSNAGELMELIRR.E Oxidation (M)	1590.7898	1589.7825	1589.7984	-0.0158	1

68 - 83	1692.8118	1691.8045	1691.7970	0.0075	0
R.MTAAQPSETTVGNMVR.R					
68 - 83	1708.7971	1707.7898	1707.7920	-0.0021	0
R.MTAAQPSETTVGNMVR.R Oxidation (M)					
68 - 83	1724.8102	1723.8029	1723.7869	0.0160	0
R.MTAAQPSETTVGNMVR.R 2 Oxidation (M)					
68 - 84	1848.9077	1847.9004	1847.8981	0.0023	1
R.MTAAQPSETTVGNMVRR.V					
68 - 84	1864.8978	1863.8906	1863.8931	-0.0025	1
R.MTAAQPSETTVGNMVRR.V Oxidation (M)					
68 - 84	1880.8934	1879.8862	1879.8880	-0.0018	1
R.MTAAQPSETTVGNMVRR.V 2 Oxidation (M)					
88 - 95	1035.5708	1034.5635	1034.5508	0.0127	1
K.IIREEYGR.L					
96 - 111	1865.9061	1864.8989	1864.8663	0.0325	1
R.LHGRSDESDQQESLHK.L					
173 - 183	1234.6865	1233.6792	1233.6717	0.0076	1
R.TVEAFLKEAAR.K					
210 - 228	2016.0383	2015.0311	2014.9703	0.0607	0
K.AGIETTVMTDAAIFAVMSR.V 2 Oxidation (M)					
260 - 273	1637.8359	1636.8287	1636.8218	0.0069	0
K.HHSTPLIVCAPMFK.L					
274 - 287	1674.7811	1673.7739	1673.7685	0.0053	0
K.LSPQFPNEEDSFHK.F					
288 - 305	2033.0482	2032.0409	2032.0404	0.0006	0
K.FVAPEEVLPPFTEGDILEK.V					
339 - 351	1568.7465	1567.7392	1567.7341	0.0051	0
R.LMSELYHPDDHVL.-					
339 - 351	1584.7410	1583.7337	1583.7290	0.0047	0
R.LMSELYHPDDHVL.- Oxidation (M)					

No match to: 860.4357, 929.5539, 1138.5446, 1169.5536, 1262.6728, 1266.6385, 1297.6648, 1318.6993, 1366.6454, 1401.6877, 1412.7589, 1421.6934, 1440.7817, 1457.7832, 1498.8264, 1518.7869, 1544.8221, 1545.8197, 1561.8121, 1573.8059, 1576.8140, 1577.8115, 1582.7640, 1603.7334, 1604.7334, 1605.7252, 1606.7190, 1619.7520, 1620.7391, 1621.7300, 1622.7015, 1656.7730, 1668.8385, 1673.8409, 1688.7958, 1689.8076, 1696.7773, 1712.7804, 1725.8190, 1732.7987, 1739.9376, 1746.8635, 1755.9044, 1799.8944, 1860.8389, 1863.8961, 1972.8615, 1986.8874, 2047.0580, 2119.0576, 2139.0005, 2149.0561, 2176.0608, 2310.1533, 2342.1492, 2704.3052, 2720.3066, 2942.4294



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P28062|PSB8_HUMAN](#) Score: **83** Expect: **0.0001**
 Proteasome subunit beta type-8 OS=Homo sapiens GN=PSMB8 PE=1 SV=2

Nominal mass (M_r): **30677**; Calculated pI value: **7.63**
 NCBI BLAST search of [sp|P28062|PSB8_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Sequence Coverage: **14%**

Matched peptides shown in **Bold Red**

1 MALLDVCGAP RGQRPESALP VAGSGRRSDP GHYSFSMRSP ELALPRGMKP
 51 TEFFQSLGGD GERNVQIEMA HGTTTLAFKF **QHGVIAAVDS RASAGSYISA**
 101 LRVNKVIEIN PYLLGTMSGC AADCQYWERL LAKECRLYYL RNGERISVSA
 151 ASKLLSNMMC QYRGMGLSMG SMICGWDK**KG PGLYYVDEHG TRLSGNMFST**
 201 GSGNTYAYGV MDSGYRPNLS PEEAYDLGRR AIAYATHRDS YSGGVVNMVH
 251 MKEDGWVK**VE STDVSDLLHQ YREANQ**

Show predicted peptides also

Sort Peptides By



Residue Number

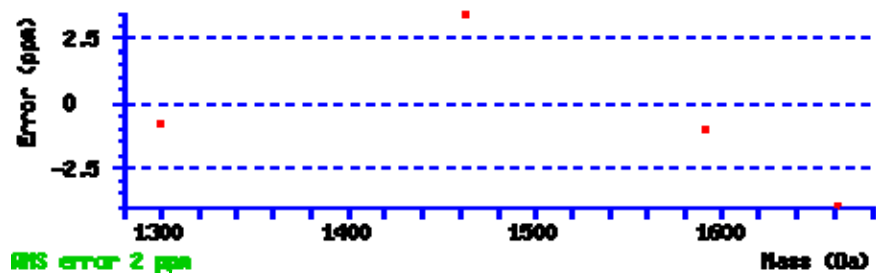


Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
80 - 91	1299.6793	1298.6720	1298.6731	-0.0011	0
K.FQHGVIAAVDSR.A (No match)					
179 - 192	1591.7847	1590.7774	1590.7790	-0.0016	1
K.KGPGLYYVDEHGTR.L (Ions score 28)					
179 - 192	1591.7847	1590.7774	1590.7790	-0.0016	1
K.KGPGLYYVDEHGTR.L (No match)					
180 - 192	1463.6964	1462.6891	1462.6840	0.0051	0
K.GPGLYYVDEHGTR.L (Ions score 38)					
180 - 192	1463.6964	1462.6891	1462.6840	0.0051	0
K.GPGLYYVDEHGTR.L (No match)					
259 - 272	1661.8063	1660.7990	1660.8056	-0.0066	0
K.VESTDVSDLLHQYR.E (No match)					





Mascot Search Results

Protein View

Match to: [sp|Q13228|SBP1_HUMAN](#) Score: **95** Expect: **6e-006**
Selenium-binding protein 1 OS=*Homo sapiens* GN=SELENBP1 PE=1 SV=2

Nominal mass (M_r): **52928**; Calculated pI value: **5.93**
NCBI BLAST search of [sp|Q13228|SBP1_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **65**
Number of mass values matched: **15**
Sequence Coverage: **37%**

Matched peptides shown in **Bold Red**

```

1  MATKCGNCGP  GYSTPLEAMK  GPREEIVYLP  CIYRNTGTEA PDYLATVDVD
51 PKSPQYCQVI HRLMPNLKD  ELHHSWNTC  SSCFGDSTKS  RTKLVLP SLI
101 SSRIYVVDVG SEPRAPKLHK  VIEPKDIHAK  CELAFLHTSH  CLASGEVMIS
151 SLGDVKGN GK  GGFVLLDGET FEVKGTWERP  GGAAPLGYDF  WYQPRHNVMI
201 STEWAAPNVL RDGFNPADVE  AGLYGSHLYV  WDWQRHEIVQ  TLSLKDGLIP
251 LEIRFLHNPD  AAQGFVGCAL SSTIQRFYKN  EGGTWSVEKV  IQVPPKKVKG
301 WLLPMPGLI  TDILLSLDDR  FLYFSNWLHG  DLRQYDISDP  QRRLTGQLF
351 LGSSIVKGGP VQVLEDEELK  SQPEPLVVKG  KRVAGGPQMI QLSLDGKRLY
401 ITTSLYSAWD  KQFYPDLIRE  GSVMLQVDVD  TVKGGCLKLN  NFLVDFGKEP
451 LGPALAHELR  YPGGDCSSDI  WI

```

Show predicted peptides also

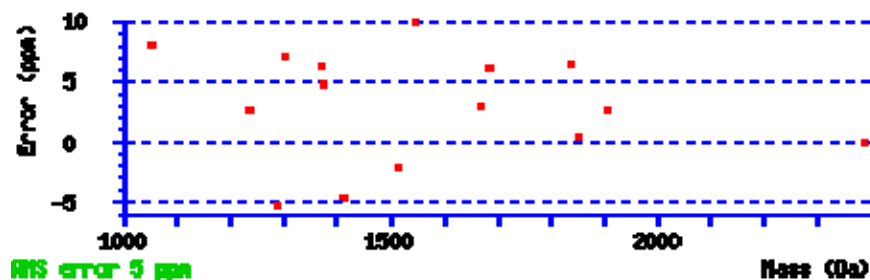
Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
35 - 52 R.NTGTEAPDYLATVDVDPK.S	1905.9128	1904.9055	1904.9003	0.0052	0
53 - 62 K.SPQYCQVIHR.L	1287.6194	1286.6121	1286.6190	-0.0068	0
104 - 114 R.IYVVDVGSEPR.A	1233.6505	1232.6432	1232.6400	0.0032	0
161 - 174 K.GGFVLLDGETFEVK.G	1510.7755	1509.7682	1509.7714	-0.0032	0
196 - 211 R.HNVMISTEWAAPNVL.R	1837.9497	1836.9424	1836.9304	0.0120	0
196 - 211 R.HNVMISTEWAAPNVL.R Oxidation (M)	1853.9335	1852.9262	1852.9254	0.0009	0
255 - 276 R.FLHNPDAAQGFVGCALSSTIQR.F	2389.1716	2388.1643	2388.1644	-0.0001	0

277 - 289	1544.7532	1543.7459	1543.7306	0.0153	1
R.FYKNEGGTWSVEK.V					
334 - 344	1374.6826	1373.6753	1373.6688	0.0066	0
R.QYDISDPQRPR.L					
358 - 370	1412.7201	1411.7128	1411.7194	-0.0066	0
K.GGPVQVLEDEELK.S					
383 - 398	1669.9102	1668.9029	1668.8981	0.0049	1
R.VAGGPQMIQLSLDGKR.L					
383 - 398	1685.9105	1684.9032	1684.8930	0.0102	1
R.VAGGPQMIQLSLDGKR.L Oxidation (M)					
412 - 419	1051.5656	1050.5583	1050.5498	0.0085	0
K.QFYPLIR.E					
449 - 460	1302.7256	1301.7183	1301.7091	0.0092	0
K.EPLGPALAEHLR.Y					
461 - 472	1369.5814	1368.5741	1368.5656	0.0085	0
R.YPGGDCSSDIWI.-					

No match to: 1010.3193, 1011.3191, 1022.3297, 1023.3279, 1036.3287, 1046.6005, 1082.0227, 1111.5631, 1136.5654, 1137.0826, 1137.5817, 1320.5781, 1399.7537, 1458.7288, 1490.7427, 1492.8047, 1642.7457, 1707.7939, 1808.9312, 2146.0688, 2161.0645, 2175.0549, 2178.0515, 2185.0432, 2201.0203, 2207.0286, 2215.9712, 2216.9922, 2223.0322, 2225.1135, 2227.9946, 2251.0205, 2255.1660, 2257.2053, 2271.1675, 2286.1418, 2287.1252, 2294.1714, 2301.1357, 2306.1091, 2311.1228, 2316.1433, 2317.1284, 2327.0886, 2333.1382, 2403.1626, 2425.1638, 2446.1995, 2514.3218, 3211.4333



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P46109|CRKL_HUMAN](#) Score: 65 Expect: 0.0067
 Crk-like protein OS=Homo sapiens GN=CRKL PE=1 SV=1

Nominal mass (M_r): 33870; Calculated pI value: 6.26
 NCBI BLAST search of [sp|P46109|CRKL_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 81
 Number of mass values matched: 11
 Sequence Coverage: 43%

Matched peptides shown in **Bold Red**

1 MSSAR**FDSSD** **RSAWYMGVPS** **RQEAQTRLQG** **QRHGMFLVRD** **SSTCPGDYVL**
 51 **SVSENSRVSH** **YIINSLPNRR** **FKIGDQEFDH** **LPALLEFYKI** **HYLDTTTLIE**
 101 **PAPRYSPPM** **GVSAPNLPT** **AEDNLEYVR**T LYDFPGNDAE DLPFKKGEIL
 151 VIIEKPEEQW WSARNKDGRV GMIPVPYVEK LVRSSPHGKH GNRNSNSYGI
 201 PEPAHAYAQP QTTTPLPAVS GSPGAAITPL PSTQNGPVFA KAIQKRVPCA
 251 YDKTALALEV GDIVKVTR**MN** **INGQWEGEVN** **GRKGLFPFTH** VKIFDPQNPD
 301 ENE

Show predicted peptides also

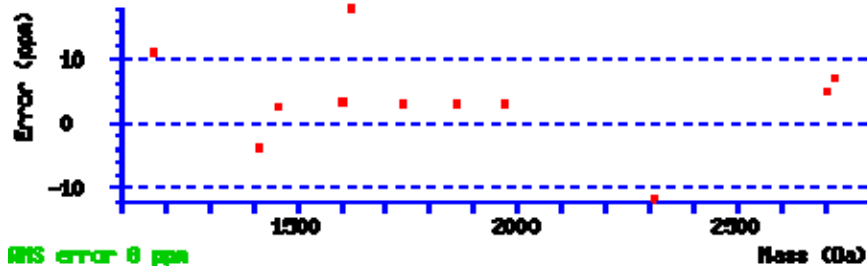
Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
6 - 21 R.FDSSDRSAWYMGVSR.Q	1860.8389	1859.8316	1859.8260	0.0056	1
12 - 21 R.SAWYMGVSR.Q Oxidation (M)	1169.5536	1168.5463	1168.5335	0.0128	0
28 - 39 R.LQGQRHGMFLVR.D Oxidation (M)	1457.7832	1456.7759	1456.7721	0.0038	1
40 - 57 R.DSSTCPGDYVLSVSENSR.V	1972.8615	1971.8542	1971.8480	0.0062	0
58 - 69 R.VSHYIINSLPNR.R	1412.7589	1411.7516	1411.7572	-0.0055	0
71 - 89 R.FKIGDQEFDHLPALLEFYK.I	2310.1533	2309.1460	2309.1731	-0.0271	1
90 - 104 K.IHYLDTTTLIEPAPR.Y	1739.9376	1738.9303	1738.9254	0.0050	0
105 - 129 R.YPSPPMGVSAPNLPTAEDNLEYVR.T	2704.3052	2703.2979	2703.2850	0.0129	0
105 - 129 R.YPSPPMGVSAPNLPTAEDNLEYVR.T Oxidation (M)	2720.3066	2719.2994	2719.2799	0.0195	0

