

The Proteome of Normal Human Chorionic Villus Sampling Cells

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Abstract. *Chorionic villi samples are widely used for prenatal diagnosis of various fetal disorders. Although, our knowledge regarding the molecular level of these disorders is extensive, little is known about the implicated proteins. In the present study, two dimensional electrophoresis (2-DE) followed by mass spectrometry (MS) was applied to reveal the proteomic profile of the CV cells. This proteomic technique was previously used successfully in the cases of amniotic fluid, follicular fluid and maternal blood, but has not yet been applied to CV. Therefore, 2-DE was combined with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS) to characterise the proteome of normal CV cultured cells. Two hundred eighty two individual gene products were identified including cytoplasmic and nuclear proteins. Although the majority of the proteins were enzymes, structural, signalling and carrier molecules were also isolated. 2D protein map elucidates 282 protein molecules expressed in the CV cells that can be used as a reference for future comparison to various pathological conditions.*

Abbreviations: CVS: chorionic villus sampling, PMF: peptide mass fingerprint, PSD: post source decay, MALDI: matrix-assisted laser desorption/ionization, IPG: immobilized pH gradient, pI: isoelectric point.

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Key Words: chorionic villus sampling, trophoblast, pregnancy, proteomics, 2-DE protein database, mass spectrometry, MALDI-MS.

Current prenatal screening methodology can be divided into invasive and non-invasive approaches. The most commonly used invasive techniques are amniocentesis and chorionic villi sampling, while non-invasive procedures include ultrasound examination and screening of maternal blood. CVS remains the method of choice when prenatal diagnosis is required during the early stages of pregnancy, between the 10th to 15th weeks of gestation.

The screening of cultured CV cells by karyotyping, fluorescence *in situ* hybridization (FISH), PCR, reverse transcription PCR (RT-PCR) and quantitative fluorescence PCR (QF-PCR) analyzes the nucleotides, DNA, RNA or/and their derivative structures (chromosomes), but diagnostic tests based on protein findings are limited. Currently, proteomic technology is being used in an attempt to identify protein biomarkers linked to pregnancy and contribute to the comprehension of the underlying pathophysiology and the discovery of molecules that mark the fetal genetic diseases or pregnancy complications (1-3). Since proteomic strategies investigate multiple molecules simultaneously, they can potentially lead to the discovery of a «panel» of markers with sufficient sensitivity and specificity for clinical application. The goal is to identify biomarkers that differ from the “normal” proteomic profile and are specific for aneuploid or disease-affected pregnancy.

Proteomic approaches have previously been applied to study Down and Turner syndromes by analysing tissues from fetuses, amniotic fluid and maternal plasma (4-7). Proteins, such as serotransferin, lumican, plasma retinol-binding protein, apolipoprotein A-I, alpha-1-microglobulin, collagen alpha 1 (I), collagen alpha 1 (III), collagen alpha 1 (V), basement membrane-specific heparin sulfate proteoglycan core protein and insulin binding protein 1 were identified demonstrating a potential link to these pathological

phenotypes, further evaluation is required to prove the diagnostic potential of these molecules and their possible future use as biomarkers. Moreover, proteomic analysis has been performed on the placental villous tissue of early pregnancy loss (8) as well as, on the human follicular fluid of recurrent spontaneous abortions (9). However, a detailed two-dimensional (2D) protein database of the normal human CV does not exist. There is an emerging medical need for such a tool, considering the implication of CV in prenatal screening and its importance for diagnosis during the early stages of pregnancy. In the present study the proteomic analysis and the identification of the proteomic profile of cultured normal chorionic villi cells are reported.

Materials and Methods

Materials and reagents. Immobilized pH-gradient (IPG) strips, IPG buffers and acrylamide/piperazine-di-acrylamide (PDA) solution (37.5:1 w/v) were purchased from Biorad Laboratories (Hercules, CA, USA). 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), and 1,4-dithioerythritol (DTE) and EDTA from Merck (Darmstadt, Germany). All the reagents were stored at 4°C, except for CHAPS which was stored at 23°C.

Cell cultures. Chorionic villi samples were obtained from 20 women, during the 11th-13th week of gestation. CVS was performed due to irregular findings during the first trimester tests of pregnancy. Written informed consent was obtained from all women. Samples were subjected to conventional cytogenetic and molecular analysis to certify the normal state of the fetuses. The tissue samples were dissected and cultured in Amniomax culture medium (Invitrogen, Carlsbad, CA, USA) at 37±0.5°C, 5% CO₂ for 9-10 days. When the cells reached confluence they were harvested by trypsinization and centrifuged at 1000 × g for 10 min. The cell pellet was washed using 0.9% NaCl and recentrifuged at 1000 × g for 10 min.

Two-dimensional electrophoresis (2-DE). The cell pellets were homogenized in TRI reagent as recommended by the manufacturer (Ambion/Applied Biosystems, Austin, TX, USA) and the RNA and DNA was removed. The protein fractions were resuspended in urea lysis buffer (20 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM 1,4-DTE, 1 mM EDTA) and a mixture of protease inhibitors [1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 tablet complete™ (Roche Diagnostics, Basel, Switzerland)] and pooled together.

The protein concentration was determined by Bradford colorimetric assay (10) using a Biorad protein assay (Biorad Laboratories). Isoelectric focusing was performed, as previously described (11). Triplicate samples of 1.0 mg total protein were applied on immobilized 3-10 pI or 4-7 pI non-linear gradient strips (17 cm) in sample cups at their basic and acidic ends. Focusing was initially performed at 250 V for 30 min and the voltage was gradually increased to 5000 V at 3 V/min and remained constant for an additional 16 h.

The second-dimensional separation was performed in 12% SDS-polyacrylamide gels (180×200×1.5 mm), running at 40 mA per gel in a Protean apparatus (Biorad Laboratories). Fixation was performed in

50% methanol, containing 10% acetic acid for 2 h; the gels were stained overnight with colloidal Coomassie blue (Novex, San Diego, CA, USA), washed twice with H₂O and scanned in a densitometer (GS-800 Calibrated Densitometer, Biorad Laboratories).

Peptide mass fingerprint (PMF). PMF analysis was essentially performed as described previously (12). Briefly, all the spots on the gels were annotated semi-automatically using Melanie 4.02 software (GeneBio, Geneva, Switzerland), excised with a Proteiner SPII robot (Bruker Daltonics, Bremen, Germany) and placed into 96-well microtiter plates. The excised spots were destained using 180 µl of 100 mM ammonium bicarbonate in 30% acetonitrile (ACN) and the gel piece was dried in a MaxiDry Plus speed vacuum concentrator (Heto-Holten A/S, Allerød, Denmark). The dried gel piece was rehydrated with 5 µL of 20 µg/mL recombinant trypsin (Proteomics grade, Roche Diagnostics, Basel, Switzerland) solution. After 16 h at room temperature, 10 µL of 50% acetonitrile containing 0.3% trifluoroacetic acid (TFA) were added and the gel pieces were incubated for 15 min with gentle shaking. Sample application to a target plate and analysis as well as peptide matching and protein searching were carried out as described previously (12). Briefly, tryptic peptide mixtures (1 µL) were applied on an anchor chip MALDI plate with 1 µL of matrix solution, consisting of 0.08% α-Cyano-4-hydroxycinnamic acid (CHCA) (Sigma-Aldrich, Taufkirchen, Germany), and the internal standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotrophic hormone fragment 18-39 (Sigma, 2465.1989 Da) in 65% ethanol, 50% ACN and 0.1% TFA. The peptide mixtures were analysed in a MALDI-TOF mass spectrometer (Ultraflex II, Bruker Daltonics). Laser shots (n=400) of intensity between 40% and 60% were collected and summarized and the peak list was created using the FlexAnalysis v2.2 software (Bruker). Peak list was created with Flexanalysis v2.2 software (Bruker). Smoothing was applied with the Savitzky-Golay algorithm (width 0.2mz, cycle number 1). A signal to noise (S/N) threshold ratio of 2.5 was allowed. The SNAP (Sophisticated Numerical Annotation Procedure) (Bruker) algorithm was used for peak picking. Tryptic autodigest as well as commonly occurring keratin contaminant peaks were filtered out by the software prior to the protein identification process. Peptide matching and protein searches were performed automatically with Mascot Server 2 (Matrix Science, London, UK). Peptide masses were compared with the theoretical peptide masses of all available proteins from *Homo sapiens* in the Swiss-Prot database. Stringent criteria were used for protein identification with a maximum allowed mass error of 10 ppm and a minimum of four matching peptides. Mascot score ≥55 indicating a probability score with *p*<0.05 was used as the criterion for affirmative protein identification. Monoisotopic masses were used, and one missed trypsin cleavage site was calculated for proteolytic products.

