APPLICATION NOTE 65957

Targeted proteomic analysis of human plasma on a discovery scale

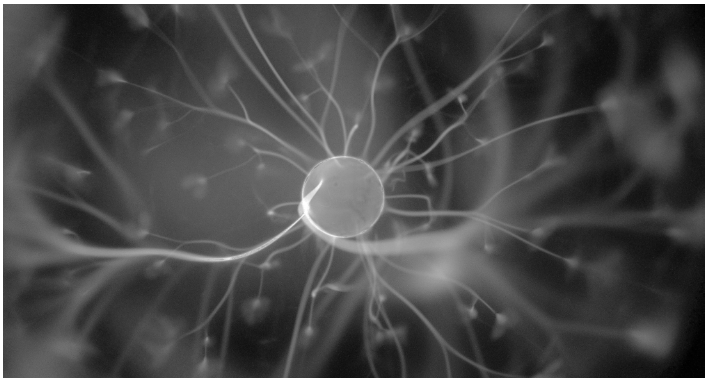
PQ500 and TSQ Altis triple quadrupole mass spectrometer

Introduction

As plasma interacts with every organ in the body it's protein composition can reflect the physiological state of these organs. This makes plasma a prominent and attractive source of protein biomarkers, which have shown utility for both disease prevention and progression monitoring.

Commonly detection and quantification of plasma proteins are performed with immunoassays which inherently lack specificity, have low multiplexing capabilities and very limited inter-assay comparability for different targets[1]. Mass spectrometry-based targeted proteomics can overcome these challenges by allowing the simultaneous measurement of hundreds of proteins in a single experiment.

Here, we present and characterize a robust clinical research workflow, that utilizes the full capabilities of the latest generation of triple quadrupole mass spectrometers. Coupled to a capillary flow LC set-up it is possible to reliably monitor more than 500 proteins in human plasma, in 70 minutes measurement time. Accurate quantification can be achieved by spiking in Biognosys' PQ500 reference peptide mix. This kit contains 804 carefully selected stable-isotope labeled standard peptides (SIS), representing more than 500 human proteins commonly found in plasma. For scheduled method set-up and data analysis, SpectroDive, Biognosys' targeted proteomics software, is used.



Methods

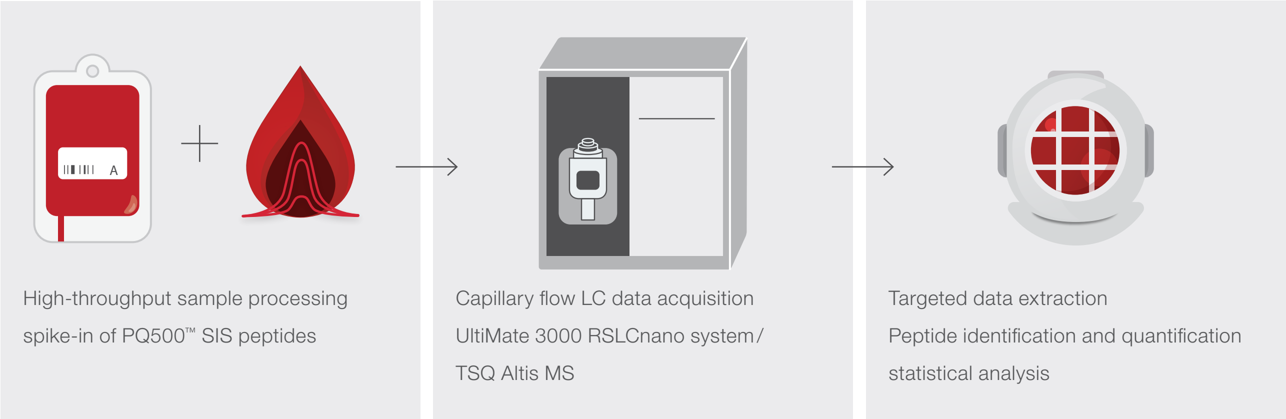
A pool of human plasma samples, as well as individual plasma samples, from a cohort of cancer patients (n = 21) and matched healthy donors (n = 15) were processed according to Biognosys' Sample Preparation Kit Pro for high-throughput proteomics. Injection-ready samples were spiked with PQ500 reference peptides before data acquisition. All samples were acquired on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer coupled to a Thermo Scientific™ UltiMate™ 3000 RSLCnano system equipped with a capillary flow-meter. Chromatographic separation was performed on a 15 cm column with CSH-C18 beads (1.7 µm; Waters) in direct injection mode without a trap column. For the cancer cohort analytical runs, a linear 60 min gradient ranging from 1 % — 40 % acetonitrile with a flow rate of 5 µl / minute was used and runs were acquired in a randomized order.