269 - 282	1603.7334	1602.7261	1602.7208	0.0053	0
R.MNINGQWEGEVNGR.K					
269 - 282	1619.7520	1618.7447	1618.7157	0.0289	0
R.MNINGQWEGEVNGR.K Oxidation (M)					

No match to: 860.4357, 929.5539, 1035.5708, 1138.5446, 1221.6931, 1234.6865, 1262.6728, 1266.6385, 1297.6648, 1318.6993, 1366.6454, 1401.6877, 1418.7170, 1421.6934, 1434.7196, 1440.7817, 1498.8264, 1518.7869, 1544.8221, 1545.8197, 1561.8121, 1568.7465, 1573.8059, 1574.8131, 1576.8140, 1577.8115, 1582.7640, 1584.7410, 1590.7898, 1591.8129, 1604.7334, 1605.7252, 1606.7190, 1607.7998, 1620.7391, 1621.7300, 1622.7015, 1637.8359, 1656.7730, 1668.8385, 1673.8409, 1674.7811, 1688.7958, 1689.8076, 1692.8118, 1696.7773, 1708.7971, 1712.7804, 1724.8102, 1725.8190, 1732.7987, 1746.8635, 1755.9044, 1799.8944, 1823.9330, 1848.9077, 1863.8961, 1864.8978, 1865.9061, 1880.8934, 1986.8874, 2016.0383, 2033.0482, 2047.0580, 2119.0576, 2139.0005, 2149.0561, 2176.0608, 2342.1492, 2942.4294



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P32119|PRDX2_HUMAN** Score: **72** Expect: **0.0013**
Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5

Nominal mass (M_r): **22049**; Calculated pI value: **5.66**
 NCBI BLAST search of [sp|P32119|PRDX2_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **65**
 Number of mass values matched: **8**
 Sequence Coverage: **36%**

Matched peptides shown in **Bold Red**

1 MASGNARIGK PAPDFK**KATAV VDGAFKEVK**L SDYKGYVVL FFYPLDFTFV
 51 CPTEIIAFSN RAEDFRKLG C EVLGVSVDSQ FTHLAWINTP R**KEGGLGPLN**
 101 **IPLLADVTRR LSEDYGLKT DEGIAYRGLF IIDGKGVLRQ ITVNDLPVGR**
 151 SVDEALRLVQ AFQYTDEHGE VCPAGWKPGS DTIKPNVDDS KEYFSKHN

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

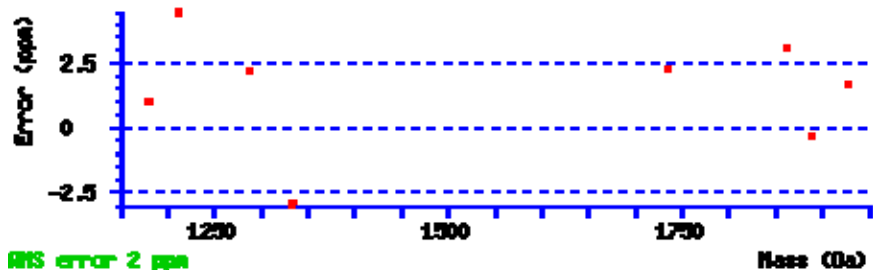


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
17 - 29 K.ATAVVDGAFKEVK.L	1334.7274	1333.7201	1333.7241	-0.0040	1
92 - 109 R.KEGGLGPLNIPLLADVTR.R	1863.0754	1862.0681	1862.0625	0.0057	1
93 - 109 K.EGGLGPLNIPLLADVTR.R	1734.9788	1733.9715	1733.9675	0.0040	0
93 - 110 K.EGGLGPLNIPLLADVTRR.L	1891.0753	1890.0680	1890.0686	-0.0006	1
110 - 119 R.RLSEDYGLK.T	1179.6379	1178.6306	1178.6295	0.0011	1
111 - 127 R.LSEDYGLKTDEGIAYR.G	1928.9631	1927.9558	1927.9527	0.0032	1
128 - 139 R.GLFIIDGKGVLR.Q	1287.7811	1286.7738	1286.7710	0.0028	1
140 - 150 R.QITVNDLPVGR.S	1211.6796	1210.6723	1210.6669	0.0054	0

No match to: 1004.3253, 1011.3088, 1017.3276, 1037.3375, 1046.6033, 1082.0197, 1111.5636, 1136.5568, 1137.0778, 1137.5767, 1145.5768, 1194.6503, 1320.5796, 1322.5789, 1362.6660, 1399.7284, 1458.7181, 1490.7501, 1520.7537, 1642.7394, 1716.9664, 1791.7384, 1895.3899,

2085.0708, 2161.0330, 2175.0486, 2178.0442, 2185.0425, 2193.9888,
2201.0071, 2207.0295, 2213.9954, 2216.9861, 2223.0212, 2225.1616,
2229.0281, 2251.0088, 2255.1514, 2256.1521, 2257.2312, 2271.1421,
2272.1531, 2287.1433, 2288.1521, 2301.1384, 2304.1477, 2311.1245,
2317.1501, 2327.0908, 2334.1021, 2350.1548, 2383.9573, 2403.0208,
2514.3428, 2515.3469, 2531.3289, 3211.4709



MASCOT SCIENCE Mascot Search Results

Significant hits: [sp|P29401|TKT_HUMAN](#) Transketolase OS=Homo sapiens
GN=TKT PE=1 SV=3

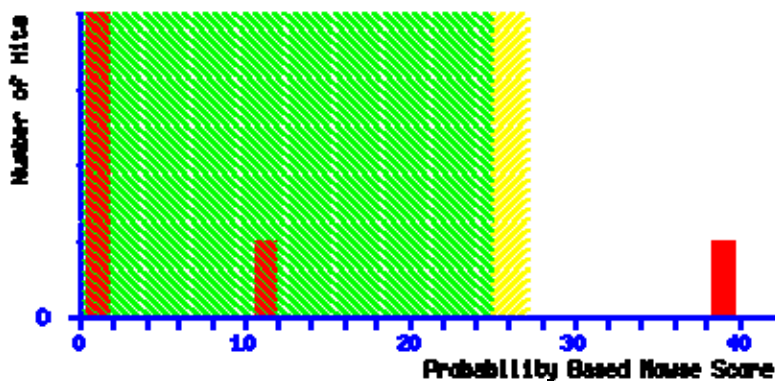
Probability Based Mowse Score

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.

Individual ions scores > 25 indicate peptides with significant homology.

Individual ions scores > 27 indicate identity or extensive homology ($p < 0.05$).

Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

Format A	<input type="text" value="Peptide Summary"/>	Help
Significance threshold p<	<input type="text" value="0.05"/>	Max. number of hits <input type="text" value="20"/>
Standard scoring <input checked="" type="checkbox"/> MudPIT scoring <input type="checkbox"/>	Ions score cut-off <input type="text" value="0"/>	Show sub-sets <input type="checkbox"/>
Show pop-ups <input checked="" type="checkbox"/> Suppress pop-ups <input type="checkbox"/>	Sort unassigned <input type="text" value="Decreasing Score"/>	Require bold red <input type="checkbox"/>

Error tolerant

1. [sp|P29401|TKT_HUMAN](#) Mass: 68519 Score: 39 Qu

eries matched: 1

Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3

- Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Mis	Score	Expect	Rank	Peptide
<u>1</u>	2020.0700	2019.0627	2019.0636	0.0009	0	39	0.0036	1	K.ILATPPQEDAPSVD IANIR.M

- 2. [sp|Q9NU38|PGM51_HUMAN](#) Mass: 24715 Score: 10 Queries matched: 1

Putative PGM5-like protein 1 OS=Homo sapiens PE=5 SV=2

- Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Mis	Score	Expect	Rank	Peptide
<u>1</u>	2020.0700	2019.0627	2019.02045	0.0382	1	10	2.7	2	R.EPQQDRPGGAAEGS RIPVR.T

- 3. [sp|O15229|KMO_HUMAN](#) Score: 2 Queries matched: 1

Kynurenine 3-monooxygenase OS=Homo sapiens GN=KMO PE=1 SV=2

- Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Mis	Score	Expect	Rank	Peptide
<u>1</u>	2020.0700	2019.0627	2019.0016	0.0611	2	18	3	3	R.LLKCNP EEGMITVLGSDK.V + Oxidation (M)

- 4. [sp|P40259|CD79B_HUMAN](#) Mass: 26316 Score: 2 Queries matched: 1

B-cell antigen receptor complex-associated protein beta chain OS=Homo sapiens GN=CD79B PE=1 SV=1

- Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Mis	Score	Expect	Rank	Peptide
<u>1</u>	2020.0700	2019.0627	2018.99042	0.0685	0	2	18	4	R.MEESQNESLATLT IQGIR.F

- 5. [sp|Q14974|IMB1_HUMAN](#) Score: 1 Queries

matched: 1

Importin subunit beta-1 OS=Homo sapiens GN=KPNB1 PE=1 SV=2

Check to include this hit in error tolerant search or archive report

Query	Observe d	Mr(expt)	Mr(calc)	Delt a	Mis s	Scor e	Expec t	Ran k	Peptide
<u>1</u>	2020.0700	2019.0627	2019.1047	0.0420	1	1	21	5	K.LVEARPMIHELLT EGRR.S

6. [sp|P02766|TTHY_HUMAN](#) Mass: 15991 Score: 1 Queries matched: 1

Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1

Check to include this hit in error tolerant search or archive report

Query	Observe d	Mr(expt)	Mr(calc)	Delt a	Mis s	Scor e	Expe ct	Ran k	Peptide
<u>1</u>	2020.0700	2019.0627	2019.1377	0.0750	1	1	22	6	K.VLDAVRGSPAINVA VHVFR.K

Search Parameters

Type of search : MS/MS Ion Search
 Enzyme : Trypsin
 Fixed modifications : Carbamidomethyl (C)
 Variable modifications : Oxidation (M)
 Mass values : Monoisotopic
 Protein Mass : Unrestricted
 Peptide Mass Tolerance : ± 100 ppm
 Fragment Mass Tolerance: ± 0.3 Da
 Max Missed Cleavages : 1
 Instrument type : MALDI-TOF-TOF
 Number of queries : 1

Mascot: <http://www.matrixscience.com/>

Top scoring peptide matches to query 1
 Score greater than 25 indicates homology
 Score greater than 27 indicates identity
 Status bar shows all hits for this peptide

Score	Delta	Hit	Protein	Peptide
39.0	-0.00	1	sp P29401 TKT_HUMAN	K.ILATPPQEDAPSVDIANIR.M
10.3	0.04	2	sp Q9NU38 PGM51_HUMAN	R.EPQQDRPGGAAEGSRIPVR.T
2.0	0.06	3	sp O15229 KMO_HUMAN	R.LLKCNP EEGMITVLGSDK.V
1.9	0.07	4	sp P40259 CD79B_HUMAN	R.MEESQNESLATLTIQGIR.F
1.3	-0.04	5	sp Q14974 IMB1_HUMAN	K.LVEARPMIHELLTEGRR.S
1.0	-0.07	6	sp P02766 TTHY_HUMAN	K.VLDAVRGSPAINVAVHVFR.K

MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P04040|CATA_HUMAN](#) Score: 140 Expect: 2e-010
 Catalase OS=Homo sapiens GN=CAT PE=1 SV=3

Nominal mass (M_r): 59947; Calculated pI value: 6.90
 NCBI BLAST search of [sp|P04040|CATA_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 65
 Number of mass values matched: 17
 Sequence Coverage: 40%

Matched peptides shown in **Bold Red**

1 MADSRDPASD QMQHWKEQRA AQK**ADVLT**TTG **AGNPVGD**KLN **VITVGPR**GPL
 51 **LVQDVVFTDE** **MAHFD**RERIP ERVVHAK**GAG** **AFGYFEV**THD **ITKYSK**AKVF
 101 EHIGKKTPIA VR**FSTVAGES** **GSADTVR**DPR GFAVKFYTED GNWDLVGNNT
 151 PIFFIRDPIL FPSFIHSQKR NPQTHLKDPD MVWDFWSLRP ESLHQVSFLF
 201 SDRGIPDGHR HMNGYGSHTF **KLVNANGEAV** **YCKFHYK**TDQ **GIKNLS**VEDA
 251 AR**LSQED**PDY **GIRDL**FNAIA TGKYPSWTFY IQVMTFNQAE TFPFNPFDLT
 301 **KVWPHKDYPL** **IPVGK**LVLNR NPVNYFAEVE QIAFDPSNMP PGIEASPKM
 351 LQGR**LFAYPD** **THRHR**LGPNY **LHIPVNC**PYR ARVANYQRDG PMCMQDNQGG
 401 APNYYPNSFG APEQQPSALE HSIQYSGEVR **RFNTANDDNV** **TQVRAFVNV**
 451 **LNEEQ**RKRLC ENIAGHLKDA QIFIQKKAVK **NFTEVHP**DYG **SHIQALL**DKY
 501 NAEKPK**NAIH** **TFVQSG**SHLA AREKANL

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

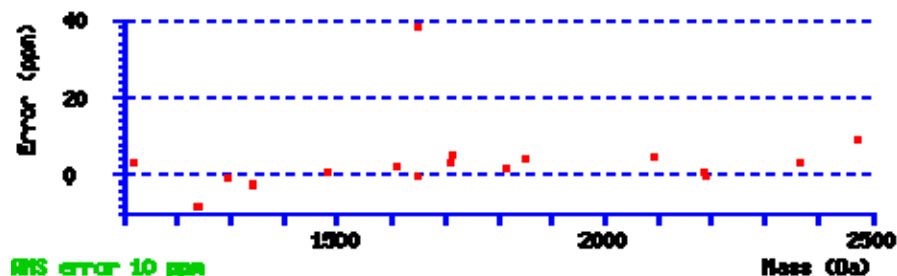


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
24 - 47 K.ADVLT TTG AGNPVGD KLN VITVGPR .G	2364.2952	2363.2879	2363.2808	0.0071	1
48 - 66 R.GP LL LVQDVVFTDE MAHFD R .E	2189.0681	2188.0608	2188.0622	-0.0014	0
48 - 68 R.GP LL LVQDVVFTDE MAHFD R .I	2474.2368	2473.2295	2473.2059	0.0236	1
78 - 93 K.GA GFYFEVTHD ITK .Y	1712.8365	1711.8292	1711.8205	0.0087	0
78 - 96 K.GA GFYFEVTHD ITKYSK .A	2091.0281	2090.0208	2090.0108	0.0100	1
113 - 130 R.F STVAGES GSADTVR DPR.G	1851.8910	1850.8837	1850.8758	0.0079	1
222 - 233 K.LV NANGEAV YCK .F	1337.6483	1336.6410	1336.6445	-0.0034	0