Results

All visible spots of all 2D gels were mapped by the 2D ImageMaster software (Amersham Biosciences). The protein gels of each pI range were almost identical, revealing the reproducibility of the applied methodology. On the 3-10 pI gels an average of 956 spots per gel were detected (Figure 1A), while ~691 spots were identified on each 4-7 pI gel (Figure 1B). All the detected spots were excised from the

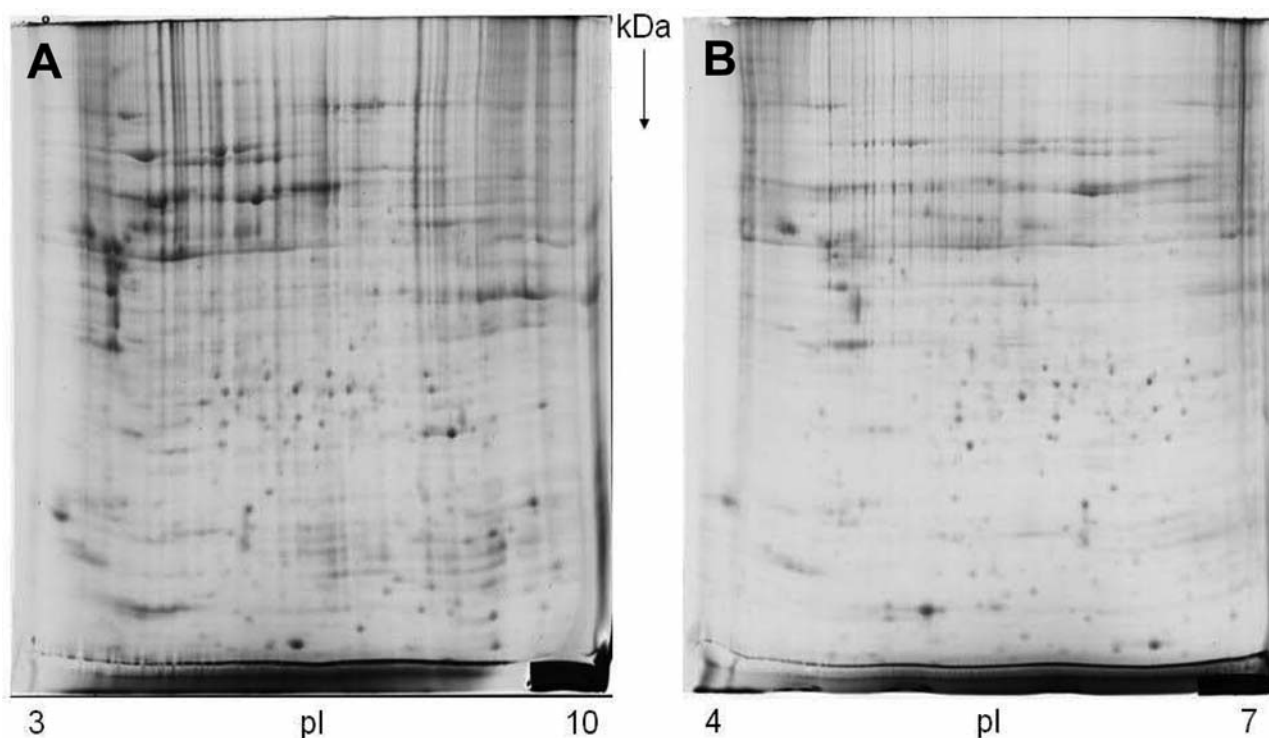


Figure 1. A representative example of the CV proteins separated on a 2-D gel. The total amount of protein applied on each strips with *pI* range from 3-10 (A) and from 4-7 (B) was 1 mg.

gels and the protein contained in the spots were identified by MALDI-MS on the basis of peptide mass matching (13), following in-gel digestion with trypsin. The peptide masses were matched to the theoretical peptide masses of all proteins from all databases, resulted in 250 and 130 discrete identifications for the 3-10 (Figure 2) and the 4-7 *pI* range gels respectively.

After comparing and combining the above results, 282 individual molecules were identified from both *pI* ranges, with an identification rate of 80%. The introduction of internal peptide standards to correct the measured peptide masses allowed the use of very narrow windows of mass tolerance (0.0025%), thus increasing the confidence of identification.

The abbreviated and full names of the proteins, their Swiss-Prot accession numbers, the theoretical molecular weight and *pI* values, as well as data from the mass spectrometry analysis, *i.e.* the Mascot scores and the protein amino acid sequence coverage by the matching peptides are listed in Table I. A minimal number of 5 matching peptides was used for protein identification. In some cases, mainly for proteins of low molecular mass that usually deliver few peptides, the identification was based on 4 matching peptides. Only human proteins were considered for the searching procedure, increasing the confidence of the

identification. Thus, Mascot scores ≥ 55 indicated that the identification may be considered as unambiguous.

The subcellular localization of the identified proteins was analyzed using publicly accessible data bases (Figure 3A). According to the Swiss-Prot database 25% of the identified proteins were located in both nucleus and cytoplasm, 5% predominantly in the nucleus and 65% were exclusively cytoplasmic. The latter can be divided into mitochondrial (21%), endoplasmic reticulum proteins (10%), cytoskeletal (10%), cytosolic (7%), ribosomal (5%), and inner-membrane proteins (7%). The remaining are either of unknown localization, secreted or extracellular.

Functional analysis (Figure 3B) according to the publicly available UniProtKB database indicated that 118 of the identified proteins were enzymes (28.3%), 60 were regulatory (14.4%), 43 were structural (10.3%), 36 were carriers (8.6%), 30 chaperons (7.2%), 26 RNA-associated (6.2%), 19 implicated in signaling (4.6%) and the rest of them in other biological functions, with smaller percentages.

Discussion

The majority of the 282 proteins identified in this study were enzymes, as expected; however, structural, signalling and carrier molecules were also isolated. Interestingly, one of the molecules

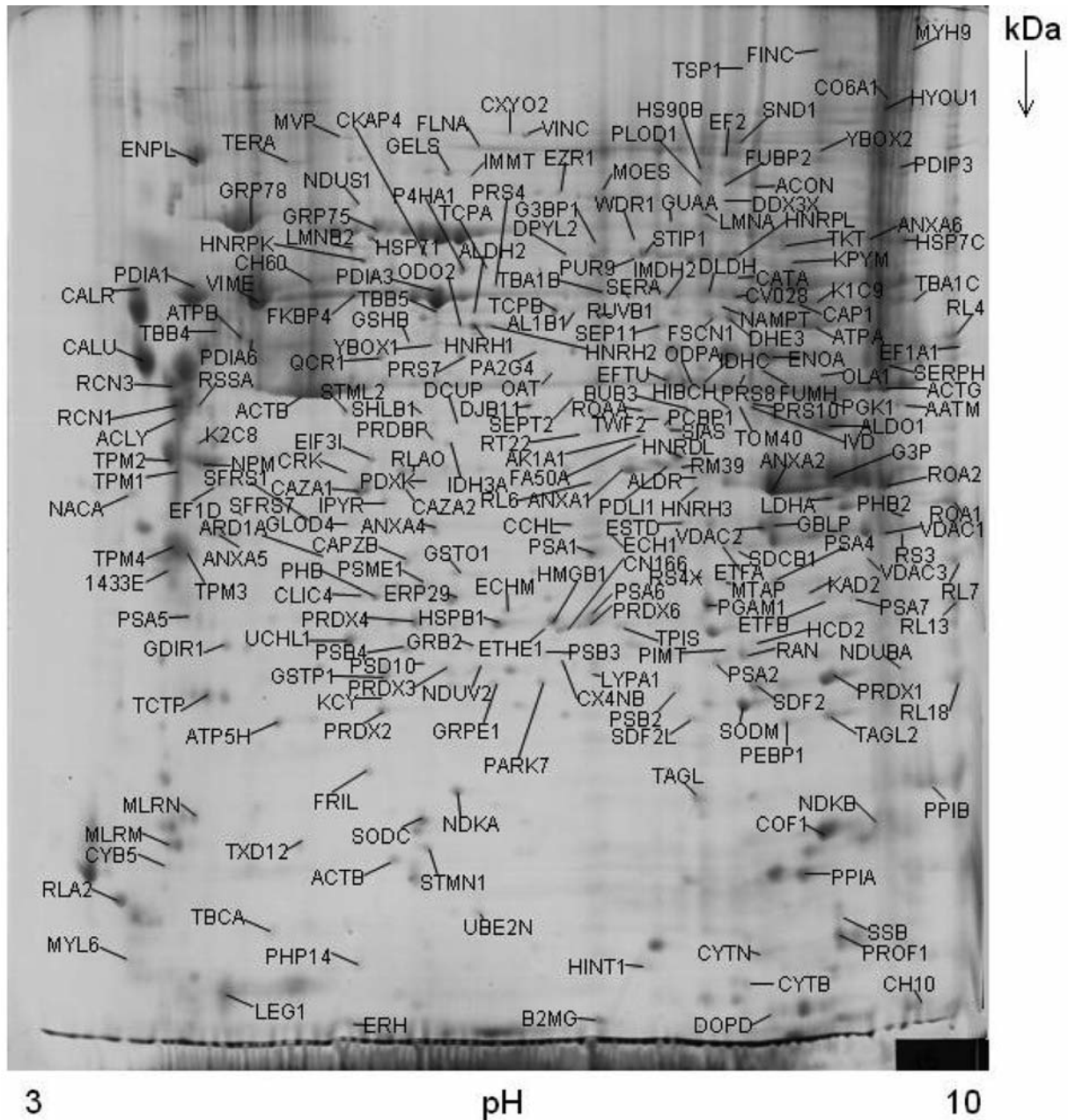


Figure 2. All identified peptides, of normal human chorionic villus cells, annotated on a two-dimensional gel with pI range from 3-10.

identified in this study is Y-box-binding protein 2, the expression of which is normally restricted to germ cells and placental trophoblasts. More specifically, it is observed in oocytes and testicular germ cells in the stage of spermatogonia to spermatocyte, as well as, in placental trophoblasts and it is up-regulated in various carcinomas and germ cell tumors (13, 14). Another interesting molecule was stomatin-like protein 2 (SLP-2), an unusual stomatin homologue of unknown functions, probably implicated in cell motility, proliferation and the cell

cycle (15). Recently, the involvement of SLP-2 in human endometrial adenocarcinoma and the effects of SLP-2 on endometrial adenocarcinoma cell growth were demonstrated (16). Another group of proteins identified in CV includes transcription factors or molecules that are involved in transcription such as protein DJ-1 and transgelin. These proteins might also be involved in the regulation of the stability and/or translation of germ cell mRNAs, as well as, in posttranslational modification. Further analysis is needed to characterize the role