Scheduled method set-up and targeted data extraction was performed with SpectroDive, based on fragment ion profile scores, with the PQ500 assay panel. This pre-defined assay panel, derived from experimental data used in the development of PQ500, was refined on this specific LC-MS set-up with SpectroDive, using three transitions per peptide for acquisition and analysis.

Results

SpectroDive utilizes all SIS peptides from the PQ500 mix as anchor points for the scheduling of the SRM/MRM method (Figure 2a). This methodology accounts for short phases of non-linearity in the gradient, leading to a median offset of measured retention times compared to scheduled retention times of 25 seconds and optimized scheduling windows of 2.5 minutes (= 4 % of the analytical gradient length). PQ500 reference peptides were also selected to be evenly spread across the elution space. This enables an unprecedented monitoring of 4857 total transitions with a maximum of 480 concurrent transitions. Using a 2.5-second cycle time results in an average of five data-points-per-peak and ~ 3 ms dwell-time per transition (Figure 2b). This acquisition speed and short overhead between analytes could not be obtained with a previous generation of triple quadrupole mass spectrometers. In total, 756 out of the 804 PQ500 SIS peptides could be reproducibly detected across the runs, with missing peptides showing high variation in elution time or high affinity to the column material leading to low signal intensity.

This optimized method was applied to individual plasma samples from a cohort of cancer patients (n = 21) and matched healthy donors (n = 15) and data filtered to a 1 % false-discovery rate (FDR) by SpectroDive. More than 300 plasma proteins could be quantified in each individual sample (Figure 3a). The quantitative precision of the assays was determined by triplicate injections from the pooled plasma sample resulting in a median coefficient of variation of 5.4 % and 271 proteins being quantified with a CV below 20 % (Figure 3b). By using SpectroDive's integrated tools for statistical testing between the cancer and healthy control group, biologically relevant inflammation markers were discovered that were significantly enriched in the cancer group (Figure 3c). For example, C-reactive protein (CRP) and S100-A9 are known secreted marker proteins that when elevated can be indicative of certain types of cancer.


High-throughput sample processing
spike-in of PQ500™ SIS peptides

Capillary flow LC data acquisition
UltiMate 3000 RSLCnano system/
TSQ Altis MS

Targeted data extraction
Peptide identification and quantification
statistical analysis