234 - 243	1236.6268	1235.6195	1235.6298	-0.0103	1
K.FHYKTDQGIK.N					
253 - 263	1292.6102	1291.6029	1291.6044	-0.0015	0
R.LSQEDPDYGIR.D					
302 - 315	1648.9203	1647.9130	1647.9137	-0.0006	1
K.VWPHKDYPLIPVGK.L					
355 - 363	1119.5614	1118.5541	1118.5509	0.0033	0
R.LFAYPDTHR.H					
366 - 380	1812.9243	1811.9170	1811.9141	0.0030	0
R.LGPNYLHIPPVNCPIR.A					
431 - 444	1649.8617	1648.8544	1648.7917	0.0627	1
R.RFNTANDDNVTQVR.A					
445 - 456	1481.7394	1480.7321	1480.7310	0.0012	0
R.AFYVNVLNNEEQR.K					
445 - 457	1609.8362	1608.8289	1608.8259	0.0030	1
R.AFYVNVLNNEEQRK.R					
481 - 499	2184.0737	2183.0664	2183.0647	0.0017	0
K.NFTEVHPDYGSHIQALLDK.Y					
507 - 522	1708.8929	1707.8856	1707.8804	0.0052	0
K.NAIHTFVQSGSHLAAR.E					

No match to: 1006.3288, 1046.6014, 1082.0273, 1082.5240, 1111.5605, 1135.5532, 1136.5569, 1137.0770, 1137.5751, 1143.5466, 1279.5518, 1295.5955, 1306.6106, 1320.5791, 1351.6663, 1352.6189, 1362.6732, 1399.7333, 1458.7101, 1474.7673, 1475.7653, 1490.7445, 1638.8531, 1642.7374, 1827.9410, 1834.8995, 1866.8868, 2185.0500, 2201.0308, 2216.9890, 2225.1626, 2227.9902, 2243.0798, 2251.0374, 2255.1689, 2257.2249, 2271.1663, 2272.1709, 2287.1414, 2309.1096, 2312.1387, 2317.1543, 2326.1497, 2514.3562, 2530.3208, 2612.2278, 2762.5310, 3211.5276



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P11413|G6PD_HUMAN](#) Score: 365 Expect: 6.4e-033
 Glucose-6-phosphate 1-dehydrogenase OS=Homo sapiens GN=G6PD PE=1 SV=4

Nominal mass (M_r): 59675; Calculated pI value: 6.39
 NCBI BLAST search of [sp|P11413|G6PD_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 65
 Number of mass values matched: 38
 Sequence Coverage: 59%

Matched peptides shown in **Bold Red**

1 MAEQVALSRT **QVCGILREEL** FQGDAFHQSD THIFIIMGAS GDLAKKKIYP
 51 TIWWLFR**DGL LPENTFIVGY ARSRLTVADI RKQSEPPFFKA TPEEKLKLED**
 101 **FFARNSYVAG QYDDAASYQR** LNSHMNALHL GSQANRLFYL **ALPPTVYEAV**
 151 **TKNIHESCMS QIGWNRIIVE KPFGRDLQSS** DRLSNHISL **FREDQIYRID**
 201 **HYLGKEMVQN LMLRFANRI FGPIWNRDNI** ACVILTFK**EP FGTEGRGGYF**
 251 **DEFGIIRDVM** QNHLLQMLCL VAMEKPASTN SDDVRDEKVK VLKCISEVQA
 301 NNVVLGQYVG NPDGEGEATK **GYLDDPTVPR** GSTTATFAAV VLYVENER**WD**
 351 **GVPFILRCGK** ALNERKAEVR **LQFHDVAGDI FHQQCKRNEL VIRVQPNEAV**
 401 **YTKMMTKKPG MFFNPEESEL DLYGNRYKN VKLPDAYERL ILDVFCGSQM**
 451 **HFVRSDELRE AWRIFTPLLH** QIELEKPKPI PYIYGSRGPT **EADELMKRVG**
 501 **FQYEGTYKWV NPHKL**

Show predicted peptides also

Sort Peptides By

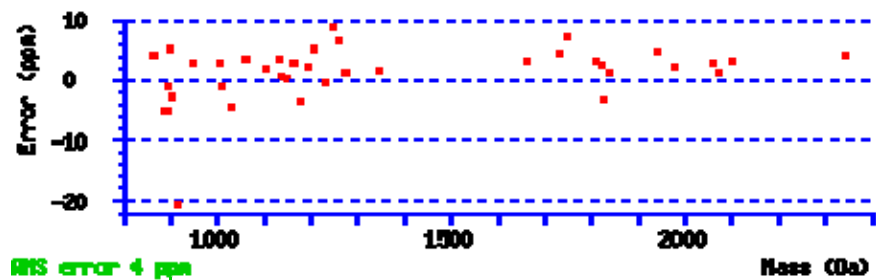
Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
10 - 17 R.TQVCGILR.E	946.5165	945.5092	945.5066	0.0027	0
58 - 72 R.DGLLPENTFIVGYAR.S	1664.8695	1663.8622	1663.8569	0.0053	0
73 - 81 R.SRLTVADIR.K	1030.5959	1029.5886	1029.5931	-0.0044	1
82 - 89 R.KQSEPPFFK.A	1010.5293	1009.5220	1009.5232	-0.0012	1
83 - 89 K.QSEPPFFK.A	882.4312	881.4239	881.4283	-0.0044	0
90 - 97 K.ATPEEKLK.L	915.4959	914.4886	914.5073	-0.0186	1
96 - 104 K.LKLEDFFAR.N	1138.6261	1137.6188	1137.6182	0.0006	1

98 - 104	897.4511	896.4438	896.4392	0.0046	0
K.LEDFFAR.N					
105 - 120	1807.7941	1806.7868	1806.7809	0.0060	0
R.NSYVAGQYDDAASYQR.L					
137 - 152	1825.0090	1824.0017	1824.0073	-0.0055	0
R.LFYALALPPTVYEAVTK.N					
153 - 166	1731.7767	1730.7694	1730.7617	0.0078	0
K.NIHESCMSQIGWNR.I					
153 - 166	1747.7767	1746.7694	1746.7566	0.0128	0
K.NIHESCMSQIGWNR.I Oxidation (M)					
167 - 175	1058.6393	1057.6320	1057.6284	0.0037	0
R.IIVEKPFGR.D					
183 - 192	1173.6337	1172.6264	1172.6302	-0.0037	0
R.LSNHISSLFR.E					
183 - 198	1978.0186	1977.0113	1977.0068	0.0045	1
R.LSNHISSLFREDQIYR.I					
199 - 215	2059.0886	2058.0813	2058.0754	0.0060	1
R.IDHYLGKEMVQNLMLR.F					
199 - 215	2075.0803	2074.0730	2074.0703	0.0027	1
R.IDHYLGKEMVQNLMLR.F Oxidation (M)					
206 - 215	1232.6484	1231.6411	1231.6416	-0.0005	0
K.EMVQNLMLR.F					
220 - 227	1002.5549	1001.5476	1001.5446	0.0030	0
R.IFGPIWNR.D					
239 - 246	892.4149	891.4076	891.4086	-0.0010	0
K.EPFGTEGR.G					
247 - 257	1273.6229	1272.6156	1272.6138	0.0018	0
R.GGYFDEFGLIR.D					
321 - 330	1132.5675	1131.5602	1131.5560	0.0042	0
K.GYLDLDDPTVPR.G					
349 - 357	1102.6063	1101.5990	1101.5971	0.0019	0
R.WDGVPFILR.C					
371 - 386	1942.9320	1941.9247	1941.9155	0.0092	0
R.LQFHDVAGDIFHQQCK.R					
371 - 387	2099.0310	2098.0237	2098.0166	0.0071	1
R.LQFHDVAGDIFHQQCKR.N					
387 - 393	899.5396	898.5323	898.5348	-0.0025	1
K.RNELVIR.V					
394 - 403	1148.5950	1147.5877	1147.5873	0.0004	0
R.VQPNEAVYTK.M					
408 - 427	2344.1013	2343.0940	2343.0841	0.0100	0
K.KPGMFFNPEESELDLTYGNY					
430 - 439	1204.6382	1203.6309	1203.6247	0.0062	1
K.NVKLPDAYER.L					
433 - 439	863.4294	862.4221	862.4185	0.0037	0
K.LPDAYER.L					
440 - 454	1821.9186	1820.9113	1820.9065	0.0048	0
R.LILDVFCGSQMHFVR.S					
440 - 454	1837.9111	1836.9038	1836.9014	0.0024	0
R.LILDVFCGSQMHFVR.S Oxidation (M)					
455 - 463	1161.5679	1160.5606	1160.5574	0.0032	1
R.SDELREAWR.I					
488 - 498	1246.6205	1245.6132	1245.6023	0.0109	1
R.GPTEADELMKR.V					
488 - 498	1262.6128	1261.6055	1261.5972	0.0083	1
R.GPTEADELMKR.V Oxidation (M)					
498 - 508	1347.6711	1346.6638	1346.6618	0.0020	1
K.RVGFQYEGTYK.W					
499 - 508	1191.5706	1190.5633	1190.5607	0.0026	0
R.VGFQYEGTYK.W					

509 - 515 893.4946 892.4873 892.4919 -0.0046 1
K.WVNPVKL.-

No match to: 851.2703, 855.0533, 860.2680, 866.3284, 867.3156,
871.0218, 872.2682, 874.3552, 920.2995, 951.2865, 1046.5909,
1136.5631, 1137.0731, 1137.5708, 1255.6122, 1320.5793, 1642.7433,
1745.7953, 1761.7911, 1763.8596, 1779.8597, 1793.9464, 1820.8978,
2185.0481, 2225.1609, 2255.1624, 2514.3506



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|Q03154|ACY1_HUMAN](#) Score: **85** Expect: **6.3e-005**
Aminoacylase-1 OS=Homo sapiens GN=ACY1 PE=1 SV=1

Nominal mass (M_r): **46084**; Calculated pI value: **5.77**
 NCBI BLAST search of [sp|Q03154|ACY1_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **65**
 Number of mass values matched: **12**
 Sequence Coverage: **36%**

Matched peptides shown in **Bold Red**

1 MTSK**GPEEEH PSVTLFR**QYL R**IRTVQPKPD YGAAVAFFEE TAR**QLGLGCQ
 51 KVEVAPGYVV TVLTWPGTNP TLSSILLNSH TDVVPVFK**EH WSHDPFEAFK**
 101 **DSEGYIYAR**G AQDMK**CVSIQ YLEAVR**RLKV EGHRFPRTIH MTFVPDEEVG
 151 GHQGMELFVQ RPEFHALR**AG FALDEGIANP TDAFTV**FYSE **RSPWVVRVTS**
 201 **TGRPGHASRF** MEDTAAEKLH **KVVNSILAFR** EKEWQRLQSN PHLKEGSVTS
 251 VNLTKLEGGV AYNVIPATMS ASFDFRVAPD VDFKAFEEQL QSWCQAAGEG
 301 VTLEFAQKWM HPQVTPTDDS NPWWAAFSRV CKDMNLTLEP EIMPAATDNR
 351 YIR**AVGVPAL GFSPMNR**TPV LLHDHDERLH EAVFLR**GVDI YTRLLPALAS**
 401 **VPALPSDS**

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

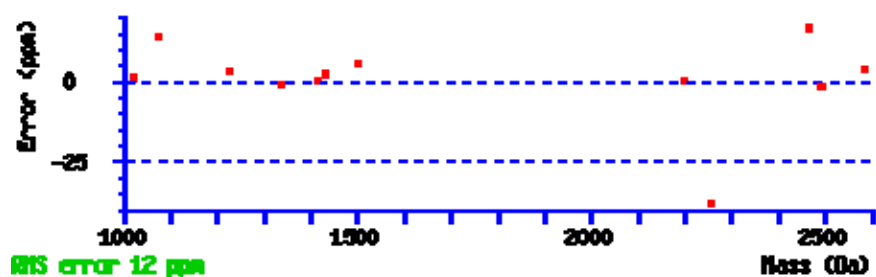


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
5 - 17 K.GPEEEHPSVTLFR.Q	1497.7415	1496.7342	1496.7259	0.0083	0
22 - 43 R.IRTVQPKPDYGAAVAFFEETAR.Q	2466.3181	2465.3108	2465.2702	0.0406	1
24 - 43 R.TVQPKPDYGAAVAFFEETAR.Q	2197.0928	2196.0855	2196.0851	0.0005	0
89 - 109 K.EHWSHDPFEAFKDSEGYIYAR.G	2584.1626	2583.1553	2583.1454	0.0099	1
101 - 109 K.DSEGYIYAR.G	1073.5052	1072.4979	1072.4825	0.0154	0
116 - 126 K.CVSIQYLEAVR.R	1337.6874	1336.6801	1336.6809	-0.0008	0
169 - 191 R.AGFALDEGIANPTDAFTVYSER.S	2491.1738	2490.1665	2490.1702	-0.0037	0
198 - 209 R.VTSTGRPGHASR.F	1225.6436	1224.6363	1224.6323	0.0040	0

222 - 230	1018.6057	1017.5984	1017.5971	0.0014	0
K.VVNSILAFR.E					
354 - 367	1415.7469	1414.7396	1414.7390	0.0006	0
R.AVGVPALGFSPMNR.T					
354 - 367	1431.7448	1430.7375	1430.7339	0.0036	0
R.AVGVPALGFSPMNR.T Oxidation (M)					
387 - 408	2255.1431	2254.1358	2254.2208	-0.0850	1
R.GVDIYTRLLPALASVPALPSDS.-					