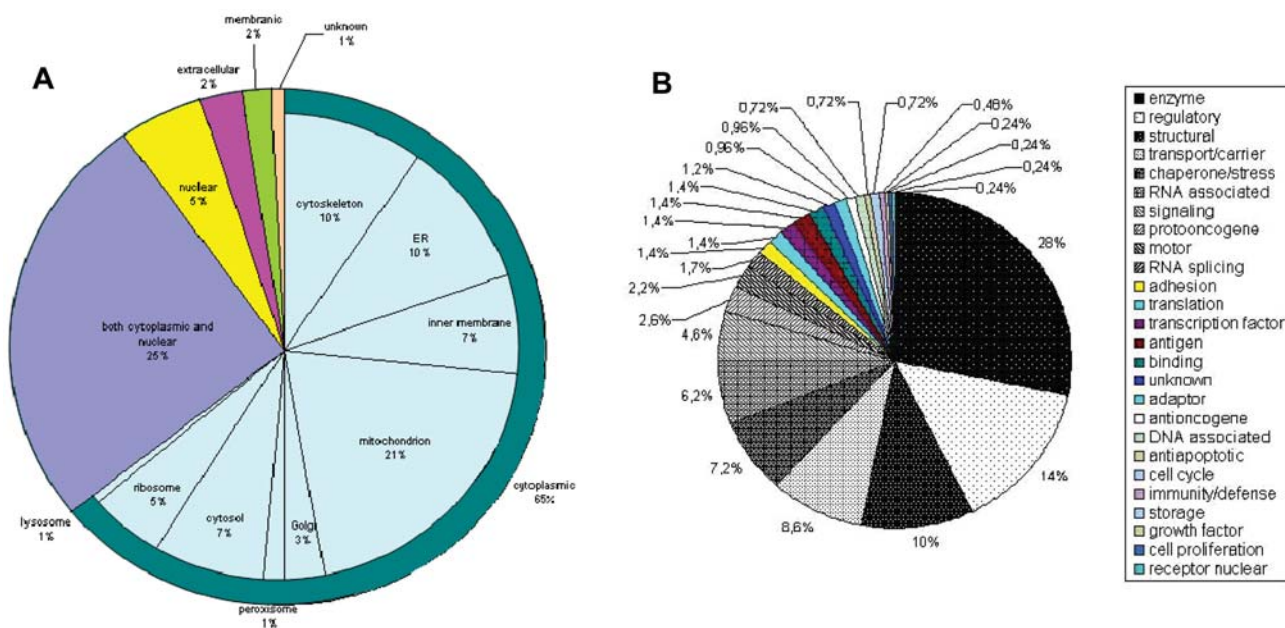


Figure 3. A) Subcellular localization and B) functional analysis of the identified proteins from chorionic villi cultured cells.

and normal function of these molecules in the chorionic villi. Interestingly, collagen alpha 1 that was identified in the CV proteomic maps, seems to play a critical role in Down syndrome since its chains (I), (III) and (V) have been found to be upregulated in amniotic fluid cells from Down syndrome cases (6). The collagen alpha 1(VI) chain was identified in this study. Comparison of the expression profiles of normal and pathological CV may lead to a more general conclusion about the role of collagen alpha 1 in Down syndrome.

Our previous studies on normal human amniotic fluid cells (AFCs) reported the identification of 432 different gene products (17). Comparative analysis of the present CV and previous AFC proteomic maps indicated that 158 proteins were common, while 124 proteins were found to be expressed only in the CV proteome. In particular, two members of the cystatin (CST or CYT) superfamily of cysteine protease inhibitors, CYTB and CYTN, were expressed only in CV. Cystatins are reversible, competitive inhibitors of cysteine proteases. Their inhibitory profiles, as well as their affinities for target enzymes, vary according to the different cysteine proteases (18). Although in recent studies the overexpression of CYTC was detected in the serum of pregnant women with clinical preeclampsia (19), there are no data regarding the expression of CYTB and CYTN in CV. Another interesting protein overexpressed in CV but not in AFCs is gelsolin (GELS), a calcium-activated actin linked to a number of pathological conditions such as inflammation, cancer and amyloidosis (20). The tight regulation of gelsolin by calcium is crucial for its activation, leading to apoptosis (21).

Interestingly, in amniotic fluid supernatant (AFS) from women carrying fetuses with Klinefelter syndrome (47,XXY karyotype) GELS was found to be downregulated (22). Another protein found to be expressed only in CV was prohibitin 2 (PHB2), while PHB was expressed in both CV and AFCs. Prohibitins are ubiquitously expressed proteins, highly conserved throughout evolution and are essential for cell proliferation and embryonic development (23, 24). They are primarily localized in mitochondria but are also found in the nucleus and in the cytoplasmic membrane (25). In mouse models, embryos lacking the PHB genes fail to develop beyond embryonic day 8.5, while depletion of PHB1 or PHB2 impairs proliferation of endothelial cells and mouse embryonic fibroblasts. Furthermore, although prohibitins have a predominantly mitochondrial function, they might also demonstrate other functions outside mitochondria, serving as a negative regulator of E2F-mediated transcription (26). Thus, prohibitins might play an essential role during human pregnancy, even from the early stages, but their exact contribution must be further elucidated. By the obtained results it is becoming obvious that the proteomic approach followed in the present study is providing a pool of information regarding the expression profile of the CV.

The proteomic analysis of chorionic villous cultured cells, isolated from first trimester pregnancies offers the first 2D protein map of CV that will supply valid and interesting leads for future studies. It also provides a reference for future comparison with various fetal pathological conditions which may help in the discovery of diagnostic markers.

Table 1. Proteins from chorionic villi cultured cells, separated by 2-DE, extracted and identified by MALDI-MS. The proteins identified by Mascot Software are designated by their Swiss-Prot accession numbers and their full names. The theoretical MW as well as the protein amino acid sequence coverage by the matching peptides are given as an indication of the confidence of the identification. Mascot Score is $-10^* \log(P)$, where P is the probability that the observed match is a random event.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P31946	1433B_HUMAN	14-3-3 protein beta/alpha	77	27	28179.00	4.60	CVS & AFC	Regulatory, signaling	Cytoplasm, perinuclear region
P62258	1433E_HUMAN	14-3-3 protein epsilon	60	39	29326.00	4.50	CVS	Adaptor, regulatory, signaling, neurotransmitter	Cytoplasm, mitochondrion, cytoskeleton, microtubule nucleus, chromatin/chromosome, kinetochore
P61981	1433G_HUMAN	14-3-3 protein gamma	55	21	28456.00	4.70	CVS	Regulatory	Intracellular
P63104	1433Z_HUMAN	14-3-3 protein zeta/delta	72	41	27899.00	4.60	CVS	Regulatory	Cytoplasm
P00505	AATM_HUMAN	Aspartate aminotransferase, mitochondrial	111	39	47844.00	9.80	CVS & AFC	Enzyme	Cytoplasm, mitochondrion matrix
P53396	ACLY_HUMAN	ATP-citrate synthase	55	11	121674.00	7.10	CVS & AFC	Enzyme	Cytoplasm
Q99798	ACON_HUMAN	Aconitate hydratase, mitochondrial	233	47	86113.00	7.90	CVS & AFC	Enzyme	Cytoplasm, mitochondrion
P60709	ACTB_HUMAN	Actin, cytoplasmic 1	146	57	42052.00	5.20	CVS	Structural	Cytoplasm
P63261	ACTG_HUMAN	Actin, cytoplasmic 2	139	61	42108.00	5.20	CVS	Structural	Cytoplasm
P14550	AK1A1_HUMAN	Alcohol dehydrogenase [NADP+]	75	23	36892.00	6.40	CVS & AFC	Enzyme	Cytoplasm, cytosolic
P30837	AL1B1_HUMAN	Aldehyde dehydrogenase X, mitochondrial	111	31	57658.00	6.40	CVS & AFC	Enzyme	Cytoplasm, mitochondrion matrix
P05091	ALDH2_HUMAN	Aldehyde dehydrogenase, mitochondrial	90	28	56859.00	6.80	CVS	Enzyme	Cytoplasm, mitochondrion
P04075	ALDOA_HUMAN	Fructose-bisphosphate aldolase A	188	70	39851.00	9.20	CVS & AFC	Enzyme	Cytoplasm, cytosolic
P15121	ALDR_HUMAN	Aldose reductase	79	28	36230.00	6.60	CVS & AFC	Enzyme	Cytoplasm, cytosolic
P04083	ANXA1_HUMAN	Annexin A1	175	56	38918.00	6.70	CVS & AFC	Regulatory, transport	Plasma membrane
P07355	ANXA2_HUMAN	Annexin A2	235	57	38808.00	8.50	CVS & AFC	Regulatory, signaling, transport	Plasma membrane
P09525	ANXA4_HUMAN	Annexin A4	109	38	36088.00	5.80	CVS & AFC	Regulatory, transport	Cytoplasm
P08758	ANXA5_HUMAN	Annexin A5	176	72	35971.00	4.80	CVS & AFC	Regulatory, transport	Plasma membrane, cytoplasm
P08133	ANXA6_HUMAN	Annexin A6	275	57	76168.00	5.30	CVS	Regulatory, transport	Cytoplasm
P41227	ARD1A_HUMAN	N-terminal acetyltransferase complex ARD1 subunit homolog A	62	33	26613.00	5.30	CVS	Enzyme	Cytoplasm, mitochondria, nucleus
P61158	ARP3_HUMAN	Actin-related protein 3	82	25	47797.00	5.50	CVS	Enzyme	Cytoplasm, cytoskeleton
O75947	ATP5H_HUMAN	ATP synthase subunit d, mitochondrial	135	81	18537.00	5.10	CVS & AFC	Enzyme	Plasma membrane, cytoplasm, mitochondrion
P25705	ATPA_HUMAN	ATP synthase subunit alpha, mitochondrial	151	45	59828.00	9.60	CVS & AFC	Enzyme, transport	Cytoplasm, mitochondrion
P06576	ATPB_HUMAN	ATP synthase subunit beta, mitochondrial	155	52	56525.00	5.10	CVS & AFC	Enzyme, transport	Cytoplasm, mitochondrion
P61769	B2MG_HUMAN	Beta-2-microglobulin precursor [Contains: Beta-2-microglobulin form pI 5.3]	55	35	13820.00	6.10	CVS	Secretory, transport carrier	Extracellular