Figure 1. Human plasma analysis workflow

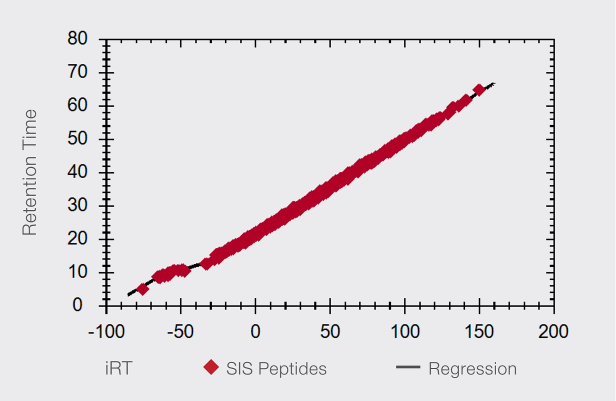


Figure 2a. RT calibration based on all SIS peptides

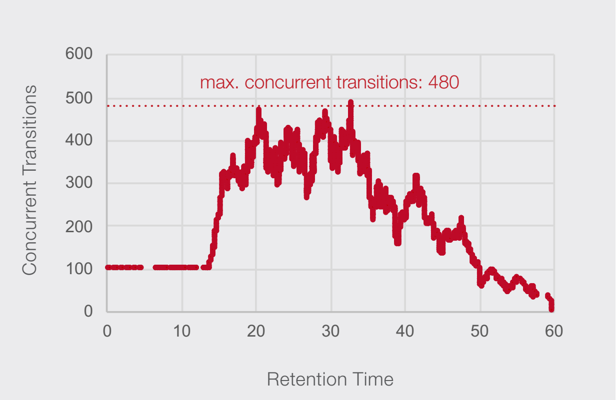


Figure 2b. PQ500 scheduling with 2.5 min RT Windows

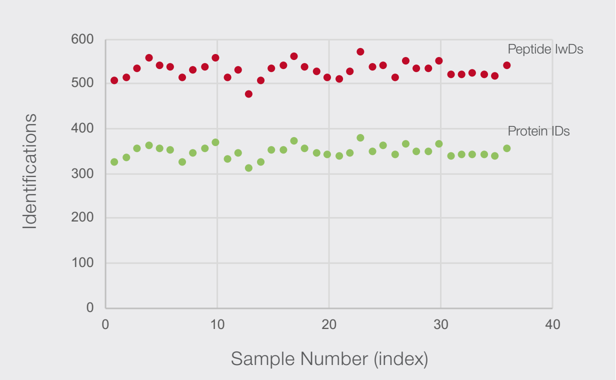


Figure 3a. Overview cancer plasma study

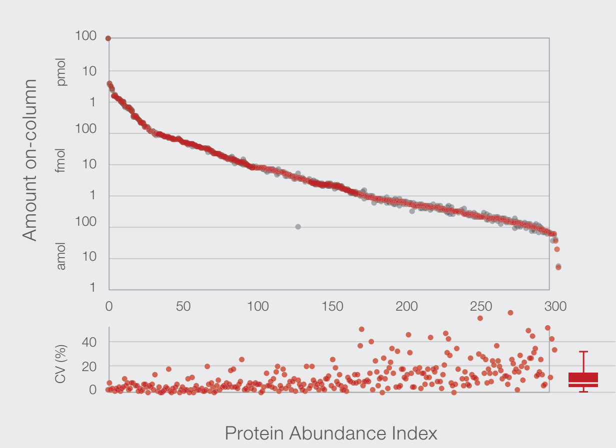


Figure 3b. Plasma protein quantities

Assessment of the limit-of-detection (LOD) and quantification linearity of the whole PQ500 panel was performed. For this, a 3-fold serial dilution of pooled plasma spiked with PQ500 into native plasma matrix was carried out. To increase coverage, the complete PQ500 panel and associated transitions were split into five equal parts and only the SIS peptides were targeted in these analytical runs. Blank plasma without PQ500 reference peptides spiked-in was also acquired with the same scheduling settings. The linearity of quantification was determined based on the SIS peptide intensities in the dilution series and the LOD as signal in the blank runs plus three times the signal variation in the blank runs. A similar approach was utilized to calculate the limit-of-quantification (LOQ) with the exception being that ten times the variation of the blank runs was used (Figure 4a). Overall, with this adjusted analytical set-up, LODs could be determined for 790 peptides of the PQ500 panel for a TSQ Altis MS capillary flow set-up. The results demonstrate excellent sensitivity with the median LOD in the atto-mole range of peptide amount on-column (Figure 4b).