No match to: 1011.3137, 1020.5219, 1046.6003, 1082.0278, 1082.5249, 1102.5535, 1111.5702, 1113.0851, 1113.5771, 1136.5621, 1137.0807, 1137.5748, 1143.5417, 1144.5558, 1251.6642, 1275.6559, 1307.6044, 1310.6112, 1320.5869, 1326.6047, 1341.7786, 1387.6494, 1399.7192, 1445.7693, 1458.7144, 1490.7443, 1642.7433, 1726.7751, 1761.8456, 1855.9227, 2146.0598, 2160.0146, 2164.7996, 2179.0474, 2185.0413, 2201.0317, 2207.0320, 2216.9788, 2225.1616, 2247.1274, 2251.0208, 2256.1995, 2271.1348, 2285.1282, 2288.1418, 2311.1221, 2312.1389, 2316.1653, 2335.1033, 2514.3372, 2530.3367, 2612.1841, 3211.4753



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|O75874|IDHC_HUMAN](#) Score: 201 Expect: 1.6e-016
 Isocitrate dehydrogenase [NADP] cytoplasmic OS=Homo sapiens GN=IDH1
 PE=1 SV=2

Nominal mass (M_r): 46915; Calculated pI value: 6.53
 NCBI BLAST search of [sp|O75874|IDHC_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 65
 Number of mass values matched: 24
 Sequence Coverage: 57%

Matched peptides shown in **Bold Red**

1 MSK**KISGGSV VEMQGD**EMTR **IIWELIKEKL IFPYVELDLH SYDLGIENRD**
 51 ATNDQVTKDA AEAIKKHNVG VKCATITPDE KRVEEFKLNQ MWKSPNGTIR
 101 NILGGTVFRE AIICKNIPRL **VSGWVKPIII GRHAYGDQYR ATDFVVPGPG**
 151 **KVEITYTPSD GTQKVTVLVH NFEEGGGVAM GMYNQDKSIE DFAHSSFQMA**
 201 **LSKGWPLYLS TKNTILKKYD GRFKDIFQEI YDKQYKSQFE AOKIWYEHRL**
 251 **IDDMVAQAMK SEGGFIWACK** NYDGDVQSDS VAQGYGSLGM MTSVLVCPDG
 301 **KTVEAEAAHG TVTRHYRMYQ KGQETSTNPI ASIFAWTRGL AHRAKLDNNK**
 351 ELAFAFANALE EVSIETIEAG FMTKDLAACI KGLPNVQRSD **YLNTFEFMDK**
 401 **LGENLKI**KLKLA QAKL

Show predicted peptides also

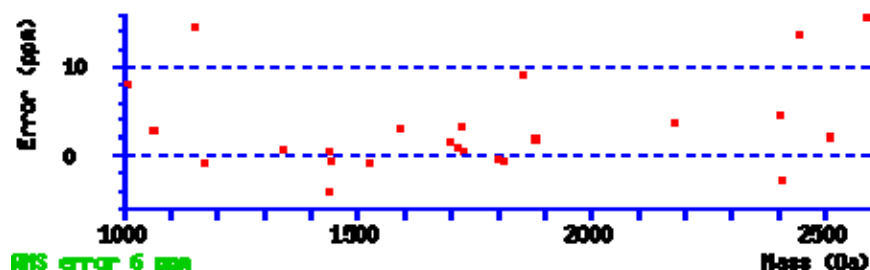
Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
4 - 20 K.KISGGSVVEMQGD EMTR.I	1855.8691	1854.8618	1854.8451	0.0167	1
5 - 20 K.ISGGSVVEMQGD EMTR.I	1695.7701	1694.7628	1694.7603	0.0025	0
5 - 20 K.ISGGSVVEMQGD EMTR.I	1711.7642	1710.7569	1710.7552	0.0017	0
5 - 20 K.ISGGSVVEMQGD EMTR.I	1727.7581	1726.7508	1726.7501	0.0007	0
21 - 29 R.IIWELIKEK.L	1171.7075	1170.7002	1170.7012	-0.0010	1
30 - 49 K.LIFPYVELDLH SYDLGIENR.D	2406.2271	2405.2198	2405.2266	-0.0068	0
120 - 132 R.LVSGWVKPIII GR.H	1437.8947	1436.8874	1436.8867	0.0007	0

133 - 140	1009.4566	1008.4493	1008.4413	0.0080	0
R.HAYGDQYR.A					
141 - 164	2507.2717	2506.2644	2506.2591	0.0054	1
R.ATDFVVPGPVKVEITYTPSDGTQK.V					
152 - 164	1438.7001	1437.6928	1437.6987	-0.0059	0
K.VEITYTPSDGTQK.V					
165 - 187	2591.1943	2590.1870	2590.1467	0.0403	0
K.VTYLVHNFEEGGVAMGMYNQDK.S					2 Oxidation (M)
188 - 203	1797.8468	1796.8395	1796.8403	-0.0008	0
K.SIEDFAHSSFQMALSK.G					
188 - 203	1813.8411	1812.8338	1812.8352	-0.0014	0
K.SIEDFAHSSFQMALSK.G					Oxidation (M)
204 - 212	1064.5803	1063.5730	1063.5702	0.0028	0
K.GWPLYLSTK.N					
223 - 233	1445.7301	1444.7228	1444.7238	-0.0010	1
R.FKDIFQEIYDK.Q					
225 - 236	1589.7892	1588.7819	1588.7773	0.0046	1
K.DIFQEIYDKQYK.S					
237 - 249	1721.8448	1720.8375	1720.8321	0.0054	1
K.SQFEAQKIWEHR.L					
250 - 270	2402.1296	2401.1223	2401.1116	0.0108	1
R.LIDDMVAQAMKSEGGFIWACK.N					2 Oxidation (M)
261 - 270	1154.5465	1153.5392	1153.5226	0.0166	0
K.SEGGFIWACK.N					
302 - 314	1341.6766	1340.6693	1340.6684	0.0009	0
K.TVEAEEAHGTVTR.H					
318 - 338	2445.2197	2444.2124	2444.1794	0.0330	1
R.MYQKGQETSTNPIASIFAWTR.G					Oxidation (M)
322 - 338	1878.9380	1877.9307	1877.9271	0.0036	0
K.QQETSTNPIASIFAWTR.G					
389 - 400	1525.6501	1524.6428	1524.6442	-0.0014	0
R.SDYLNTEFMDK.L					Oxidation (M)
389 - 406	2180.0295	2179.0222	2179.0143	0.0080	1
R.SDYLNTEFMDKLGLENLK.I					Oxidation (M)

No match to: 1021.3962, 1024.3447, 1066.4818, 1136.5548, 1137.0792, 1137.5798, 1155.5409, 1270.6652, 1318.6552, 1334.6584, 1398.7018, 1399.7140, 1490.7441, 1606.7451, 1611.8743, 1642.7367, 1647.7623, 1736.8501, 1749.8605, 1774.8517, 1778.8629, 1822.8905, 1835.8915, 1864.9465, 1893.9136, 1921.9747, 2162.0557, 2179.0474, 2185.0427, 2195.0178, 2201.0061, 2207.0107, 2213.9854, 2216.9805, 2225.1643, 2251.0352, 2255.1162, 2256.1453, 2287.1208, 2328.0762, 2514.3328



Mascot Search Results

Protein View

Match to: **sp|P37837|TALDO_HUMAN** Score: **71** Expect: **0.0017**
Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2

Nominal mass (M_r): **37688**; Calculated pI value: **6.36**
NCBI BLAST search of [sp|P37837|TALDO_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **21%**

Matched peptides shown in **Bold Red**

1 MSSSPVKRQR MESALDQLKQ FTTVVADTGD FHAIDEYKPQ DATTNPSLIL
51 AAAQMPAYQE LVEEAIAYGR KLGGSQEDQI KNAIDKLFVL FGAEILKKIP
101 GRVSTEV DAR **LSFDKDAMVA** RARRLIELYK EAGISKDRIL IKLSSTWEGI
151 QAGKELEEQH GIHCNMTLLF SFAQAVACAE AGVTLISPFV GR**ILDWHVAN**
201 **TDKKSYEPL**E **DPGVK**SVTKI YNYKKFSYK TIVMGASFRN TGEIK**ALAGC**
251 **DFLTISP**PKLL **GELLQ**DNAL VPVLSAK**AAQ** **ASDLEK**IHL D **EK**SFRWLHNE
301 DQMAVEKLS D GIRKFAADAV KLERMLTERM FNAENGK

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
111 - 121 R.LSFDKDAMVAR.A	1252.6328 (No match)	1251.6255	1251.6281	-0.0026	1
111 - 121 R.LSFDKDAMVAR.A	1268.6292 Oxidation (M) (No match)	1267.6219	1267.6230	-0.0011	1
193 - 204 R.ILDWHVANTDKK.S	1439.7706 (Ions score 19)	1438.7633	1438.7568	0.0065	1
193 - 204 R.ILDWHVANTDKK.S	1439.7706 (No match)	1438.7633	1438.7568	0.0065	1
205 - 215 K.SYEPLEDPGVK.S	1233.6023 (Ions score 19)	1232.5950	1232.5924	0.0026	0
205 - 215 K.SYEPLEDPGVK.S	1233.6023 (No match)	1232.5950	1232.5924	0.0026	0
246 - 258 K.ALAGCDFLTISPK.L	1392.7196 (Ions score 4)	1391.7123	1391.7119	0.0005	0
246 - 258 K.ALAGCDFLTISPK.L	1392.7196 (No match)	1391.7123	1391.7119	0.0005	0
259 - 269 K.LLGELLQDNAL.L	1213.6689 (No match)	1212.6616	1212.6714	-0.0097	0
278 - 292 K.AAQASDLEKIHLDLDEK.S	1667.8674 (No match)	1666.8601	1666.8526	0.0076	1

MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P53004|BIEA_HUMAN** Score: **93** Expect: **0.00026**
Biliverdin reductase A OS=Homo sapiens GN=BLVRA PE=1 SV=2

Nominal mass (M_r): **33692**; Calculated pI value: **6.06**
 NCBI BLAST search of [sp|P53004|BIEA_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **112**
 Number of mass values matched: **15**
 Sequence Coverage: **46%**

Matched peptides shown in **Bold Red**

1 MNAEPER**KFG VVVVGVGR**AG SVRMRDLR**NP HPSSAFNLNI GFVSR**RELGS
 51 IDGVQQISLE DALSSQEV**EV AYICSESSSH EDYIRQFLNA GKHLV**EYPM
 101 TSLSLAAQ**EL WELAEQKGK V LHEEHVELLM EEF**AF**LKKEV** VGK**DLKGS**L
 151 **LFTAGPLEEE RFGFP**AFSGI **SRLTWLVSLF GELS**LVSATL **EERKEDQYMK**
 201 **MTVCLETEKK SPLSWIEEK G**PGLKR**NR YLS FHF**KSGS**LEN V**PNVGV**NKNI**
 251 **FLK**DQ**NI**FVQ **KLLGQFSEKE LAE**KKRILH **CLGLAE**EIQK **YCCSR**K

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

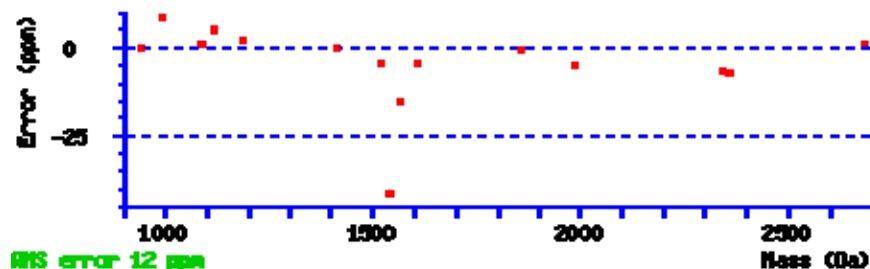


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
8 - 18 R.KFGVVVVGVGR.A	1116.6943	1115.6871	1115.6814	0.0057	1
9 - 18 K.FGVVVGVGR.A	988.6021	987.5948	987.5865	0.0084	0
29 - 45 R.NPHPSSAFNLNIGFVSR.R	1855.9805	1854.9732	1854.9740	-0.0008	0
120 - 138 K.VLHEEHVELLMEEFAFLKK.E	2341.2097	2340.2024	2340.2187	-0.0162	1
120 - 138 K.VLHEEHVELLMEEFAFLKK.E Oxidation (M)	2357.2039	2356.1966	2356.2136	-0.0170	1
144 - 161 K.DLLKGSLLFTAGPLEEER.F	1988.0603	1987.0530	1987.0625	-0.0095	1
148 - 161 K.GSLLFTAGPLEEER.F	1518.7733	1517.7660	1517.7725	-0.0065	0
148 - 172 K.GSLLFTAGPLEEERFGFP AFSGISR.L	2685.3704	2684.3631	2684.3597	0.0034	1
162 - 172 R.FGFP AFSGISR.L	1185.6078	1184.6005	1184.5978	0.0027	0

211 - 219	1088.5636	1087.5563	1087.5549	0.0014	0
K.SPLSWIEEK.G					
211 - 224	1540.7731	1539.7658	1539.8296	-0.0638	1
K.SPLSWIEEKGPGLK.R					
228 - 234	941.4882	940.4809	940.4807	0.0002	0
R.YLSFHFK.S					
235 - 248	1413.7332	1412.7259	1412.7259	-0.0000	0
K.SGSLENVNPVGVNK.N					
249 - 261	1606.8879	1605.8807	1605.8878	-0.0072	1
K.NIFLKDQNIQVQK.L					
262 - 275	1562.8185	1561.8112	1561.8351	-0.0239	1
K.LLGQFSEKELAAEK.K					

No match to: 860.4363, 896.4192, 981.5276, 1010.5091, 1120.5293, 1138.5376, 1145.5635, 1180.5470, 1189.6477, 1197.5617, 1205.6350, 1213.6758, 1233.5968, 1252.6339, 1261.6659, 1262.6615, 1266.6243, 1269.6342, 1301.6511, 1318.6924, 1359.7045, 1362.6708, 1366.6378, 1382.6392, 1383.6537, 1392.7189, 1420.6943, 1421.6831, 1436.6989, 1437.7221, 1439.7577, 1472.7191, 1499.7629, 1501.7991, 1527.7882, 1532.7994, 1543.8159, 1544.8160, 1545.8103, 1558.8027, 1559.7859, 1560.8087, 1561.8040, 1566.7858, 1576.8087, 1577.7997, 1599.7582, 1629.8471, 1634.8919, 1638.8474, 1642.7367, 1655.8911, 1669.8485, 1696.9360, 1724.8284, 1737.8959, 1740.8546, 1742.8497, 1753.8925, 1770.8738, 1795.8611, 1848.0291, 1865.9373, 2016.0665, 2032.0538, 2058.0520, 2083.0332, 2139.0662, 2140.0510, 2194.0115, 2256.1519, 2310.1863, 2342.2109, 2384.3061, 2403.0559, 2419.0410, 2470.2722, 2485.2634, 2510.2075, 2572.2302, 2638.3623, 2654.3672, 2671.3494, 2716.4199, 2717.4165, 2724.3814, 2732.4370, 2733.4231, 2748.4131, 2765.4055, 2925.6138, 2942.4648, 2956.4390, 3225.7378, 3574.7668, 3575.7937, 3576.7830