Table 1. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
O43684	BUB3_HUMAN	Mitotic checkpoint protein BUB3	117	38	37587.00	6.40	CVS & AFC	Enzyme, regulatory	Nucleus
P27797	CALR_HUMAN	Calreticulin	180	40	48283.00	4.10	CVS & AFC	Chaperone/stress, antigen	Cytoplasm, endoplasmic reticulum
O43852	CALU_HUMAN	Calumenin	143	54	37198.00	4.30	CVS & AFC	Regulatory, transport	Cytoplasm, endoplasmic reticulum, Golgi
Q01518	CAP1_HUMAN	Adenylyl cyclase-associated protein 1	60	21	52222.00	9.10	CVS & AFC	Enzyme	Cytoplasm, nucleus
P47756	CAPZB_HUMAN	F-actin-capping protein subunit beta	139	46	31616.00	5.20	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P04040	CATA_HUMAN	Catalase	55	14	59947.00	7.00	CVS	Enzyme	Cytoplasm, peroxisome
P07858	CATB_HUMAN	Cathepsin B	56	23	38766.00	5.90	CVS & AFC	Enzyme	Plasma membrane, cytoplasm, lysosome
P52907	CAZA1_HUMAN	F-actin-capping protein subunit alpha-1	145	72	33073.00	5.40	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P47755	CAZA2_HUMAN	F-actin-capping protein subunit alpha-2	105	44	33157.00	5.50	CVS & AFC	Structural	Cytoplasm
P53701	CCHL_HUMAN	Cytochrome c-type heme lyase	57	28	30981.00	6.30	CVS	Enzyme, transport carrier	Cytoplasm, mitochondrion
P61604	CH10_HUMAN	10 kDa heat shock protein, mitochondrial	82	56	10925.00	9.40	CVS	Chaperone/stress, antigen	Cytoplasm, mitochondrion matrix
P10809	CH60_HUMAN	[Early-pregnancy factor, EPF] 60 kDa heat shock protein, mitochondrial	118	46	61187.00	5.60	CVS & AFC	Chaperone/stress	Cytoplasm, mitochondrion matrix
Q99653	CHP1_HUMAN	Calcium-binding protein p22	143	73	22442.00	4.80	CVS & AFC	Regulatory, transport	Cytoplasm
Q07065	CKAP4_HUMAN	Cytoskeleton-associated protein 4	124	27	66097.00	5.60	CVS & AFC	Structural	Cytoplasm, endoplasmic reticulum
O00299	CLIC1_HUMAN	Chloride intracellular channel protein 1	68	43	27248.00	4.90	CVS & AFC	Transport channel	Nucleus
Q9Y696	CLIC4_HUMAN	Chloride intracellular channel protein 4	70	46	28982.00	5.30	CVS & AFC	Chaperone/stress, regulatory, transport, channel	Plasma membrane
Q9Y224	CN166_HUMAN	UPF0568 protein C14orf166	55	20	28165.00	6.20	CVS	Regulatory	Cytoplasm, mitochondria
P12109	C06A1_HUMAN	Collagen alpha-1(VI) chain	65	9	109602.00	5.10	CVS & AFC	Structural	Extracellular
P12110	C06A2_HUMAN	Collagen alpha-2(VI) chain	58	10	109709.00	5.80	CVS	Structural	Extracellular
P23528	COF1_HUMAN	Cofilin-1	123	65	18719.00	9.10	CVS	Structural	Cytoplasm, mitochondria
P46108	CRK_HUMAN	Proto-oncogene C-crk	60	29	33867.00	5.30	CVS & AFC	Adaptor, transcription factor, protooncogene	Cytoplasm, nucleus
Q9BT78	CSN4_HUMAN	COP9 signalosome complex subunit 4	89	43	46525.00	5.50	CVS	Regulatory	Cytoplasm, nucleus
Q9Y310	CV028_HUMAN	UPF0027 protein C22orf28	77	39	55688.00	6.90	CVS	Unknown	Cytoplasm, cytoskeleton
O43402	CX4NB_HUMAN	Neighbor of COX4	65	29	24214.00	5.90	CVS	Unknown	Cytoplasm, mitochondrion, nucleus
Q9H8Q0	CXY02_HUMAN	Uncharacterized protein CXYorf2	55	28	20999.00	11.60	CVS	Unknown	Unknown

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P00167	CYB5_HUMAN	Cytochrome b5	55	33	15321.00	4.70	CVS	Enzyme	Cytoplasm, mitochondrial outer membrane, endoplasmic reticulum membrane
P04080	CYTB_HUMAN	Cystatin-B	48	45	11190.00	7.90	CVS	Enzyme	Cytoplasm, nucleus
P01037	CYTN_HUMAN	Cystatin-SN	97	63	16579.00	7.70	CVS	Enzyme, secretory	Extracellular
P06132	DCUP_HUMAN	Uroporphyrinogen decarboxylase	56	17	41103.00	5.70	CVS	Enzyme	Cytoplasm, cytosolic
O00571	DDX3X_HUMAN	ATP-dependent RNA helicase DDX3X	55	11	73597.00	6.80	CVS	Enzyme, RNA associated	Cytoplasm, nucleus
P00367	DHE3_HUMAN	Glutamate dehydrogenase 1, mitochondrial	157	41	61701.00	8.50	CVS & AFC	Enzyme, signaling neurotransmitter	Mitochondrion matrix
Q9UBS4	DJB11_HUMAN	DnaJ homolog subfamily B member 11	83	33	40774.00	5.80	CVS & AFC	Chaperone/stress, protein binding	Endoplasmic reticulum
P09622	DLDH_HUMAN	Dihydrolipoyl dehydrogenase, mitochondrial	64	26	54686.00	8.60	CVS & AFC	Enzyme	Mitochondrion
P30046	DOPD_HUMAN	D-dopachrome decarboxylase	57	43	12818.00	7.80	CVS	Enzyme	Cytoplasm
Q16555	DPYL2_HUMAN	Dihydropyrimidinase-related protein 2	103	25	62711.00	5.90	CVS	Enzyme, signaling	Cytoplasm, mitochondrion
Q13011	ECHI_HUMAN	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	55	19	36136.00	9.20	CVS & AFC	Enzyme, regulatory	Mitochondrion, peroxisomes
P30084	ECHM_HUMAN	Enoyl-CoA hydratase, mitochondrial	84	28	31823.00	9.40	CVS & AFC	Enzyme	Mitochondrion matrix
P68104	EF1A1_HUMAN	Elongation factor 1-alpha 1	64	19	50451.00	9.70	CVS	Translation, RNA associated	Cytoplasm, ribosome
P29692	EF1D_HUMAN	Elongation factor 1-delta	141	50	31217.00	4.80	CVS & AFC	Translation, RNA associated	Cytoplasm, ribosome, mitochondrion, nucleus
P13639	EF2_HUMAN	Elongation factor 2	150	28	96246.00	6.40	CVS & AFC	Translation, RNA associated	Cytoplasm, ribosome
P49411	EFTU_HUMAN	Elongation factor Tu, mitochondrial	171	55	49852.00	7.90	CVS & AFC	Regulatory, transcription factor	Cytoplasm, mitochondrion
Q13347	EIF3L_HUMAN	Eukaryotic translation initiation factor 3 subunit I	144	56	36878.00	5.30	CVS & AFC	RNA associated, translation, antioncogene	Cytoplasm, ribosome
P06733	ENOA_HUMAN	Alpha-enolase	265	64	47481.00	7.70	CVS & AFC	Enzyme, structural, antigen, antioncogene	Cytoplasm
P14625	ENPL_HUMAN	Endoplasmic	209	38	92696.00	4.60	CVS & AFC	Chaperone/stress, antigen, antiapoptotic protein	Endoplasmic reticulum
P84090	ERH_HUMAN	Enhancer of rudimentary homolog	57	38	12422.00	5.60	CVS	Regulatory, cell cycle	Cytoplasm, nucleus
P30040	ERP29_HUMAN	Endoplasmic reticulum protein ERp29	99	34	29032.00	7.50	CVS & AFC	Chaperone/stress, enzyme, transport	Cytoplasm, endoplasmic reticulum
P10768	ESTD_HUMAN	S-formylglutathione hydrolase	120	48	31956.00	6.60	CVS & AFC	Enzyme	Cytoplasm
P13804	ETFA_HUMAN	Electron transfer flavoprotein subunit alpha, mitochondrial	80	41	35400.00	9.50	CVS & AFC	Transport, carrier	Mitochondrion
P38117	ETFB_HUMAN	Electron transfer flavoprotein subunit beta	74	32	28054.00	9.20	CVS & AFC	Transport, carrier	Mitochondrion
O95571	ETHE1_HUMAN	ETHE1 protein, mitochondrial	89	36	28368.00	6.40	CVS	Regulatory	Cytoplasm, nucleus, mitochondrion