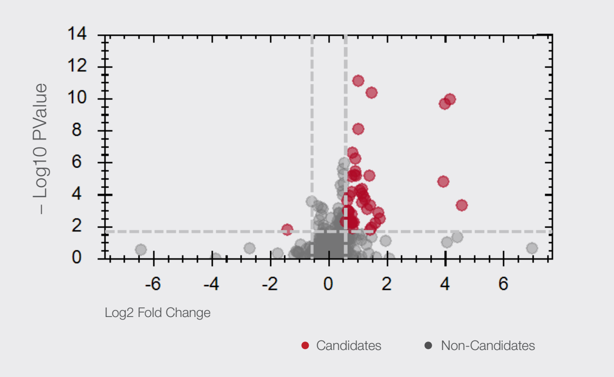


Figure 3c. Cancer vs healthy

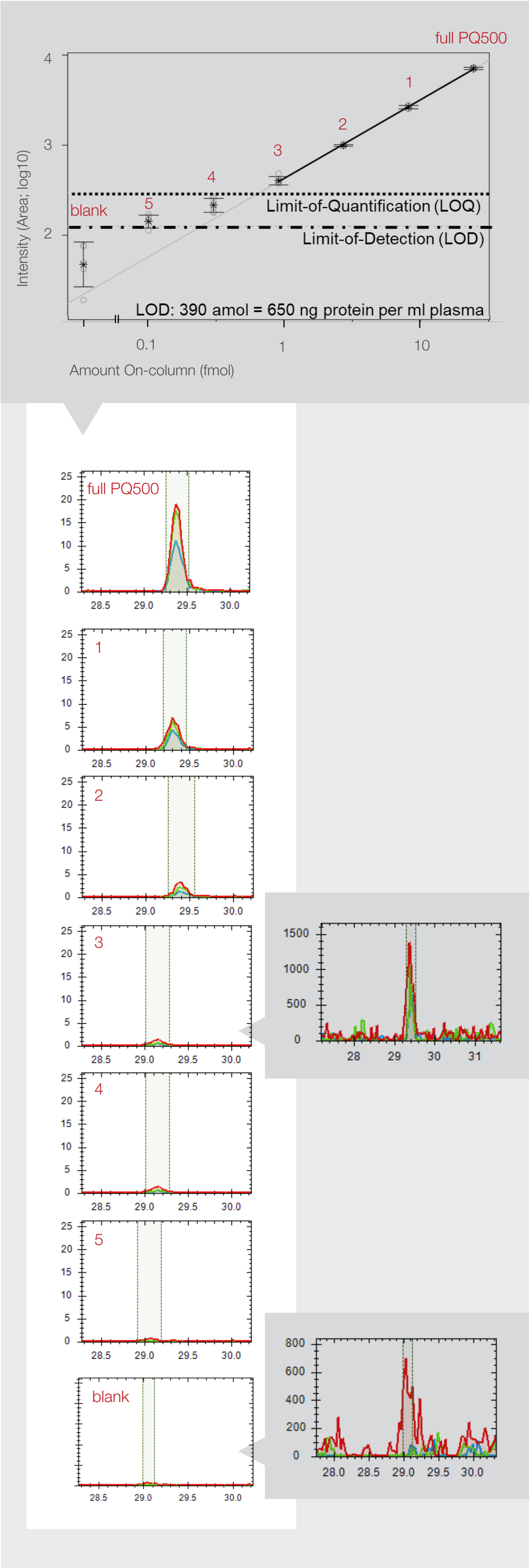


Figure 4a. Assay for Pigment Epithelium-derived Factor (PEDF), P36955-Peptide: ELLDTVTAPOK

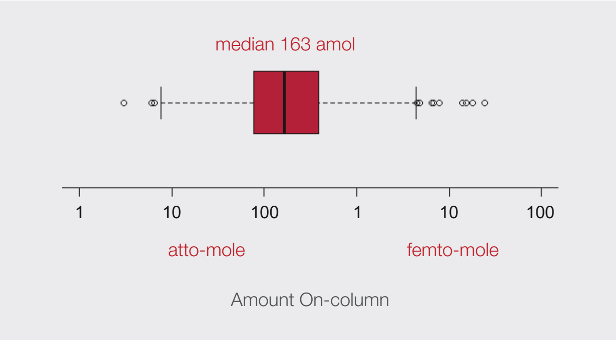


Figure 4b. PQ Assay LODs

Conclusion

The combination of the TSQ Altis MS and selected PQ500 reference peptides enables the reliable targeted quantification of several hundreds of proteins in plasma, matching the coverage of discovery proteomics experiments in human plasma. SpectroDive software streamlines the method set-up and data analysis to achieve unprecedented depth, sensitivity and quantitative precision with an overall analytical throughput of 20 samples per day.

Meaningful biological results were obtained from a small cohort of 36 cancer patients and healthy donors, confirming the potential of the method for clinical research such as patient stratification and personalized medicine.