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P04406|G3P_HUMAN** Score: **73** Expect: **0.00093**
Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3

Nominal mass (M_r): **36201**; Calculated pI value: **8.57**
 NCBI BLAST search of [sp|P04406|G3P_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **15**
 Number of mass values matched: **6**
 Sequence Coverage: **24%**

Matched peptides shown in **Bold Red**

1 MGKVKVGVNG FGRIGRLVTR AAFNSGKVDI VAINDPFIDL NYMVYMFQYD
 51 STHGKFGHTV KAENGLVIN GNPITIFQER DPSKIKWGDA GAEYVVESTG
 101 VFTTMEK**AGA HLQGGAKRVI** ISAPSADAPM FVMGVNHEKY DNSLK**IISNA**
 151 **SCTTNCLAPL AKVIHDNFGI** VEGLMTTVHA ITATQK**TVDG PSGKLWRDGR**
 201 **GALQNIIPAS TGAAK**AVGKV IPELNGKLTG MAFR**VPTANV SVVDLTCRLE**
 251 KPAKYDDIKK VVKQASEGPL KGILGYTEHQ VVSSDFNSDT HSSTFDAGAG
 301 IALNDHFVK**L ISWYDNEFGY SNR**VVDLMAH MASKE

Show predicted peptides also

Sort Peptides By



Residue Number



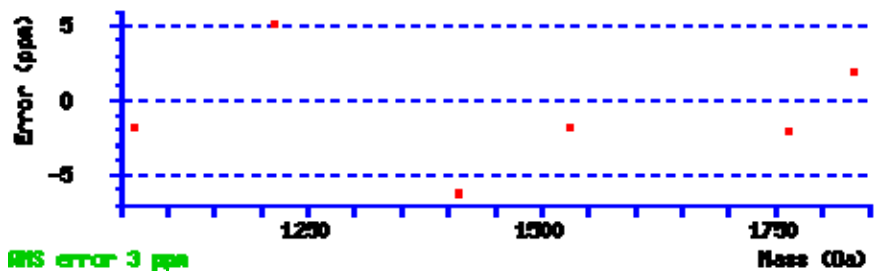
Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
108 - 118 K.AGAHLQGGAKR.V	1065.5892	1064.5819	1064.5839	-0.0019	1
146 - 162 K.IISNASCTTNCLAPLAK.V	1833.9231	1832.9158	1832.9124	0.0034	0
187 - 197 K.TVDGPSGKLWR.D	1215.6542	1214.6469	1214.6407	0.0062	1
201 - 215 R.GALQNIIPASTGAAK.A	1411.7814	1410.7741	1410.7830	-0.0089	0
235 - 248 R.VPTANVSVVDLTCR.L	1530.7915	1529.7842	1529.7871	-0.0029	0
310 - 323 K.LISWYDNEFGYSNR.V	1763.7985	1762.7912	1762.7950	-0.0038	0

No match to: 1137.5868, 1138.0890, 1225.1395, 1614.8851, 1642.7218, 2042.0958, 2185.0588, 2216.9822, 2277.1519



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P42330|AK1C3_HUMAN](#) Score: 132 Expect: 1.3e-009
 Aldo-keto reductase family 1 member C3 OS=Homo sapiens GN=AKR1C3 PE=1 SV=3

Nominal mass (M_r): 37220; Calculated pI value: 8.05
 NCBI BLAST search of [sp|P42330|AK1C3_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 53
 Number of mass values matched: 16
 Sequence Coverage: 55%

Matched peptides shown in **Bold Red**

1 MDSKQQCVKL **NDGHFMPVLG FGTYPPEVP RSKALEVTKL AIEAGFRHID**
 51 **SAHLYNNEEQ VGLAIRSKIA** DGSVK**REDIF YTSK**LWSTFH RPELVRLPALE
 101 NSLKKAQLDY VDLYLHSPM SLKPGEELSP TDENGKVIFD IVDLCTTWEA
 151 MEKCKDAGLA **KSIGVSNFNR RQLEMILNKP GLKYKPCVNCQ VECHPYFNRS**
 201 KLLDFCK**SKD IVLVAYSALG SQ**DKRWVDP NSPVLLEDPV **LCALAKKHKR**
 251 TPALIALRYQ LQRGVVVLAK SYNEQR**IRQN VQVFEFQLTA EDMKAIDGLD**
 301 **RNLHYFNSDS FASHPNYPYS DEY**

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

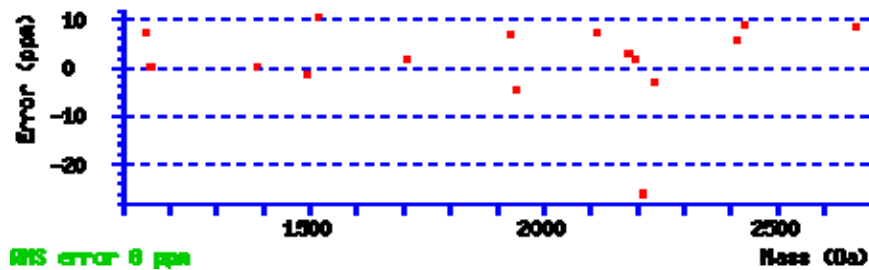


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
10 - 31 K.LNDGHFMPVLGFGTYAPPEVPR.S	2414.2100	2413.2027	2413.1888	0.0139	0
10 - 31 K.LNDGHFMPVLGFGTYAPPEVPR.S	2430.2122	2429.2049	2429.1837	0.0212	0
34 - 47 K.ALEVTKLAIEAGFR.H	1517.8845	1516.8772	1516.8613	0.0160	1
48 - 66 R.HIDSAHLYNNEEQVGLAIR.S	2179.0959	2178.0886	2178.0817	0.0069	0
76 - 84 K.REDIFYTSK.L	1158.5793	1157.5720	1157.5717	0.0004	1
162 - 171 K.SIGVSNFNRR.Q	1149.6208	1148.6135	1148.6050	0.0085	1
172 - 183 R.QLEMILNKPGLK.Y	1383.8032	1382.7959	1382.7955	0.0004	0
184 - 199 K.YKPCVNCQVECHPYFNRS.S	2110.9739	2109.9666	2109.9512	0.0154	0

208 - 223	1706.9468	1705.9395	1705.9362	0.0033	1
K.SKDIVLVAYSALGSQR.D					
210 - 223	1491.8143	1490.8070	1490.8092	-0.0022	0
K.DIVLVAYSALGSQR.D					
227 - 246	2236.1616	2235.1543	2235.1609	-0.0066	0
R.WVDPNSPVLLEDPVLCALAK.K					
277 - 294	2196.1160	2195.1087	2195.1044	0.0043	1
R.IRQNVQVFEFQLTAEDMK.A					
277 - 294	2212.0488	2211.0415	2211.0993	-0.0578	1
R.IRQNVQVFEFQLTAEDMK.A Oxidation (M)					
279 - 294	1926.9397	1925.9324	1925.9192	0.0132	0
R.QNVQVFEFQLTAEDMK.A					
279 - 294	1942.9121	1941.9048	1941.9142	-0.0093	0
R.QNVQVFEFQLTAEDMK.A Oxidation (M)					
302 - 323	2667.1282	2666.1209	2666.0986	0.0224	0
R.NLHYFNSDSFASHPNYPYSDEY.-					

No match to: 1044.1211, 1066.0701, 1137.5701, 1225.1541, 1300.0314, 1301.0414, 1320.5925, 1490.7473, 1614.9006, 1642.7332, 1733.0149, 1763.8092, 2019.9579, 2042.1285, 2125.9729, 2146.0596, 2185.0574, 2188.0283, 2192.1331, 2195.0698, 2199.1201, 2201.0793, 2215.0232, 2216.9841, 2225.1147, 2233.0154, 2240.0264, 2251.0925, 2256.1653, 2266.9082, 2270.8965, 2297.1516, 2311.1118, 2323.1350, 2327.0867, 2514.3408, 2531.3479



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P40925|MDHC_HUMAN](#) Score: **66** Expect: **0.0047**
Malate dehydrogenase, cytoplasmic OS=Homo sapiens GN=MDH1 PE=1 SV=4

Nominal mass (M_r): **36631**; Calculated pI value: **6.91**
 NCBI BLAST search of [sp|P40925|MDHC_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Sequence Coverage: **15%**

Matched peptides shown in **Bold Red**

```

1 MSEPIRVLVT GAAGQIAYSL LYSIGNGSVF GKDQPIILVL LDITPMMGVL
51 DGVLMEQLQDC ALPLLKDVIA TDKEDVAFKD LDVAILVGSM PRREGMERKD
101 LLKANVKIFK SQGAALDKYA KKSVKVIVVG NPANTNCLTA SKSAPSIPKE
151 NFSCLTRLDH NRAKAQIALK LGVTANDVKN VIIWGNHSST QYPDVNHAKV
201 KLQKKEVGVY EALKDDSWLK GEFVTTVQQR GAAVIKARKL SSAMSAAKAI
251 CDHVRDIWFG TPEGEFVSMG VISDGNSYGV PDDLLYSFPV VIKNKTWKFV
301 EGLPINDFSR EKMDLTAKEL TEEKESAFEF LSSA
  
```

Show predicted peptides also

Sort Peptides By



Residue Number

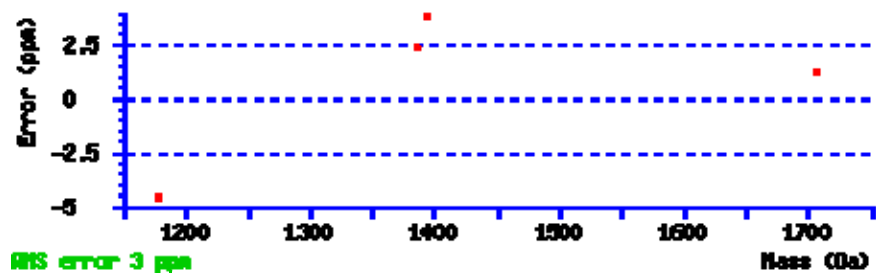


Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
80 - 92	1385.7490	1384.7417	1384.7384	0.0033	0
K.DLDVAILVGSMR.R	(No match)				
108 - 118	1177.6521	1176.6448	1176.6502	-0.0054	1
K.IFKSQGAALDK.Y	(No match)				
143 - 157	1706.8551	1705.8478	1705.8457	0.0021	1
K.SAPSIPKENFSCLTR.L	(No match)				
299 - 310	1393.7164	1392.7091	1392.7037	0.0054	0
K.FVEGLPINDFSR.E	(Ions score 52)				
299 - 310	1393.7164	1392.7091	1392.7037	0.0054	0
K.FVEGLPINDFSR.E	(No match)				



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P52895|AK1C2_HUMAN** Score: **165** Expect: **6.4e-013**
Aldo-keto reductase family 1 member C2 OS=Homo sapiens GN=AKR1C2 PE=1 SV=3

Nominal mass (M_r): **37111**; Calculated pI value: **7.13**
 NCBI BLAST search of [sp|P52895|AK1C2_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **55**
 Number of mass values matched: **20**
 Sequence Coverage: **52%**

Matched peptides shown in **Bold Red**

1 MDSKYQCVKL **NDGHFMPVLG FGTYPAEVP KSKALEAVKL** AIEAGFHHID
 51 SAHVYNNNEEQ VGLAIRSKIA DGSVK**REDIF YTSKLWSNSH RPELVRPALE**
 101 **RSLKNLQLDY** VDLYLHFPV SVKPGEEVIP **KDENGKILFD TVDLCATWEA**
 151 **MEKCKDAGLA KSIGVSNFNH RLLEMILNKP GLKYKPCNQ VECHPYFNQR**
 201 KLLDFCKSKD **IVLVAYSALG SHREEPWVDP** NSPVLLEDPV LCALAKKHKR
 251 **TPALIALRYQ LQRGVVVLAK SYNEQIRQN VQVFEFQLTS EEMKAIDGLN**
 301 **RNVRYLTLDI FAGPPNYPPS** DEY

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

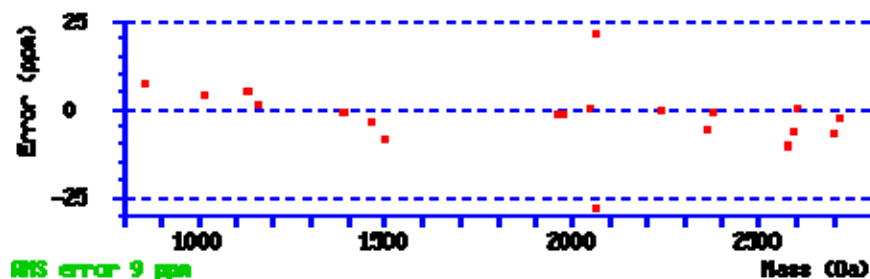


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
10 - 31 K.LNDGHFMPVLGFGTYAPAEVPK.S	2360.1606	2359.1534	2359.1670	-0.0136	0
10 - 31 K.LNDGHFMPVLGFGTYAPAEVPK.S Oxidation (M)	2376.1670	2375.1597	2375.1619	-0.0022	0
10 - 33 K.LNDGHFMPVLGFGTYAPAEVPKSK.A	2575.2747	2574.2674	2574.2940	-0.0266	1
10 - 33 K.LNDGHFMPVLGFGTYAPAEVPKSK.A Oxidation (M)	2591.2798	2590.2725	2590.2889	-0.0164	1
76 - 84 K.REDIFYTSK.L	1158.5804	1157.5732	1157.5717	0.0015	1
85 - 101 K.LWSNSHRPELVRPALER.S	2060.0574	2059.0501	2059.1075	-0.0574	0
132 - 153 K.DENGKILFDTVDLCATWEAMEK.C Oxidation (M)	2601.1860	2600.1788	2600.1774	0.0014	1
137 - 153 K.ILFDTVDLCATWEAMEK.C	2041.9614	2040.9542	2040.9536	0.0005	0