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P15311	EZRI_HUMAN	Ezrin	72	25	69484.00	5.90	CVS & AFC	Structural	Membrane, cytoplasm, cytoskeleton
Q14320	FA50A_HUMAN	Protein FAM50A	56	16	40216.00	6.40	CVS	DNA associated, transcriptional factor	Nucleus
P02751	FINC_HUMAN	Fibronectin	55	4	266034.00	5.40	CVS	Cell adhesion, cell motility	Secreted, cytoplasm
Q02790	FKBP4_HUMAN	FK506-binding protein 4	80	27	52057.00	5.20	CVS & AFC	Chaperone/stress, enzyme, regulatory, protooncogene	Cytoplasm, nucleus
P21333	FLNA_HUMAN	Filamin-A	74	9	283301.00	5.70	CVS	Structural, motor/contractile, signaling	Cytoplasm, cytoskeleton
P02792	FRIL_HUMAN	Ferritin light chain	72	42	20064.00	5.40	CVS	Storage, carrier	Cytoplasm, cytosol
Q16658	FSCN1_HUMAN	Fascin	179	53	55123.00	7.00	CVS & AFC	Structural	Cytoplasm, cytoskeleton
Q92945	FUBP2_HUMAN	Far upstream element-binding protein 2	98	18	73443.00	7.00	CVS	Regulatory, RNA associated, RNA splicing, transcription	Cytoplasm, nucleus
P07954	FUMH_HUMAN	Fumarate hydratase, mitochondrial	91	29	54773.00	9.40	CVS & AFC	Enzyme	Cytoplasm, mitochondrion
Q13283	G3BP1_HUMAN	Ras GTPase-activating protein-binding protein 1	69	25	52189.00	5.30	CVS	Enzyme	Cytoplasm, nucleus
P04406	G3P_HUMAN	Glyceroldehyde-3-phosphate dehydrogenase	136	60	36201.00	9.30	CVS & AFC	Enzyme	Cytoplasm, nucleus
Q14697	GANAB_HUMAN	Neutral alpha-glucosidase AB	167	33	107263.00	5.70	CVS & AFC	Enzyme	Endoplasmic reticulum, Golgi apparatus
P62873	GBBI_HUMAN	Guanine nucleotide-binding protein G(I)/G(S)/G(T)	94	37	38151.00	5.60	CVS	Signal transduction	Membrane
P62879	GBB2_HUMAN	subunit beta-1 Guanine nucleotide-binding protein G(I)/G(S)/G(T)	55	20	38048.00	5.60	CVS	Signal transduction	Unknown
P63244	GBLP_HUMAN	subunit beta-2 Guanine nucleotide-binding protein subunit beta-2-like 1	179	85	35511.00	8.90	CVS	Signal transduction	Membrane, cytoplasm
P52565	GDRI1_HUMAN	Rho GDP-dissociation inhibitor 1	82	38	23250.00	4.90	CVS	Signal transduction, anti-apoptosis	Cytoplasm
P06396	GELS_HUMAN	Gelsolin	93	27	86043.00	5.90	CVS & AFC	Regulatory, structural protein	Structural, cytoplasm, extracellular
Q9HC38	GLOD4_HUMAN	Glyoxalase domain-containing protein 4	48	16	35170.00	5.30	CVS	Enzyme	Mitochondrion
P62993	GRB2_HUMAN	Growth factor receptor-bound protein 2	102	38	25304.00	5.90	CVS	Regulatory, signal transduction, growth factor	Membrane, cytosol, Golgi apparatus
P38646	GRP75_HUMAN	Stress-70 protein, mitochondrial	180	49	73920.00	5.80	CVS & AFC	Chaperone/stress, regulatory, protooncogene factor, transcription	Mitochondrion
P11021	GRP78_HUMAN	78 kDa glucose-regulated protein	255	49	72402.00	4.90	CVS & AFC	Chaperone/stress	Endoplasmic reticulum
Q9HAV7	GRPE1_HUMAN	GrpE protein homolog 1, mitochondrial	76	31	24492.00	9.20	CVS	Chaperone/stress	Mitochondrion

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P48637	GSHB_HUMAN	Glutathione synthetase	80	29	52523.00	5.60	CVS & AFC	Enzyme	Cytoplasm, nucleus
P78417	GSTO1_HUMAN	Glutathione transferase omega-1	57	24	27833.00	6.30	CVS & AFC	Enzyme	Cytoplasm
P09211	GSTP1_HUMAN	Glutathione S-transferase P	158	70	23569.00	5.30	CVS & AFC	Enzyme	Cytoplasm
P49915	GUAH_HUMAN	GMP synthase [glutamine-hydrolyzing]	125	30	77408.00	6.40	CVS	Enzyme	Cytoplasm
Q99714	HCD2_HUMAN	3-hydroxyacyl-CoA dehydrogenase type-2	148	68	27134.00	9.10	CVS & AFC	Enzyme	Cytoplasm, mitochondrion, endoplasmic reticulum
Q6NVY1	HIBCH_HUMAN	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial	55	17	43797.00	9.20	CVS	Enzyme	Mitochondrion
P49773	HINT1_HUMAN	Histidine triad nucleotide-binding protein 1	60	71	13907.00	6.50	CVS & AFC	Enzyme, regulatory	Cytoplasm, nucleus
P09429	HMGBl_HUMAN	High mobility group protein B1	76	34	25049.00	5.50	CVS	Transcription factor, DNA binding	Nucleus
O14979	HNRDL_HUMAN	Heterogeneous nuclear ribonucleoprotein D-like	55	32	46580.00	10.00	CVS	Transcription regulator, RNA associated	Nucleus, cytoplasm
P31943	HNRH1_HUMAN	Heterogeneous nuclear ribonucleoprotein H	100	50	49484.00	5.90	CVS & AFC	RNA binding, RNA splicing	Nucleus
P55795	HNRH2_HUMAN	Heterogeneous nuclear ribonucleoprotein H2	67	33	49517.00	5.90	CVS & AFC	RNA binding, RNA splicing	Nucleus
P31942	HNRH3_HUMAN	Heterogeneous nuclear ribonucleoprotein H3	58	32	36960.00	6.40	CVS	RNA binding, RNA splicing	Nucleus
P61978	HNRPK_HUMAN	Heterogeneous nuclear ribonucleoprotein K	67	25	51230.00	5.30	CVS	RNA binding, RNA splicing, signal transduction	Nucleus, cytoplasm
P14866	HNRPL_HUMAN	Heterogeneous nuclear ribonucleoprotein L	129	34	60719.00	6.70	CVS & AFC	RNA binding, RNA splicing	Nucleus, cytoplasm
O60506	HNRPQ_HUMAN	Heterogeneous nuclear ribonucleoprotein Q	55	16	69788.00	9.10	CVS	RNA binding, RNA splicing	Cytoplasm, nucleus, endoplasmic reticulum
P08238	HS90B_HUMAN	Heat shock protein HSP 90-beta	156	36	83554.00	4.80	CVS	Chaperone/stress	Cytoplasm
P08107	HSP71_HUMAN	Heat shock 70 kDa protein 1	166	36	70294.00	5.40	CVS & AFC	Chaperone/stress, anti-apoptosis	Cytoplasm, mitochondrion, endoplasmic reticulum, nucleus
P11142	HSP7C_HUMAN	Heat shock cognate 71 kDa protein	179	37	71082.00	5.20	CVS & AFC	Chaperone/stress	Cytoplasm, nucleus
P04792	HSPB1_HUMAN	Heat shock protein beta-1	154	71	22826.00	6.00	CVS	Chaperone/stress	Cytoplasm, cytoskeleton, nucleus
Q9Y4L1	HYOU1_HUMAN	Hypoxia up-regulated protein 1	76	11	111494.00	5.00	CVS & AFC	Chaperone/stress, protooncogene	Cytoplasm, endoplasmic reticulum
P50213	IDH3A_HUMAN	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	96	20	40022.00	6.50	CVS & AFC	Enzyme	Mitochondrion
O75874	IDHC_HUMAN	Isocitrate dehydrogenase [NADP] cytoplasmic	112	46	46915.00	6.60	CVS & AFC	Enzyme	Cytoplasm, peroxisome
P12268	IMDH2_HUMAN	Inosine-5'-monophosphate dehydrogenase 2	61	20	56226.00	6.50	CVS	Enzyme	Cytoplasm