References

1. Baker, M. Antibody anarchy: A call to order. Nature 527, 545–551 (2015) doi:10.1038/527545a

Determined pq500 LODs

Protein ID	Protein Name	LOD (fmol)
A0M8Q6	Immunoglobulin lambda constant 7	0.4802
B1AJZ9	Forkhead-associated domain-containing protein 1	0.4253
O00151	PDZ and LIM domain protein 1 (Elfin)	0.0801
O00187	Mannan-binding lectin serine protease 2	0.1470
O00299	Chloride intracellular channel protein 1	0.0385
O00300	Osteoprotegerin	0.0757
O00391	Sulfhydryl oxidase 1 (Quiescin Q6)	0.0878
O00533	Neural cell adhesion molecule L1-like protein	0.0107
O14498	Ig superfamily containing leucine-rich repeat protein	0.1979
O14786	Neuropilin-1 (CD antigen CD304)	0.2438
O14791	Apolipoprotein L1 (Apo-L)	0.0504
O15031	Plexin-B2	0.9922
O15240	Neurosecretory protein VGF	0.1525
O15394	Neural cell adhesion molecule 2 (NCAM-2)	0.1997
O43157	Plexin-B1 (Semaphorin receptor SEP)	0.0383
O43301	Heat shock 70 kDa protein 12A	0.0836
O43493	Trans-Golgi network integral membrane protein 2	0.0415
O43505	Beta-1,4-glucuronyltransferase 1	0.0077
O43653	Prostate stem cell antigen	0.0445
O43866	CD5 antigen-like	0.0457
O75015	CD antigen CD16b	4.4125
O75083	WD repeat-containing protein 1	1.2312
O75144	ICOS ligand (CD antigen CD275)	0.0426
O75173	ADAM-TS4	0.1223
O75326	Semaphorin-7A (CD antigen CD108)	0.4532
O75460	IRE1 (IRE1a)	0.3043
O75636	Ficolin-3	0.0700
O75874	Isocitrate dehydrogenase cytoplasmic (IDH)	0.2772
O75882	Attractin (DPPT-L)	0.1433
O94985	Calsyntenin-1	0.2607
O95445	Apolipoprotein M (Apo-M)	0.1058
O95479	GDH/6PGL endoplasmic bifunctional protein	0.7923
O95497	Pantetheinase (Vanin-1)	0.3835
O95757	Heat shock 70 kDa protein 4L	0.1512
O95810	Caveolae-associated protein 2 (Cavin-2)	0.2050
O95998	Interleukin-18-binding protein (IL-18BP)	0.3677
P00338	L-lactate dehydrogenase A chain (LDH-A)	2.9757
P00441	Superoxide dismutase (hSod1)	0.1846
P00450	Ceruloplasmin (Ferroxidase)	0.3040
P00488	Coagulation factor XIII A chain	0.0279
P00491	Purine nucleoside phosphorylase	0.3899
P00533	Epidermal growth factor receptor	0.1117
P00558	Phosphoglycerate kinase 1	0.1500
P00734	Prothrombin (Coagulation factor II)	0.0261
P00736	Complement C1r subcomponent	0.1481
P00738	Haptoglobin	0.0146
P00739	Haptoglobin-related protein	0.0176
P00740	Coagulation factor IX	0.0339
P00742	Coagulation factor X	0.0171
P00746	Complement factor D (Adipsin)	0.0480
P00747	Plasminogen	0.1681
P00748	Coagulation factor XII	0.0940
P00751	Complement factor B (C3/C5 convertase)	0.7424
P00915	Carbonic anhydrase 1 (CA-I)	0.1087
P00918	Carbonic anhydrase 2 (CA-II)	0.0661
P00966	Argininosuccinate synthase	1.9102
P01008	Antithrombin-III (ATIII) (Serpin C1)	0.0179
P01009	Alpha-1-antitrypsin (Serpin A1)	0.0534
P01011	Alpha-1-antichymotrypsin (Serpin A3)	0.1382
P01019	Angiotensinogen (Serpin A8)	0.0333
P01023	Alpha-2-macroglobulin	0.0372
P01024	Complement C3	0.0913
P01031	Complement C5	0.0254
P01033	Metalloproteinase inhibitor 1 (TIMP-1)	0.0273
P01034	Cystatin-C (Cystatin-3)	1.5553
P01042	Kininogen-1	0.0871
P01133	Pro-epidermal growth factor (EGF)	0.1907
P01138	Beta-nerve growth factor (Beta-NGF)	0.0730