137 - 153	2058.0000	2056.9927	2056.9485	0.0442	0
K.IILFDITVDLCATWEAMEK.C	Oxidation (M)				
162 - 171	1130.5753	1129.5680	1129.5628	0.0052	0
K.SIGVSNFNHR.L					
172 - 183	1384.8221	1383.8149	1383.8159	-0.0010	0
R.LLEMILNKPGLK.Y	Oxidation (M)				
184 - 200	2239.0164	2238.0091	2238.0098	-0.0007	0
K.YKPVCNQVECHPYFNQR.K					
210 - 223	1500.8040	1499.7967	1499.8096	-0.0129	0
K.DIVLVAYSALGSHR.E					
250 - 258	1010.6505	1009.6432	1009.6396	0.0036	1
K.RTPALIALR.Y					
251 - 258	854.5521	853.5448	853.5385	0.0063	0
R.TPALIALR.Y					
264 - 276	1462.7957	1461.7884	1461.7939	-0.0055	1
R.GVVVLAKSYNEQR.I					
279 - 294	1956.9347	1955.9274	1955.9298	-0.0024	0
R.QNVQVFEFQLTSEEMK.A					
279 - 294	1972.9288	1971.9216	1971.9247	-0.0032	0
R.QNVQVFEFQLTSEEMK.A	Oxidation (M)				
279 - 301	2696.3157	2695.3084	2695.3275	-0.0191	1
R.QNVQVFEFQLTSEEMKAIDGLNR.N					
279 - 301	2712.3227	2711.3155	2711.3224	-0.0069	1
R.QNVQVFEFQLTSEEMKAIDGLNR.N	Oxidation (M)				

No match to: 1352.5454, 1477.6580, 1491.7449, 1707.7732, 1719.8556, 1763.7992, 1810.9309, 1828.9383, 1939.9127, 1955.9156, 1963.9001, 1971.9386, 1978.9039, 1994.8948, 2010.8846, 2044.0406, 2045.0724, 2055.9731, 2056.9790, 2072.9734, 2075.0581, 2090.9592, 2122.9529, 2194.0271, 2200.0801, 2253.0334, 2267.0427, 2293.9548, 2296.1148, 2368.1169, 2391.0195, 2405.0212, 2586.1885, 2679.3862, 3004.4988



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|O75390|CISY_HUMAN](#) Score: 108 Expect: 3.2e-007
 Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2

Nominal mass (M_r): 51908; Calculated pI value: 8.45
 NCBI BLAST search of [sp|O75390|CISY_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 8
 Number of mass values matched: 7
 Sequence Coverage: 16%

Matched peptides shown in **Bold Red**

```

1 MALLTAAARL LGTKNASCLV LAARHASASS TNLKDILADL IPKEQARIKT
51 FRQQHGKTVV GQITVDMMYG GMRGMKGLVY ETSVLDPDEG IRFRGFSIPE
101 CQKLLPKAKG GEEPLPEGLF WLLVTGHIPT EEQVSWLSKE WAKRAALPSH
151 VVTMLDNFPT NLHPMSQLSA AVTALNSESF FARAYAQGIS RTKYWELIYE
201 DSMDLIAKLP CVAAKIYRNL YREGSGIGAI DSNLDWSHNF TNMLGYTDHQ
251 FTELTRLVLT IHSDEHGGNV SAHTSHLVGS ALSDPYLSFA AAMNGLAGPL
301 HGLANQEVLV WLTQLQKEVG KDVSDEKLRD YIWNTLNLSGR VVPGYGHAVL
351 RKTDPRYTCQ REFALKHLPN DPMFKLVAQL YKIVPNVLE QGKAKNPWPN
401 VDAHSGVLLQ YYGTEMNYY TVLFGVSRAL GVLAQLWSR ALGFPLERPK
451 SMSTEGLMKF VDSKSG
  
```

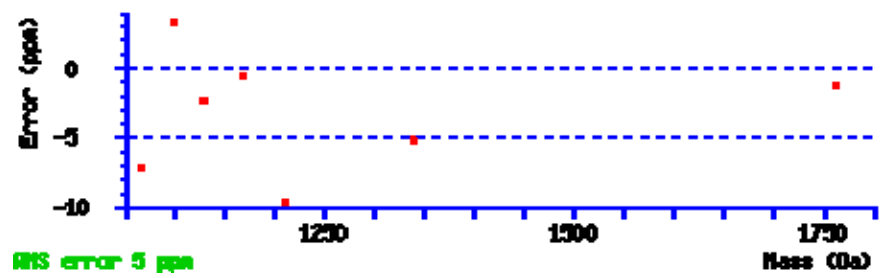
Show predicted peptides also

Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
77 - 92 K.GLVYETSVLDPDEGIR.F	1762.8835	1761.8762	1761.8784	-0.0022	0
95 - 103 R.GFSIPECQK.L	1065.4956	1064.4883	1064.4960	-0.0077	0
330 - 340 R.DYIWNTLNLSGR.V	1338.6368	1337.6295	1337.6364	-0.0069	0
341 - 351 R.VVPGYGHAVLR.K	1167.6626	1166.6553	1166.6560	-0.0006	0
367 - 375 K.HLPNDPMFK.L	1098.5438	1097.5365	1097.5328	0.0037	0
383 - 393 K.IVPNVLLEQ GK.A	1209.7084	1208.7011	1208.7128	-0.0117	0
441 - 450 R.ALGFPLERP.K.S	1127.6545	1126.6472	1126.6498	-0.0026	0

No match to: 1320.5737



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: **sp|P17174|AATC_HUMAN** Score: **142** Expect: **1.3e-010**
Aspartate aminotransferase, cytoplasmic OS=Homo sapiens GN=GOT1 PE=1 SV=3

Nominal mass (M_r): **46447**; Calculated pI value: **6.52**
 NCBI BLAST search of [sp|P17174|AATC_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **65**
 Number of mass values matched: **16**
 Sequence Coverage: **54%**

Matched peptides shown in **Bold Red**

1 **MAPPSVFAEV PQAQPVLVFK LTADFREDPD PRKVNLGVGA YRTDDCHPWV**
 51 **LPVVKKVEQK IANDNSLNHE YLPILGLAEF RSCASRLALG DDSPALKEKR**
 101 **VGGVQSLGGT GALRIGADFL ARWYNGTNNK NTPVYVSSPT WENHNAVFSFA**
 151 **AGFKDIRSYR YWDAEKRLD LQGFLNDLEN APEFSIVVLH ACAHNPTGID**
 201 **PTPEQWKQIA SVMKHRFLFP FFDSAYQGFA SGNLERDAWA IRYFVSEGF**
 251 **FFCAQSFASKN FGLYNERVGN LTVVGKEPES ILQVLSQMEK IVRITWSNPP**
 301 **AQGARIVAST LSNPELFEEW TGNVKTMDR ILTMRSELRA RLEALKTPGT**
 351 **WNHITDQIGM FSFTGLNPKQ VEYLVNEKHI YLLPSGRINV SGLTTKNLDY**
 401 **VATSIHEAVT KIQ**

Show predicted peptides also

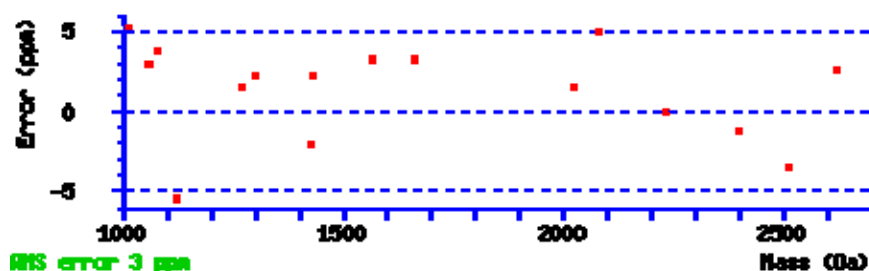
Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
2 - 20 M.APPSVFAEVPQAQPVLVFK.L	2024.1245	2023.1172	2023.1142	0.0031	0
21 - 32 K.LTADFREDPDPR.K	1431.6895	1430.6822	1430.6790	0.0032	1
33 - 42 R.KVN LGVGAYR.T	1076.6250	1075.6177	1075.6138	0.0040	1
43 - 55 R.TDDCHPWVLPVVK.K	1565.7830	1564.7757	1564.7708	0.0049	0
61 - 81 K.IANDNSLNHEYLPILGLAEFR.S	2399.2324	2398.2251	2398.2280	-0.0029	0
100 - 114 K.RVGGVQSLGGTGALR.I	1427.8047	1426.7974	1426.8004	-0.0029	1
101 - 114 R.VGGVQSLGGTGALR.I	1271.7084	1270.7011	1270.6993	0.0019	0

131 - 154	2623.2644	2622.2571	2622.2502	0.0069	0
K.NTPVYVSSPTWENHNAVFSAGFK.D					
243 - 259	2079.9260	2078.9187	2078.9083	0.0104	0
R.YFVSEGFEEFFCAQSFSK.N					
260 - 267	1012.4899	1011.4826	1011.4773	0.0053	0
K.NFGLYNER.V					
268 - 290	2514.3396	2513.3323	2513.3410	-0.0087	1
R.VGNLTVVGKEPESILQVLSQMEK.I Oxidation (M)					
294 - 305	1297.6676	1296.6603	1296.6575	0.0029	0
R.ITWSNPPAQGAR.I					
306 - 325	2234.1340	2233.1267	2233.1266	0.0001	0
R.IVASTLSNPELFEETGTVK.T					
370 - 378	1121.5776	1120.5703	1120.5764	-0.0061	0
K.QVEYLVNEK.H					
379 - 387	1055.6027	1054.5954	1054.5923	0.0031	0
K.HIYLLPSGR.I					
397 - 411	1660.8594	1659.8521	1659.8468	0.0054	0
K.NLDYVATSIHEAVTK.I					

No match to: 1007.3352, 1024.4900, 1046.5989, 1082.0226, 1104.5610, 1111.5660, 1113.0837, 1113.5891, 1136.5662, 1137.0791, 1137.5778, 1312.6394, 1320.5945, 1329.6720, 1373.6609, 1399.7295, 1458.7198, 1475.7651, 1490.7429, 1638.8547, 1642.7384, 1765.7413, 2160.1252, 2162.0264, 2164.8035, 2185.0420, 2198.1506, 2201.0105, 2207.0413, 2216.9919, 2220.0662, 2225.1628, 2227.4856, 2231.1230, 2240.0447, 2247.1379, 2251.0750, 2255.1238, 2256.1868, 2271.1423, 2279.1453, 2290.1584, 2312.1406, 2328.0999, 2331.1980, 2343.0549, 2456.2671, 2467.3179, 3211.4783





Mascot Search Results

Protein View

Match to: [sp|P17174|AATC_HUMAN](#) Score: **61** Expect: **0.018**
Aspartate aminotransferase, cytoplasmic OS=Homo sapiens GN=GOT1 PE=1 SV=3

Nominal mass (M_r): **46447**; Calculated pI value: **6.52**
NCBI BLAST search of [sp|P17174|AATC_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **11**
Number of mass values matched: **5**
Sequence Coverage: **14%**

Matched peptides shown in **Bold Red**

1 MAPPSVFAEV PQAQPVLVFK **LTADFREDPD** **PRK**VNLGVGA YRTDDCHPWV
51 LPVVKKVEQK IANDNSLNHE YLPILGLAEF RSCASRLALG DDSPALKEKR
101 **VGGVQSLGGT** **GALR**IGADFL ARWYNGTNNK NTPVYVSSPT WENHNAVFS
151 AGFKDIRSYR YWDAEKRLD LQGFLNDLEN APEFSIVVLH ACAHNPTGID
201 PTPEQWKQIA SVMKHRFLFP FFDSAYQGFA SGNLERDAWA IRYFVSEGFE
251 FFCAQSF~~SKN~~ **FGLYNER**VGN LTVVGKEPES ILQVLSQMEK IVR**ITWSNPP**
301 **AQGAR**IVAST LSNPELFEEW TGNVKTADR ILTMRSELRA RLEALKTPGT
351 WNHITDQIGM FSFTGLNPKQ VEYLVNEKHI YLLPSGRINV SGLTTK**NLDY**
401 **VATSIHEAVT** **KIQ**

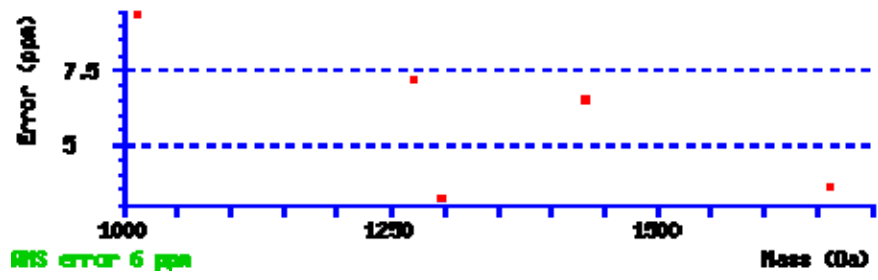
Show predicted peptides also

Sort Peptides By

Residue Number Increasing Mass Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
21 - 32 K.LTADFREDPDPR.K	1431.6956	1430.6883	1430.6790	0.0093	1
101 - 114 R.VGGVQSLGGTGALR.I	1271.7157	1270.7084	1270.6993	0.0092	0
260 - 267 K.NFGLYNER.V	1012.4941	1011.4868	1011.4773	0.0095	0
294 - 305 R.ITWSNPPAQGAR.I	1297.6689	1296.6616	1296.6575	0.0042	0
397 - 411 K.NLDYVATSIHEAVTK.I	1660.8600	1659.8527	1659.8468	0.0060	0

No match to: 1400.7400, 1451.7200, 1566.7800, 1737.8500, 2272.1394, 2288.1360





Mascot Search Results

Protein View

Match to: **sp|P50213|IDH3A_HUMAN** Score: **95** Expect: **7.2e-006**
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial OS=Homo sapiens GN=IDH3A PE=1 SV=1

Nominal mass (M_r): **40022**; Calculated pI value: **6.47**
 NCBI BLAST search of [sp|P50213|IDH3A_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **34**
 Number of mass values matched: **10**
 Sequence Coverage: **25%**