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
Q16891	IMMT_HUMAN	Mitochondrial inner membrane protein	94	17	84026.00	6.10	CVS & AFC	Structural	Mitochondrion
Q15181	IPYR_HUMAN	Inorganic pyrophosphatase	128	55	33095.00	5.50	CVS & AFC	Enzyme	Cytoplasm
P26440	IVD_HUMAN	Isovaleryl-CoA dehydrogenase, mitochondrial	55	18	46803.00	9.30	CVS & AFC	Enzyme	Mitochondrion
P35527	K1C9_HUMAN	Keratin, type I cytoskeletal 9	163	44	62320.00	5.10	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P05787	K2C8_HUMAN	Keratin, type II cytoskeletal 8	118	38	53671.00	5.40	CVS & AFC	Structural	Cytoplasm, cytoskeleton,
P54819	KAD2_HUMAN	Adenylate kinase isoenzyme 2, mitochondrial	71	28	26689.00	8.90	CVS & AFC	Enzyme	Cytoplasm, mitochondrion
P30085	KCY_HUMAN	UMP-CMP kinase	55	23	22436.00	5.30	CVS & AFC	Enzyme	Cytoplasm, nucleus
P14618	KPYM_HUMAN	Pyruvate kinase isozymes M1/M2	163	47	58470.00	9.00	CVS & AFC	Enzyme	Cytoplasm,cytosol
P00338	LDHA_HUMAN	L-lactate dehydrogenase A chain	139	43	36950.00	9.30	CVS & AFC	Enzyme	Cytoplasm,cytosol
P07195	LDHB_HUMAN	L-lactate dehydrogenase B chain	125	44	36900.00	5.70	CVS & AFC	Enzyme	Cytoplasm,cytosolic
P09382	LEGI_HUMAN	Galectin-1	115	74	15048.00	5.20	CVS & AFC	Adhesion, signal transduction	Cytoplasm
P02545	LMNA_HUMAN	Lamin-A/C	238	48	74380.00	6.60	CVS & AFC	Structural	Cytoplasm, nucleus, endoplasmic reticulum, cytoskeleton
Q03252	LMNB2_HUMAN	Lamin-B2	191	44	67762.00	5.20	CVS	Structural	Nucleus, intermediate filament
O75608	LYP1_HUMAN	Acyl-protein thioesterase 1	55	20	24996.00	6.30	CVS	Enzyme	Cytoplasm
P26038	MOES_HUMAN	Moesin	109	39	67892.00	6.00	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P19105	MRLC3_HUMAN	Myosin regulatory light chain MRLC3	86	63	19839.00	4.50	CVS & AFC	Binding	Cytoplasm
Q13126	MTAP_HUMAN	S-methyl-5'-thioadenosine phosphorylase	94	49	31729.00	6.90	CVS	Enzyme	Cytoplasm
Q14764	MVP_HUMAN	Major vault protein	79	18	99551.00	5.20	CVS & AFC	Signal transduction, structural, RNA associated, transport	Cytoplasm,nucleus
P35579	MYH9_HUMAN	Myosin-9	58	6	227646.00	5.40	CVS	Structural, motor/contractile, transport	Cytoplasm
P60660	MYL6_HUMAN	Myosin light polypeptide 6	92	45	17090.00	4.40	CVS	Structural	Cytoplasm
P24844	MYL9_HUMAN	Myosin regulatory light chain 2, smooth muscle isoform	66	39	19871.00	4.60	CVS & AFC	Regulatory, structural	Cytoplasm
Q13765	NACA_HUMAN	Nascent polypeptide-associated complex subunit alpha	55	25	23370.00	4.40	CVS	Transcription, translation, transport	Cytoplasm, mitochondrion, nucleus
P43490	NAMPT_HUMAN	Nicotinamide phosphoribosyltransferase	139	36	55772.00	6.80	CVS & AFC	Enzyme	Cytoplasm,cytosolic
P15531	NDKA_HUMAN	Nucleoside diphosphate kinase A	122	70	17309.00	5.80	CVS & AFC	Enzyme	Cytoplasm, nucleus
P22392	NDKB_HUMAN	Nucleoside diphosphate kinase B	82	76	17401.00	9.40	CVS & AFC	Enzyme	Cytoplasm, nucleus
O96000	NDUBA_HUMAN	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	70	38	21048.00	9.40	CVS	Enzyme, transport	Mitochondrion, membrane

Table I. *continued*

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P28331	NDUS1_HUMAN	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	118	32	80443.00	5.80	CVS & AFC	Enzyme	Mitochondrion
P19404	NDUV2_HUMAN	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	63	26	27659.00	9.20	CVS & AFC	Enzyme	Mitochondrion
P06748	NPM_HUMAN	Nucleophosmin	64	28	32726.00	4.50	CVS	Protooncogene, transcription, transport, regulation	Nucleus, nucleolus
Q02818	NUCB1_HUMAN	Nucleobindin-1 precursor	144	50	53846.00	5.00	CVS	DNA binding, binding	Golgi apparatus, cytoplasm
P04181	OAT_HUMAN	Ornithine aminotransferase, mitochondrial	60	24	48846.00	6.60	CVS & AFC	Enzyme	Mitochondrion
P36957	ODO2_HUMAN	Dihydrodipicolylsine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	79	21	48952.00	9.80	CVS & AFC	Enzyme	Mitochondrion
P08559	ODPA_HUMAN	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	67	28	43952.00	9.30	CVS & AFC	Enzyme	Mitochondrion
Q9NTK5	OLA1_HUMAN	Obg-like ATPase 1	102	40	44943.00	8.60	CVS & AFC	Regulatory	Cytoplasm
Q32P28	P3HI_HUMAN	Prolyl 3-hydroxylase 1	72	14	84196.00	4.90	CVS	Enzyme, regulatory	Endoplasmic reticulum, extracellular matrix, secreted
P13674	P4HA1_HUMAN	Prolyl 4-hydroxylase subunit alpha-1	109	37	61296.00	5.60	CVS & AFC	Enzyme	Endoplasmic reticulum
P68402	PA1B2_HUMAN	Platelet-activating factor acetylhydrolase IB subunit beta	55	24	25724.00	5.50	CVS	Enzyme	Cytoplasm
Q9UQ80	PA2G4_HUMAN	Proliferation-associated protein 2G4	55	13	44101.00	6.10	CVS & AFC	Transcription, translation, cell proliferation, regulatory	Cytoplasm, nucleus, nucleolus
P05121	PA1I1_HUMAN	Plasminogen activator inhibitor 1	127	49	45088.00	6.80	CVS	Enzyme, regulatory	Extracellular
Q99497	PARK7_HUMAN	Protein DJ-1	103	51	20050.00	6.40	CVS	Chaperone, protooncogene, signaling	Cytoplasm.nucleus
Q15365	PCBP1_HUMAN	Poly(rC)-binding protein 1	110	44	37987.00	6.80	CVS & AFC	RNA associated	Cytoplasm, nucleus
O75340	PDCD6_HUMAN	Programmed cell death protein 6	55	26	21912.00	5.00	CVS	Regulatory, binding	Membrane, cytoplasm, nucleus endoplasmic reticulum, nuclear membrane
P07237	PDIA1_HUMAN	Protein disulfide-isomerase	255	63	57480.00	4.60	CVS & AFC	Enzyme, protooncogene	Cytoplasm, endoplasmic reticulum
P30101	PDIA3_HUMAN	Protein disulfide-isomerase A3	208	53	57146.00	5.90	CVS & AFC	Enzyme, chaperone/stress	Endoplasmic reticulum
Q15084	PDIA6_HUMAN	Protein disulfide-isomerase A6	100	40	48490.00	4.80	CVS & AFC	Enzyme, chaperone/stress	Endoplasmic reticulum
Q9BY77	PDIP3_HUMAN	Polymerase delta-interacting protein 3	55	13	46289.00	10.60	CVS	Unknown/unspecified	Nucleus
O00151	PDL1I1_HUMAN	PDZ and LIM domain protein 1	187	62	36505.00	6.60	CVS & AFC	Adaptor, transport	Cytoplasm, cytoskeleton
O00764	PDXK_HUMAN	Pyridoxal kinase	59	25	35308.00	5.70	CVS & AFC	Enzyme	Cytoplasm
P30086	PEBP1_HUMAN	Phosphatidylethanolamine-binding protein 1	97	71	21158.00	7.80	CVS & AFC	Protein binding, regulatory	Cytoplasm