Protein ID	Protein Name	LOD (fmol)
P01303	Pro-neuropeptide Y	0.0671
P01344	Insulin-like growth factor II (IGF-II)	0.0266
P01591	Immunoglobulin J chain	0.0337
P01602	Immunoglobulin kappa variable 1-5	0.3247
P01833	Polymeric immunoglobulin receptor	0.1951
P01834	Immunoglobulin kappa constant	0.0323
P01857	Immunoglobulin heavy constant gamma 1	0.3284
P01859	Immunoglobulin heavy constant gamma 2	0.0309
P01860	Immunoglobulin heavy constant gamma 3	0.3283
P01861	Immunoglobulin heavy constant gamma 4	0.2342
P01871	Immunoglobulin heavy constant mu	0.0846
P01876	Immunoglobulin heavy constant alpha 1	0.1036
P01877	Immunoglobulin heavy constant alpha 2	0.0317
P01880	Immunoglobulin heavy constant delta	0.0559
P02042	Hemoglobin subunit delta (Delta-globin)	0.3025
P02100	Hemoglobin subunit epsilon (Epsilon-globin)	0.2333
P02144	Myoglobin	0.0506
P02549	Spectrin alpha chain, erythrocytic 1	0.1733
P02647	Apolipoprotein A-I (Apo-AI)	0.0394
P02649	Apolipoprotein E (Apo-E)	0.0312
P02652	Apolipoprotein A-II (Apo-AII)	0.1940
P02654	Apolipoprotein C-I (Apo-CI)	0.2730
P02655	Apolipoprotein C-II (Apo-CII)	0.1455
P02656	Apolipoprotein C-III (Apo-CIII)	0.0339
P02671	Fibrinogen alpha chain	0.0865
P02675	Fibrinogen beta chain	0.0677
P55290	Cadherin-13	0.0522
P58335	Anthrax toxin receptor 2 (CMG-2)	0.2602
P60174	Triosephosphate isomerase	0.0330
P60484	PTEN	0.1020
P60660	Myosin light polypeptide 6 (Myosin light chain 3)	0.1812
P60985	Keratinocyte differentiation-associated protein	6.7672

Protein ID	Protein Name	LOD (fmol)
P61626	Lysozyme C	0.2710
P61769	Beta-2-microglobulin	0.3994
P61981	14-3-3 protein gamma	0.5181
P62258	14-3-3 protein epsilon	0.7076
P62328	Thymosin beta-4	0.1603
P63104	14-3-3 protein zeta/delta	2.0270
P67936	Tropomyosin alpha-4 chain (Tropomyosin-4)	0.3050
P68871	Hemoglobin subunit beta (Beta-globin)	0.0709
P69905	Hemoglobin subunit alpha (Alpha-globin)	0.0521
P78417	Glutathione S-transferase omega-1	0.0464
P78509	Reelin	0.0064
P78563	Double-stranded RNA-specific editase 1	0.3069
P80108	Phosphatidylinositol-glycan-specific phospholipase D	0.0227
P80188	Neutrophil gelatinase-associated lipocalin	0.0916
P80370	Protein delta homolog 1 (DLK-1)	0.7403
P80511	Protein S100-A12(Calgranulin-C)	3.8371
P80723	Brain acid soluble protein 1 (NAP-22)	0.1549
P81605	Dermcidin	0.1256
P98160	Perlecan	0.0205
P98164	Low-density lipoprotein receptor-related protein 2	0.2689
Q01459	Di-N-acetylchitobiase	0.1566
Q01518	Adenylyl cyclase-associated protein 1 (CAP 1)	0.0991
Q02985	Complement factor H-related protein 3 (FHR-3)	0.0242
Q03167	Transforming growth factor beta receptor type 3	0.0355
Q03591	Complement factor H-related protein 1 (FHR-1)	0.1224
Q04721	Neurogenic locus notch homolog protein 2 (Notch 2)	0.2506
Q04756	Hepatocyte growth factor activator (HGFA)	0.1154
Q04760	Lactoylglutathione lyase	0.0915
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	0.1400
Q06830	Peroxiredoxin-1 (NKEF-A)	1.3683
Q07954	Prolow-density lipoprotein receptor-related protein 1	0.4915
Q08380	Galectin-3-binding protein	0.0341