Matched peptides shown in **Bold Red**

```

1  MAGPAWISKV  SRLLGAFHNP  KQVTRGFTGG  VQTVTLIPGD  GIGPEISAAV
51  MKIFDAAKAP IQWEERNVTA  IQGPGGKWMI  PSEAKESMDK  NKMGLKGPLK
101 TPIAAGHPSM  NLLLRKTFDL  YANVRPCVSI  EGYKTPYTDV NIVTIRENTE
151 GEYSGIEHVI VDGVVQSIKL  ITEGASKRIA  EFAFEYARNN  HRSNVTAVHK
201 ANIMRMSDGL  FLQKCREVAE  SCKDIKFNEM  YLDTVCLNMV  QDPSQFDVLV
251 MPNLYGDILS  DLCAGLIGGL  GVTPSGNIGA  NGVAIFESVH  GTAPDIAGKD
301 MANPTALLLS  AVMLLRHMGL  FDHAARIEAA CFATIKDGKS LTKDLGGNAK
351 CSDFTTEEICR RVKDLD

```

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass

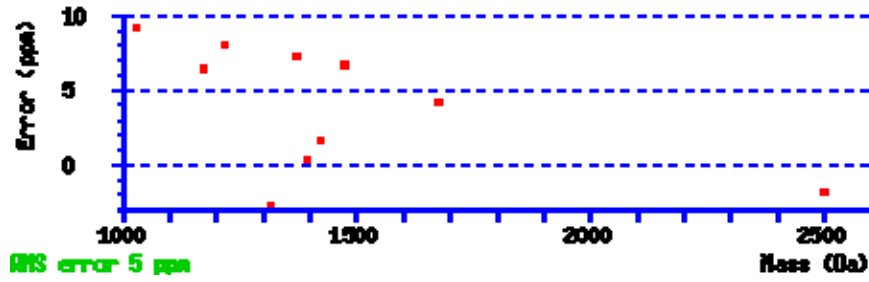


Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
53 - 66 K.IFDAAKAPIQWEER.N	1673.8716	1672.8643	1672.8573	0.0071	1
59 - 66 K.APIQWEER.N	1028.5254	1027.5181	1027.5087	0.0095	0
135 - 146 K.TPYTDVNIVTIR.E	1391.7533	1390.7460	1390.7456	0.0004	0
147 - 169 R.ENTEGEYSGIEHVIVDGVVQSIK.L	2502.2312	2501.2239	2501.2285	-0.0045	0
178 - 188 K.RIAEFAFEYAR.N	1372.7107	1371.7034	1371.6935	0.0100	1
179 - 188 R.IAEFAFEYAR.N	1216.6094	1215.6021	1215.5924	0.0098	0
317 - 326 R.HMGLFDHAAR.I	1170.5548	1169.5475	1169.5400	0.0075	0
327 - 339 R.IEAACFATIKDGK.S	1423.7273	1422.7200	1422.7177	0.0024	1

351 - 360	1316.5209	1315.5136	1315.5173	-0.0036	0
K.CSDFTEEICR.R					
351 - 361	1472.6356	1471.6283	1471.6184	0.0099	1
K.CSDFTEEICRR.V					

No match to: 1001.3287, 1006.3261, 1010.3149, 1011.3268, 1013.3229,
 1021.3257, 1023.3337, 1046.6008, 1137.5754, 1399.7310, 1458.7183,
 1490.7502, 1642.7401, 2185.0442, 2201.0088, 2207.0144, 2216.9822,
 2221.0603, 2225.1594, 2251.0320, 2255.1489, 2257.1599, 2296.1472,
 2515.3503



MASCOT SCIENCE Mascot Search Results

Protein View

Match to: [sp|P11142|HSP7C_HUMAN](#) Score: **54** Expect: **0.074**
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1

Nominal mass (M_r): **71082**; Calculated pI value: **5.37**
 NCBI BLAST search of [sp|P11142|HSP7C_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: **71**
 Number of mass values matched: **12**
 Sequence Coverage: **22%**

Matched peptides shown in **Bold Red**

```

1 MSKGPVAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL
51 IGDAAKNQVA MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR
101 PKVQVEYKGE TKSFYPEEVS SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF
151 NDSQRQATKD AGTIAGLNVL RIINEPTAAA IAYGLDKKVG AERNVLIFDL
201 GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH FIAEFKRKHK
251 KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQLL
351 QDFFNKGELN KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS
401 LGIETAGGVM TVLIKRNTTI PTKQTQTFTT YSDNQPGVLI QVYEGERAMT
451 KDNLLGKFE LTGIPPAPRG VPQIEVTFDI DANGILNVSA VDKSTGKENK
501 ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN SLESYAFNMK
551 ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGGMPGG MPGGFPGGGA PPSGASSGP TIEEVD
  
```

Show predicted peptides also

Sort Peptides By



Residue Number



Increasing Mass



Decreasing Mass

Start - End Sequence	Observed	Mr(expt)	Mr(calc)	Delta	Miss
37 - 49 R.TTPSYVAFTDTER.L	1487.6952	1486.6879	1486.6940	-0.0061	0
57 - 72 K.NQVAMNPTNTVFDAKR.L	1805.8793	1804.8720	1804.8890	-0.0170	1
89 - 102 K.HWPFMVVNDAGRPK.V	1653.8372	1652.8299	1652.8245	0.0054	0
113 - 126 K.SFYPEEVSSMVLTK.M	1616.7672	1615.7599	1615.7803	-0.0204	0
138 - 155 K.TVTNAVVTVPAYFNDSQR.Q	1982.0103	1981.0030	1980.9904	0.0125	0
172 - 188 R.IINEPTAAAIAYGLDKK.V	1787.9456	1786.9383	1786.9828	-0.0445	1

221 - 236	1691.7117	1690.7044	1690.7183	-0.0139	0
K.STAGDTHLGGEDFDNR.M					
237 - 246	1251.6227	1250.6154	1250.6117	0.0037	0
R.MVNHFIAEFK.R Oxidation (M)					
237 - 247	1391.7073	1390.7000	1390.7179	-0.0179	1
R.MVNHFIAEFKR.K					
237 - 247	1407.7191	1406.7118	1406.7128	-0.0010	1
R.MVNHFIAEFKR.K Oxidation (M)					
300 - 311	1480.7518	1479.7446	1479.7470	-0.0024	1
R.ARFEELNADLFR.G					
326 - 342	1837.9275	1836.9202	1837.0057	-0.0855	1
K.LDKSQIHDIIVLVGGSTR.I					

No match to: 1145.5675, 1334.6069, 1358.7142, 1393.7199, 1417.7304, 1431.7078, 1434.7300, 1465.7087, 1521.7516, 1529.7046, 1539.7914, 1556.7244, 1562.7950, 1564.7987, 1566.7902, 1567.7548, 1570.8195, 1590.7831, 1606.7422, 1628.8868, 1638.8558, 1651.8195, 1656.7396, 1658.7323, 1671.8671, 1674.7290, 1687.8832, 1690.7231, 1701.8927, 1703.9197, 1718.8568, 1740.8617, 1750.8823, 1756.9430, 1771.8404, 1817.9147, 1827.8980, 1830.9373, 1851.9396, 1878.8190, 1885.9940, 1894.8064, 1901.0007, 1918.8909, 1941.9677, 1964.0448, 1992.9533, 2020.0671, 2049.9717, 2069.0117, 2103.9807, 2119.0481, 2128.0256, 2175.0503, 2403.0073, 2566.2751, 2572.2398, 2582.2341, 3412.5830

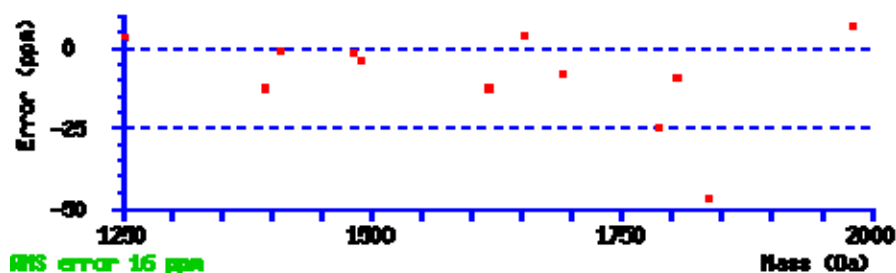
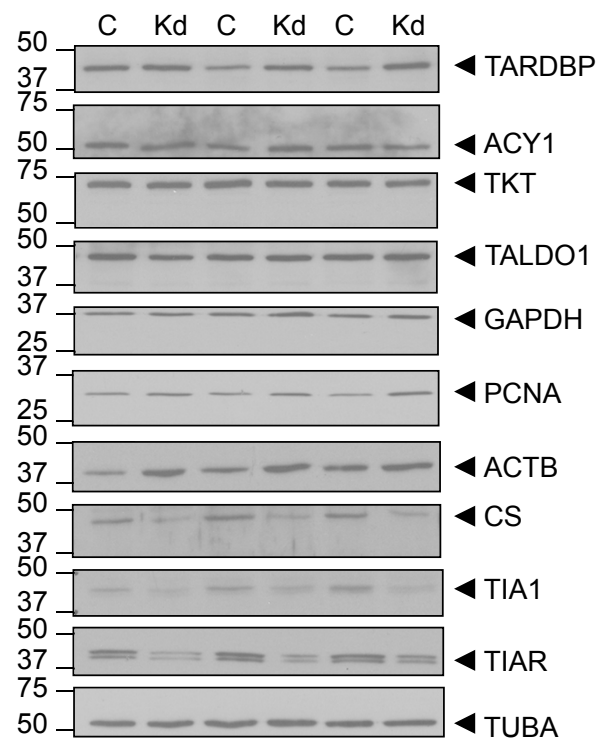