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P18669	PGAM1_HUMAN	Phosphoglycerate mutase 1	127	51	28900.00	6.80	CVS & AFC	Enzyme	Cytoplasm, cytosol
P00558	PGK1_HUMAN	Phosphoglycerate kinase 1	204	66	44985.00	9.20	CVS & AFC	Enzyme	cytoplasm
P35232	PHB_HUMAN	Prohibitin	142	74	29843.00	5.50	CVS & AFC	Proliferation, signal transduction, transcription, regulatory, antioncogene	Cytoplasm, mitochondrion, nucleus
Q99623	PHB2_HUMAN	Prohibitin-2	87	41	33276.00	10.20	CVS	Transcription, regulatory, receptor nuclear	Cytoplasm, mitochondrion, nucleus
Q9NRX4	PHP14_HUMAN	14 kDa phosphohistidine phosphatase	81	40	13995.00	5.60	CVS	Enzyme	Cytoplasm
P22061	PIMT_HUMAN	Protein-L-isoaspartate (D-aspartate) O-methyltransferase	58	39	24806.00	6.90	CVS	Enzyme, protein repairer	Cytoplasm, endoplasmic reticulum
Q02809	PLOD1_HUMAN	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	72	14	84068.00	6.50	CVS	Enzyme, regulation	Cytoplasm, endoplasmic reticulum
O00469	PLOD2_HUMAN	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	84	22	85373.00	6.20	CVS & AFC	Enzyme, regulation	Cytoplasm, endoplasmic reticulum
O60568	PLOD3_HUMAN	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	80	18	85302.00	5.60	CVS & AFC	Enzyme	Cytoplasm, endoplasmic reticulum
P62937	PPIA_HUMAN	Peptidyl-prolyl cis-trans isomerase A	114	74	18229.00	9.00	CVS	Enzyme, immunity/defense	Cytoplasm, nucleus
P23284	PPIB_HUMAN	Peptidyl-prolyl cis-trans isomerase B	120	54	22785.00	9.90	CVS	Enzyme, immunity/defense	Endoplasmic reticulum
Q969G5	PRDBP_HUMAN	Protein kinase C delta-binding protein	70	23	27625.00	5.80	CVS	Regulatory, antioncogene	Nucleus
Q06830	PRDX1_HUMAN	Peroxiredoxin-1	181	67	22324.00	9.20	CVS & AFC	Enzyme	Cytoplasm,nucleus
P32119	PRDX2_HUMAN	Peroxiredoxin-2	89	45	22049.00	5.60	CVS & AFC	Enzyme	Cytoplasm
P30048	PRDX3_HUMAN	Thioredoxin-dependent peroxide reductase, mitochondrial	58	21	28017.00	8.90	CVS & AFC	Enzyme, regulatory	Mitochondrion
Q13162	PRDX4_HUMAN	Peroxiredoxin-4	122	61	30749.00	5.80	CVS	Enzyme	Cytoplasm
P30041	PRDX6_HUMAN	Peroxiredoxin-6	174	74	25133.00	6.00	CVS & AFC	Enzyme, regulatory	Cytoplasm, lysosome
P07737	PROF1_HUMAN	Profilin-1	102	65	15216.00	9.40	CVS & AFC	Structural, regulatory	Cytoplasm, cytoskeleton
P62333	PRS10_HUMAN	26S protease regulatory subunit S10B	151	57	44430.00	7.80	CVS & AFC	Chaperone/stress, enzyme	Cytoplasm, nucleus
P62191	PRS4_HUMAN	26S protease regulatory subunit 4	95	34	49325.00	5.80	CVS	Chaperone/stress, enzyme	Cytoplasm, nucleus
P35998	PRS7_HUMAN	26S protease regulatory subunit 7	148	41	49002.00	5.60	CVS & AFC	Chaperone/stress , enzyme	Cytoplasm, cytosolic
P62195	PRS8_HUMAN	26S protease regulatory subunit 8	69	24	45768.00	7.80	CVS	Chaperone/stress, enzyme, regulatory	Cytoplasm, nucleus
P25786	PSA1_HUMAN	Proteasome subunit alpha type-1	75	39	29822.00	6.20	CVS & AFC	Enzyme	cytoplasm, nucleus
P25787	PSA2_HUMAN	Proteasome subunit alpha type-2	56	44	25996.00	7.70	CVS & AFC	Enzyme	Cytoplasm, nucleus
P25789	PSA4_HUMAN	Proteasome subunit alpha type-4	62	26	29750.00	8.70	CVS & AFC	Enzyme	Cytoplasm, cytosolic
P28066	PSA5_HUMAN	Proteasome subunit alpha type-5	74	44	26565.00	4.60	CVS & AFC	Enzyme	Cytoplasm, nucleus
P60900	PSA6_HUMAN	Proteasome subunit alpha type-6	73	38	27838.00	6.40	CVS	Enzyme	Cytoplasm, nucleus
O14818	PSA7_HUMAN	Proteasome subunit alpha type-7	85	49	28041.00	9.30	CVS	Enzyme	Cytoplasm, nucleus
P49721	PSB2_HUMAN	Proteasome subunit beta type-2	66	45	22993.00	6.60	CVS & AFC	Enzyme	Cytoplasm, nucleus
P49720	PSB3_HUMAN	Proteasome subunit beta type-3	71	35	23219.00	6.20	CVS	Enzyme	Cytoplasm, nucleus

Table I. *continued*

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
P28070	PSB4_HUMAN	Proteasome subunit beta type-4	56	21	29243.00	5.60	CVS	Enzyme	Cytoplasm, nucleus
Q99436	PSB7_HUMAN	Proteasome subunit beta type-7	59	30	30288.00	8.80	CVS	Enzyme	Cytoplasm, nucleus
O75832	PSD10_HUMAN	26S proteasome non-ATPase regulatory subunit 10	76	32	24697.00	5.70	CVS	Regulatory , protooncogene	Extracellular, cytoplasm
Q9UNM6	PSD13_HUMAN	26S proteasome non-ATPase regulatory subunit 13	76	36	43176.00	5.50	CVS & AFC	Enzyme	Cytoplasm, cytosolic
Q06323	PSME1_HUMAN	Proteasome activator complex subunit 1	55	22	28876.00	5.70	CVS & AFC	Chaperone/stress, regulatory	Cytosol, nucleus
P31939	PUR9_HUMAN	Bifunctional purine biosynthesis protein PURH	107	27	65089.00	6.30	CVS & AFC	Enzyme	Cytoplasm
P31930	QCR1_HUMAN	Cytochrome b-c1 complex subunit, mitochondrial	215	60	53297.00	5.90	CVS & AFC	Enzyme	Cytoplasm, mitochondrion
P62826	RAN_HUMAN	GTP-binding nuclear protein Ran	130	53	24579.00	7.80	CVS	Regulatory, DNA associated, transport	Cytoplasm, nucleus, chromatin/chromosome, nuclear envelope, nuclear pore
Q15293	RCN1_HUMAN	Reticulocalbin-1	76	35	38866.00	4.70	CVS & AFC	Regulatory	Cytoplasm, endoplasmic reticulum
Q96D15	RCN3_HUMAN	Reticulocalbin-3	106	50	37470.00	4.60	CVS & AFC	Signaling	Cytoplasm, endoplasmic reticulum
P26373	RL13_HUMAN	60S ribosomal protein L13	55	19	24304.00	12.20	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
Q07020	RL18_HUMAN	60S ribosomal protein L18	76	38	21735.00	12.30	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
P36578	RL4_HUMAN	60S ribosomal protein L4	60	31	47953.00	11.80	CVS & AFC	RNA associated	Cytoplasm, cytosolic, ribosome
Q02878	RL6_HUMAN	60S ribosomal protein L6	55	17	32765.00	11.10	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
P18124	RL7_HUMAN	60S ribosomal protein L7	66	30	29264.00	11.10	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
P05388	RLA0_HUMAN	60S acidic ribosomal protein P0	140	57	34423.00	5.60	CVS & AFC	RNA associated	Cytoplasm, cytosolic, ribosome
P05387	RLA2_HUMAN	60S acidic ribosomal protein P2	74	69	11658.00	4.20	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
Q9NYK5	RM39_HUMAN	Mitochondrial 39S ribosomal protein L39	55	29	39210.00	8.70	CVS	Structural protein, RNA associated	Cytoplasm, mitochondrion
P09651	ROA1_HUMAN	Heterogeneous nuclear ribonucleoprotein A1	128	50	38936.00	9.70	CVS & AFC	RNA associated	Nucleus, nucleoplasm
P22626	ROA2_HUMAN	Heterogeneous nuclear ribonucleoproteins A2/B1	136	57	37464.00	9.30	CVS & AFC	RNA associated	Nucleus
Q99729	ROAA_HUMAN	Heterogeneous nuclear ribonucleoprotein A/B	55	15	36316.00	8.80	CVS	mRNA binding, protein binding	Cytoplasm, nucleus
P25398	RS12_HUMAN	40S ribosomal protein S12	55	28	14859.00	6.40	CVS	RNA associated	Cytoplasm, cytosolic, ribosome
P23396	RS3_HUMAN	40S ribosomal protein S3	71	43	26842.00	10.30	CVS	Enzyme , RNA associated	Cytoplasm, cytosolic, ribosome, nucleus
P62701	RS4X_HUMAN	40S ribosomal protein S4, X isoform	58	22	29807.00	10.80	CVS	Structural, RNA associated	Cytoplasm, cytosolic, ribosome
P08865	RSSA_HUMAN	40S ribosomal protein SA	95	43	32947.00	4.60	CVS & AFC	Adhesion , signaling , receptor	Cytoplasm, cytosolic, ribosome
P82650	RT22_HUMAN	Mitochondrial 28S ribosomal protein S22	55	26	41425.00	8.70	CVS & AFC	Structural, RNA associated	Cytoplasm, mitochondrion
Q9Y265	RUVB1_HUMAN	RuvB-like 1	58	16	50538.00	6.00	CVS	Enzyme , DNA associated, antigen	Cytoplasm, nucleus, nucleoplasm, membrane
O00560	SDCB1_HUMAN	Syntenin-1	55	25	32595.00	7.90	CVS	Regulatory	Cytoplasm, endoplasmic reticulum, cytoskeleton, melanosome, nucleus, cell membrane