Figure S3

A



B

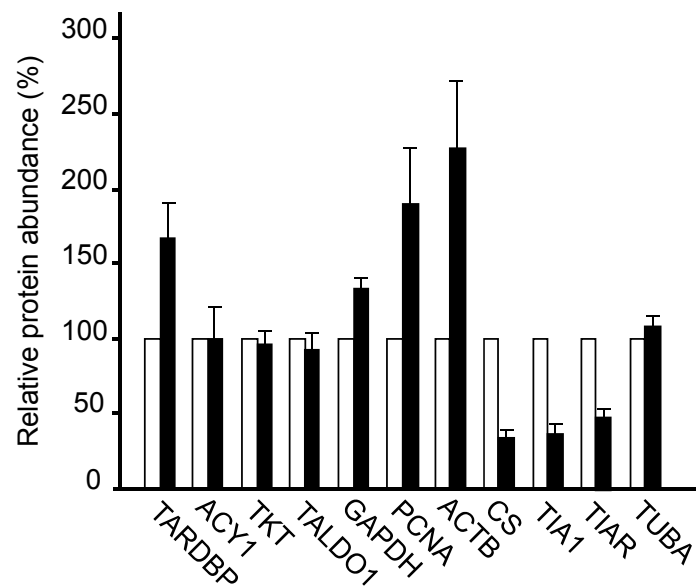


Figure S4

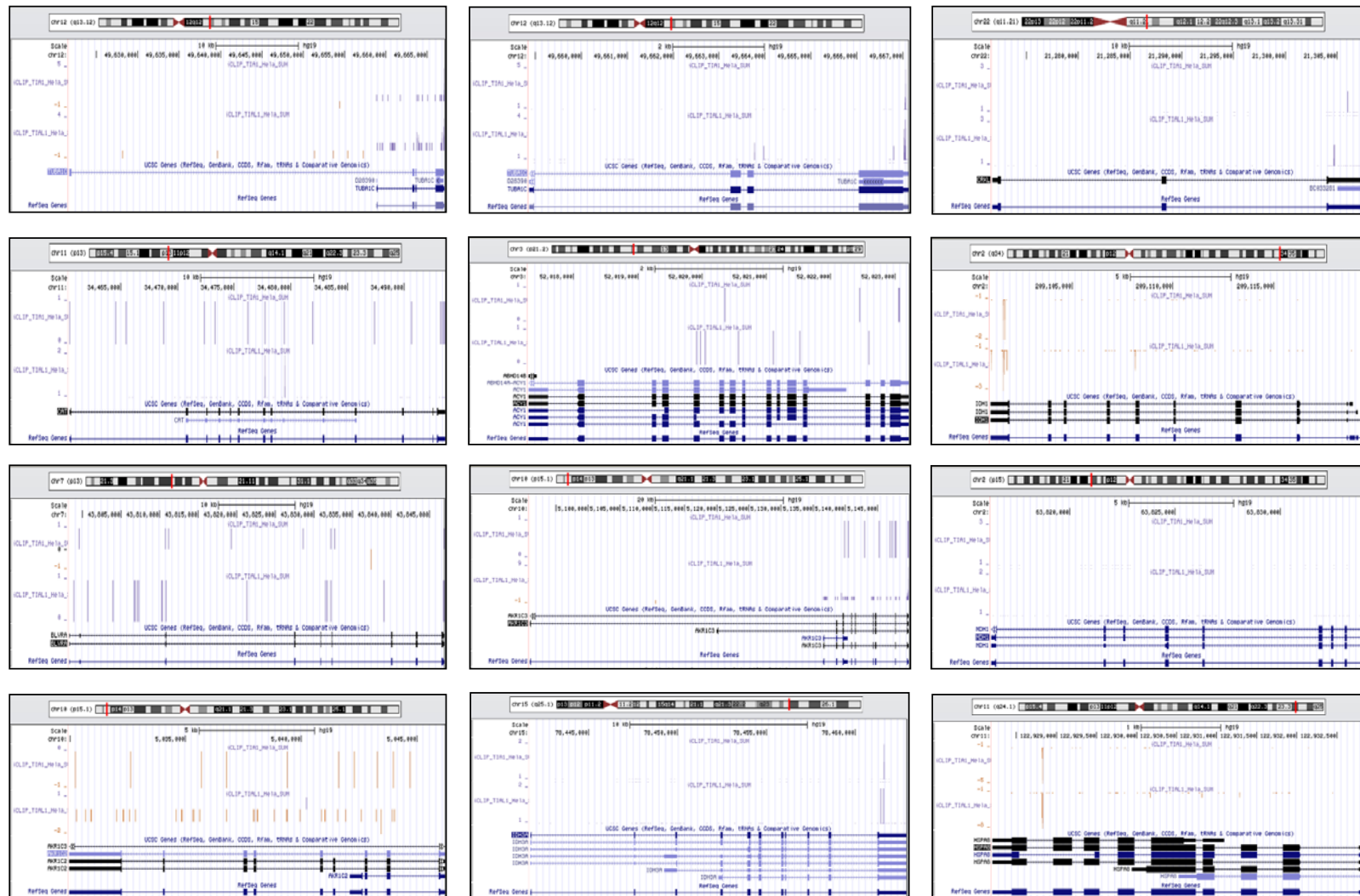
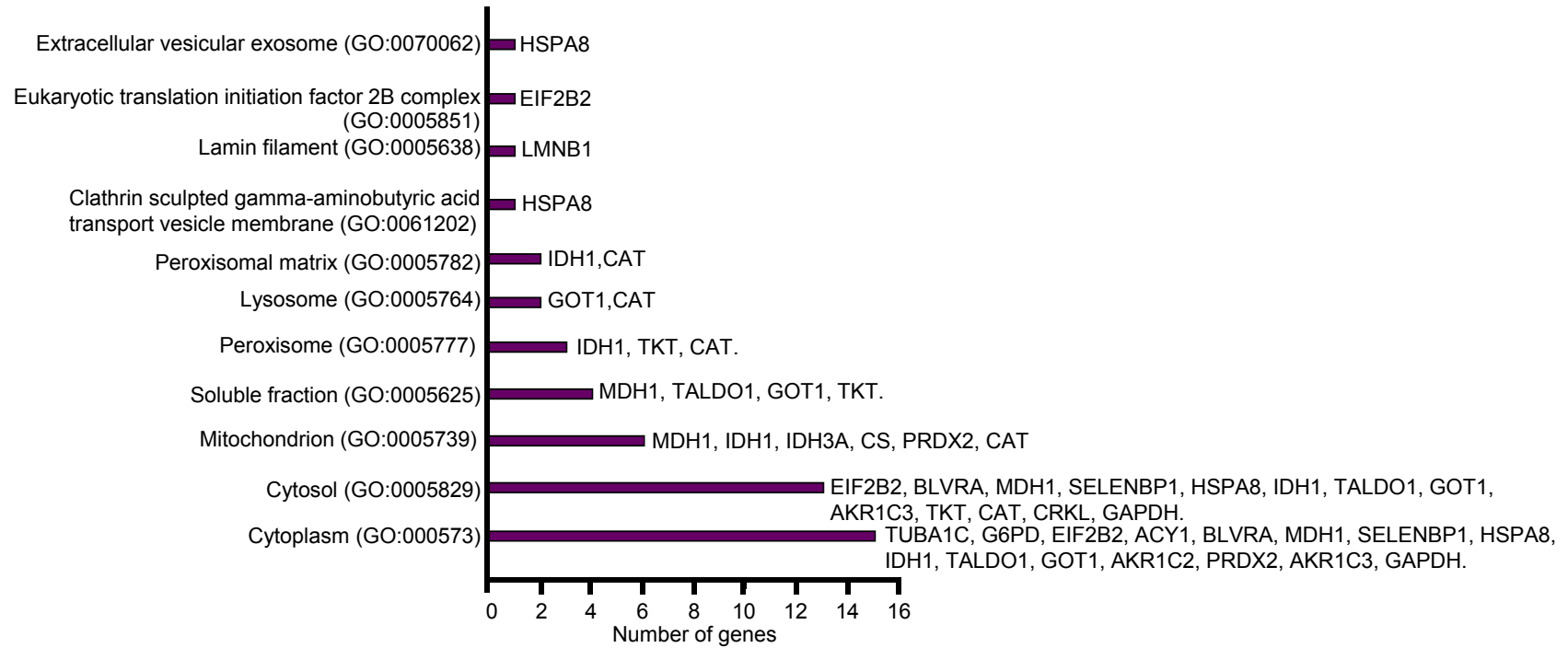


Figure S5

A



B

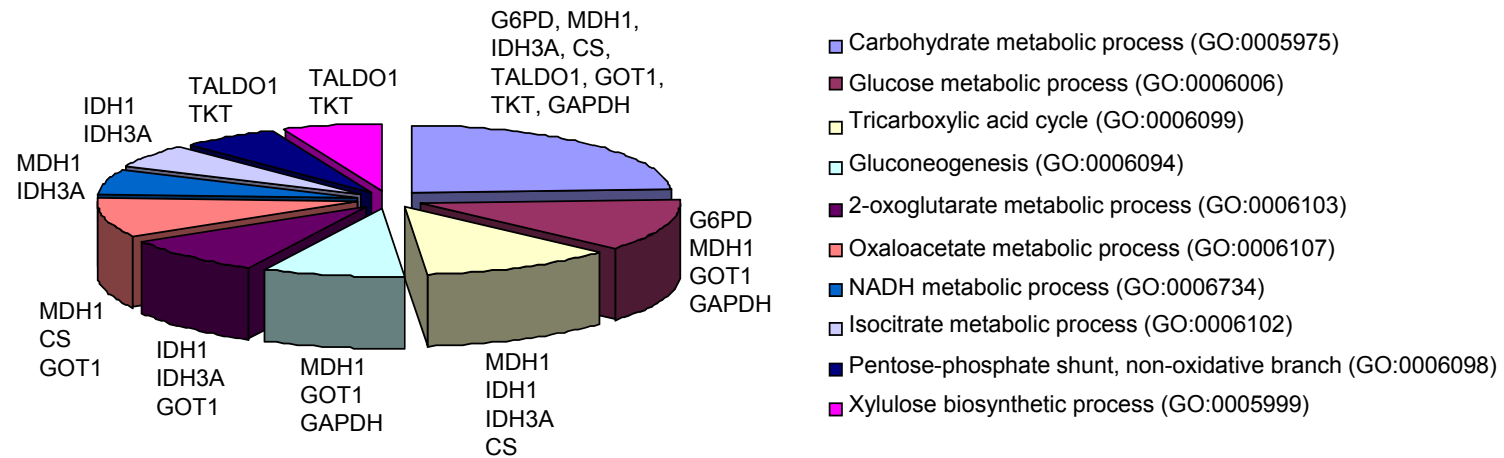
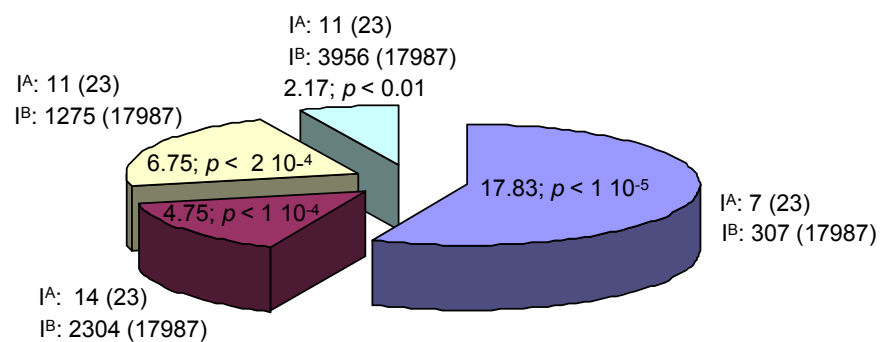
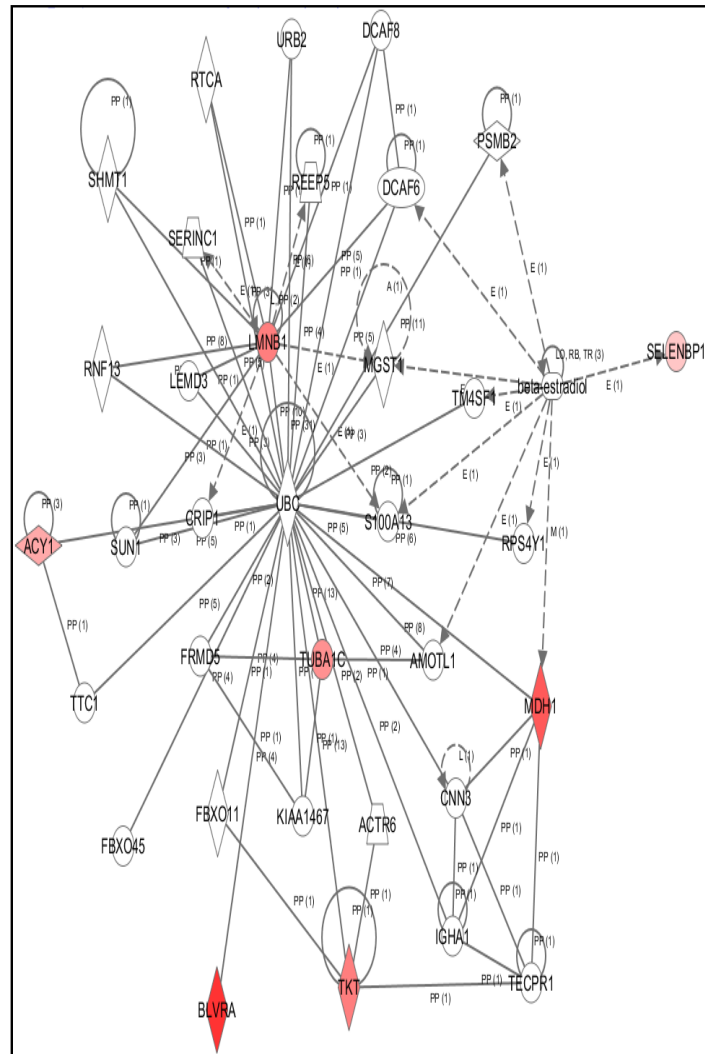


Figure S6



- Carbohydrate metabolic process: CS, G6PD, GAPDH, GOT1, IDH3A, MDH1 & TALDO1.
- Small molecule metabolic process: BLVRA, CAT, CS, G6PD, GAPDH, GOT1, IDH1, IDH3A, MDH1, PSMB8 & TALDO1.
- Cytosol: ACY1, BLVRA, CAT, CRKL, G6PD, GAPDH, GOT1, HSPA8, IDH1, MDH1, PSMB8, TALDO1, EIF2B2 & SELENBP1.
- Cytoplasm: AKR1C2, G6PD, GAPDH, GOT1, IDH1, MDH1, TALDO1, PRDX2, AKR1C3, EIF2B2 & TUBA1C.

Figure S7



Biological Function	Symbol	Swiss Prot	Name	Av. Ratio (a)	T-test (a)	Biological Process	MW	pI	Score (b)	Peptides (c)	% Cover (d)	App.
<i>Structural & cytoskeleton-related</i>												
	TUBA1C	Q9BQE3	Tubulin alpha-1C chain	3.36	0.05	Microtubule formation	50,548	4.96	51	1	3	12 (12)
<i>Transcription/splicing/protein synthesis & turnover</i>												
	LMNB1	P20700	Lamin-B1	3.97	0.0077	Nuclear lamina matrix	66,653	5.11	75	15	28	12 (12)
	TARDBP	Q13148	TAR DNA-binding protein 43	2.62	0.0084	Transcription and splicing	45,053	5.85	72	3	6	12 (12)
	EIF2B2	P49770	eIF-2B subunit beta	2.65	0.003	Protein synthesis	39,193	5.77	119	23	49	12 (12)
	PSMB8	P28062	Proteasome subunit b type-8	5.59	0.023	Multicatalytic proteinase complex	30,677	7.63	83	3	14	9 (12)
<i>Transport</i>												
	SELENBP1	Q13228	Selenium-binding protein 1	1.73	0.021	Intra-Golgi protein transport	52,928	5.93	95	15	37	12 (12)
<i>Signal transduction</i>												
	CRKL	P46109	Crk-like protein	2.65	0.003	RAS & JUN kinase signaling path	33,870	6.26	65	11	43	12 (12)
	PRDX2	P32119	Peroxiredoxin-2	1.82	0.0017	Signaling cascades of GF&TNF-a	22,049	5.66	72	8	36	12 (12)
<i>Metabolism</i>												
	TKT	P29401	Transketolase	3.97	0.0077	Pentose phosphate pathway						12 (12)
	CAT	P04040	Catalase	-1.66	0.022	Removing of hydrogen peroxide	59,947	6.90	140	17	40	12 (12)
	G6PD	P11413	Glucose-6-phosphate 1-DH	-1.87	0.012	Synthesis of pentose sugars	59,675	6.39	365	38	59	12 (12)
	ACY1	Q03154	Aminoacylase-1	2.62	0.0084	Hydrolysis of N-acetylated proteins	46,084	5.77	85	12	36	12 (12)
	IDH1	O75874	Isocitrate DH [NADP] cytopl.	-1.96	0.0014	NADPH production	46,915	6.53	201	24	57	12 (12)
	TALDO1	P37837	Transaldolase	1.59	0.018	Synthesis of ribose-5-phosphate	37,688	6.36	71	4	21	12 (12)
	BLVRA	P53004	Biliverdin reductase A	6.28	0.0019	Heme catabolic process	33,692	6.06	93	15	46	12 (12)
	GAPDH	P04406	Glyceraldehyde-3-P DH	1.69	0.027	Carbohydrate metabolism	36,201	8.57	73	6	24	12 (12)
	AKR1C3	P42330	Aldo-keto reductase 1 C3	-2.0	0.024	Prostaglandin metabolism	37,220	8.05	132	16	55	12 (12)
	MDH1	P40925	Malate DH, cytoplasmic	5.08	0.018	Malate-aspartate shuttle	36,631	6.91	66	4	15	12 (12)
	AKR1C2	P52895	Aldo-keto reductase 1 C2	-5.88	0.0017	Prostaglandin metabolism	37,111	7.13	165	20	52	12 (12)
<i>Energy production</i>												
	CS	O75390	Citrate synthase, mitochon.	-1.59	0.0026	Tricarboxylic acid cycle	51,908	8.45	108	7	16	12 (12)
	GOT1	P17174	Aspartate aminotransferase	1.55	0.026	Amino acid metabolism	46,447	6.52	142	16	54	12 (12)
	GOT1	P17174	Aspartate aminotransferase	1.57	0.02	Amino acid metabolism	46,447	6,52	61	5	14	12 (12)
	IDH3A	P50213	Isocitrate DH [NAD] alpha	1.73	0.0051	Tricarboxylic acid cycle	40,022	6.47	95	10	25	12 (12)
<i>Chaperones/Stress</i>												
	HSPA8	P11142	Heat shock cognate 71 kDa	3.97	0.0077	Protein folding	71,082	5.37	54	12	22	12 (12)