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
Q99470	SDF2_HUMAN	Stromal cell-derived factor 2	55	28	23240.00	7.00	CVS	Enzyme , secretory	Extracellular
Q9HCN8	SDF2L_HUMAN	Stromal cell-derived factor 2-like protein 1	87	52	23812.00	6.60	CVS	Structural	Cytoplasm, endoplasmic reticulum
Q9NVA2	SEPI1_HUMAN	Septin-11	163	44	49652.00	6.40	CVS & AFC	Regulatory, cell cycle, binding	Cytoplasm, nucleus
Q15019	SEPT2_HUMAN	Septin-2	56	19	41689.00	6.10	CVS & AFC	Motor/contractile, regulatory, cell cycle	Cytoplasm, nucleus
O43175	SERA_HUMAN	D-3-phosphoglycerate dehydrogenase	102	26	57356.00	6.30	CVS	Enzyme , transport carrier	Cytoplasm, mitochondrion, endoplasmic reticulum
P50454	SERP1_HUMAN	Serpin H1 precursor	131	57	46525.00	9.30	CVS & AFC	Chaperone, regulatory	Cytoplasm, endoplasmic reticulum
Q07955	SFRS1_HUMAN	Splicing factor, arginine/serine-rich 1	93	39	27842.00	10.80	CVS & AFC	RNA associated, protooncogene	Cytoplasm, nucleus
Q16629	SFRS7_HUMAN	serine-rich 1 Splicing factor, arginine/serine-rich 7	55	16	27578.00	12.40	CVS	RNA associated, binding	Nucleus
Q9Y371	SHLB1_HUMAN	Endophilin-B1	55	14	41056.00	5.70	CVS	Regulatory	Cytoplasm, Golgi apparatus, Mitochondrion
Q9NR45	SIAS_HUMAN	Sialic acid synthase	91	34	40738.00	6.30	CVS	Enzyme	Cytoplasm
Q7KZF4	SND1_HUMAN	Staphylococcal nuclease domain-containing protein 1	162	35	102618.00	6.80	CVS	Antigen	Nucleus
P00441	SODC_HUMAN	Superoxide dismutase [Cu-Zn]	67	47	16154.00	5.70	CVS	Chaperone/stress, enzyme	Cytoplasm, endoplasmic reticulum, cytosolic, extracellular, mitochondrion, nucleus, peroxisome
P04179	SODM_HUMAN	Superoxide dismutase [Mn], mitochondrial	101	68	24878.00	9.10	CVS & AFC	Enzyme	Cytoplasm, mitochondrion
Q04837	SSB_HUMAN	Single-stranded DNA-binding protein, mitochondrial	77	53	17249.00	9.90	CVS	Regulatory	Mitochondrion
P31948	STIP1_HUMAN	Stress-induced-phosphoprotein 1	197	39	63227.00	6.40	CVS & AFC	Adaptor, chaperone/stress	Cytoplasm, nucleus, Golgi apparatus
Q9UIJ1	STML2_HUMAN	Stomatatin-like protein 2	70	33	38624.00	7.70	CVS	Regulatory, transport	Membrane, cytoplasm, mitochondrion
P16949	STMN1_HUMAN	Stathmin	58	34	17292.00	5.70	CVS	Regulatory, protooncogene, signaling	Cytoplasm, Golgi, cytosolic, granule, cytoskeleton, microtubule, nucleus, nucleoplasm,
P41250	SYG_HUMAN	Glycyl-tRNA synthetase	101	26	83828.00	6.70	CVS & AFC	Enzyme	nuclear bodies,coiled bodies (Cajal)
Q01995	TAGL_HUMAN	Transgelin	111	55	22653.00	9.40	CVS	Motor/contractile, regulatory, binding	Cytoplasm, mitochondrion
P37802	TAGL2_HUMAN	Transgelin-2	145	81	22548.00	9.30	CVS & AFC	Unknown/unspecified, transcription factor	Membrane, cytoplasm, cytoskeleton, nuclear
P37837	TALDO_HUMAN	Transaldolase	55	16	37688.00	6.40	CVS	Enzyme	Cytoplasm
P68363	TBA1B_HUMAN	Tubulin alpha-1B chain	119	41	50804.00	4.80	CVS	Structural	Cytoplasm, cytoskeleton, microtubule
Q9BQE3	TBA1C_HUMAN	Tubulin alpha-1C chain	109	42	50548.00	4.80	CVS	Structural	Cytoplasm, mitochondrion
Q13885	TBB2A_HUMAN	Tubulin beta-2A chain	197	59	50274.00	4.60	CVS	Structural	Cytoplasm, microtubule
P68371	TBB2C_HUMAN	Tubulin beta-2C chain	157	48	50255.00	4.60	CVS	Structural, cell motion	Cytoplasm, microtubule
P04350	TBB4_HUMAN	Tubulin beta-4 chain	77	18	50010.00	4.60	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P07437	TBB5_HUMAN	Tubulin beta chain	180	55	50095.00	4.60	CVS & AFC	Structural	Cytoplasm

Table I. continued

Table I.

SwissProt Acc. No	Accession name	Title	Mascot score	Coverage	Protein MW	pI-Value	Reproductive fluid	Function	Localization
O75347	TBCA_HUMAN	Tubulin-specific chaperone A	56	37	12904.00	5.10	CVS	Chaperone/stress	Cytoplasm, cytoskeleton, microtubule
P17987	TCPA_HUMAN	T-complex protein 1 subunit alpha	55	10	60819.00	5.70	CVS & AFC	Chaperone	Cytoplasm
P78371	TCPB_HUMAN	T-complex protein 1 subunit beta	147	47	57794.00	6.00	CVS & AFC	Chaperone/stress	Cytoplasm
P48643	TCPE_HUMAN	T-complex protein 1 subunit epsilon	55	15	60089.00	5.30	CVS & AFC	Chaperone/stress	Cytoplasm
P40227	TCPZ_HUMAN	T-complex protein 1 subunit zeta	66	21	58444.00	6.20	CVS & AFC	Chaperone/stress	Cytoplasm
P13693	TCTP_HUMAN	Translationally-controlled tumor protein	75	56	19697.00	4.70	CVS	Enzyme, protooncogene	Extracellular, cytoplasm
P55072	TERA_HUMAN	Transitional endoplasmic reticulum ATPase	178	44	89950.00	5.00	CVS & AFC	Chaperone/stress, structural protein	Cytoplasm, endoplasmic reticulum, Golgi, nucleus
P29401	TKT_HUMAN	Transketolase	111	26	68519.00	8.50	CVS & AFC	Enzyme	Cytosol
O96008	TOM40_HUMAN	Mitochondrial import receptor subunit TOM40 homolog	78	40	38211.00	7.00	CVS & AFC	Transport channel	Mitochondrion
P60174	TPIS_HUMAN	Triosephosphate isomerase	172	63	26938.00	6.50	CVS	Enzyme	Cytoplasm, cytosolic
P09493	TPM1_HUMAN	Tropomyosin alpha-1 chain	101	34	32746.00	4.50	CVS & AFC	Structural, motor/contractile	Cytoplasm, cytoskeleton
P07951	TPM2_HUMAN	Tropomyosin beta chain	94	23	32945.00	4.50	CVS & AFC	Structural, motor/contractile	Cytoplasm, cytoskeleton
P06753	TPM3_HUMAN	Tropomyosin alpha-3 chain	70	31	32856.00	4.50	CVS & AFC	Structural, motor/contractile	Cytoplasm, cytoskeleton, microfilament
P67936	TPM4_HUMAN	Tropomyosin alpha-4 chain	101	34	28619.00	4.50	CVS	Structural, motor/contractile	Cytoplasm, cytoskeleton, microfilament
P07996	TSPI_HUMAN	Thrombospondin-1 precursor	140	24	133291.00	4.60	CVS	Adhesion, signaling	Membrane, extracellular matrix
Q61BS0	TWF2_HUMAN	Twinfilin-2	67	23	39751.00	6.40	CVS	Regulatory, binding	Cytoplasm, cytoskeleton
O95881	TXD12_HUMAN	Thioredoxin domain-containing protein 12	70	34	19365.00	5.10	CVS	Enzyme, transport	Cytoplasm, endoplasmic reticulum
Q8NBS9	TXND5_HUMAN	Thioredoxin domain-containing protein 5	55	18	48283.00	5.60	CVS	Regulatory	Cytoplasm, endoplasmic reticulum
P61088	UBE2N_HUMAN	Ubiquitin-conjugating enzyme E2 N	73	39	17184.00	6.20	CVS	Enzyme, regulatory	Cytoplasm, mitochondrion, nucleus
P09936	UCHL1_HUMAN	Ubiquitin carboxyl-terminal hydrolase isozyme L1	123	60	25151.00	5.20	CVS & AFC	Enzyme, transcription	Cytoplasm, nucleus
P21796	VDAC1_HUMAN	Voltage-dependent anion-selective channel protein 1	153	61	30868.00	9.20	CVS & AFC	Transport, channel, regulatory	Cytoplasm, mitochondrion, membrane
P45880	VDAC2_HUMAN	Voltage-dependent anion-selective channel protein 2	116	55	32060.00	8.70	CVS & AFC	Transport channel	Mitochondrion
Q9Y277	VDAC3_HUMAN	Voltage-dependent anion-selective channel protein 3	55	25	30981.00	9.50	CVS & AFC	Transport channel	Mitochondrion
P08670	VIME_HUMAN	Vimentin	303	69	53676.00	4.90	CVS & AFC	Structural	Cytoplasm, cytoskeleton, intermediate filament
P18206	VINC_HUMAN	Vinculin	114	17	124292.00	5.40	CVS & AFC	Adhesion, structural	Cytoplasm, cytoskeleton
O75083	WDRI_HUMAN	WD repeat-containing protein 1	157	41	66836.00	6.20	CVS & AFC	Structural	Cytoplasm, cytoskeleton
P67809	YBOX1_HUMAN	Nuclease-sensitive element-binding protein 1	55	18	35903.00	0.00	CVS	RNA associated, transcription factor	Cytoplasm, nucleus
Q9Y2T7	YBOX2_HUMAN	Y-box-binding protein 2	55	15	38552.00	11.40	CVS	Regulatory, traslation	Cytoplasm, nucleus

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Received April 13, 2011

Revised July 7, 2011

Accepted July 8, 2011