Protein ID	Protein Name	LOD (fmol)
Q08629	Testican-1	0.0314
Q08830	Fibrinogen-like protein 1	0.7219
Q0VDF9	Heat shock 70 kDa protein 14 (HSP60)	0.1171
Q10588	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2	0.2465
Q12794	Hyaluronidase-1 (Lung carcinoma protein 1)	0.1710
Q12805	Fibulin-3	0.1188
Q12860	Contactin-1 (Glycoprotein gp135)	0.0862
Q12884	Prolyl endopeptidase FAP (Seprase)	0.4573
Q12912	Lymphoid-restricted membrane protein	0.4817
Q12913	Receptor-type tyrosine-protein phosphatase eta	0.0650
Q13093	Platelet-activating factor acetylhydrolase	0.1624
Q13103	Secreted phosphoprotein 24 (Spp-24)	0.0423
Q13201	Multimerin-1 (EMILIN-4)	0.1337
Q13228	Methanethiol oxidase (Selenium-binding protein 1)	0.0650
Q13231	Chitotriosidase-1 (Chitinase-1)	0.2433
Q13332	Receptor-type tyrosine-protein phosphatase S	0.5877
Q13418	Integrin-linked protein kinase (ILK-1; ILK-2)	0.6104
Q13421	Mesothelin (CAK1 antigen)	0.6963
Q13449	Limbic system-associated membrane protein	0.3120
Q13740	CD166 antigen	0.0895
Q13790	Apolipoprotein F (Apo-F; LTIP)	0.0566
Q13822	E-NPP 2; Autotaxin	0.1194
Q14118	Dystroglycan (Dystrophin-associated glycoprotein 1)	0.2632
Q14126	Desmoglein-2	0.1112
Q14247	Src substrate cortactin (Amplaxin; Oncogene EMS1)	n/a
Q14393	Growth arrest-specific protein 6 (GAS-6)	0.2512
Q14508	WAP four-disulfide core domain protein 2	0.0110
Q14515	SPARC-like protein 1	0.1519
Q14520	Hyaluronan-binding protein 2	0.0725
Q14623	Indian hedgehog protein (IHH)	0.1837
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	0.3453

Protein ID	Protein Name	LOD (fmol)
Q14643	Inositol 1,4,5-trisphosphate receptor type 1	0.1965
Q14697	Neutral alpha-glucosidase AB	0.2819
Q14766	LTBP-1; TGF-beta1-BP-1	0.1517
Q15063	Periostin (PN; OSF-2)	0.6659
Q15113	Procollagen C-endopeptidase enhancer 1	0.0617
Q15166	Serum paraoxonase/lactonase 3	0.1951
Q15465	Sonic hedgehog protein (SHH)	0.2928
Q15485	Ficolin-2	0.1813
Q15582	Beta ig-h3	0.0104
Q15848	Adiponectin	0.0898
Q15942	Zyxin (Zyxin-2)	0.1274
Q16270	Insulin-like growth factor-binding protein 7 (IGFBP-7)	0.2505
Q16563	Synaptophysin-like protein 1 (Pantophysin)	0.1083
Q16610	Extracellular matrix protein 1	0.0613
Q16627	C-C motif chemokine 14	0.3806
Q16706	Alpha-mannosidase 2	1.4220
Q16853	Membrane primary amine oxidase (VAP-1)	0.0718
Q2KHT4	Germ cell-specific gene 1 protein	n/a
Q53RD9	Fibulin-7 (FIBL-7)	0.1634
Q5SRH9	Tetratricopeptide repeat protein 39A	0.8524
Q5T5C0	Syntaxin-binding protein 5 (Tomosyn-1)	0.1625
Q6B0K9	Hemoglobin subunit mu (Mu-globin)	0.0124
Q6EMK4	Vasorin (Protein slit-like 2)	0.1397
Q6GTX8	LAIR-1; CD antigen CD305	0.0466
Q6Q788	Apolipoprotein A-V (Apo-AV)	0.0317
Q6UX71	Plexin domain-containing protein 2	0.0566
Q6UXB8	Peptidase inhibitor 16	0.0867
Q6UXD5	Seizure 6-like protein 2	0.3949
Q6UY14	ADAMTS-like protein 4 (ADAMTSL-4)	0.1159
Q6YHK3	CD109 antigen (CD antigen CD109)	0.1679
Q76LX8	ADAM-TS13	0.1030
Q7L4I2	Arginine/serine-rich coiled-coil protein 2	1.2032
Q7Z7D3	V-set domain-containing T-cell activation inhibitor 1	1.2438
Q7Z7G0	Target of Nesh-SH3	0.0867

Protein ID	Protein Name	LOD (fmol)
Q7Z7M0	EGF-like protein 4	0.2609
Q86SQ4	Adhesion G-protein coupled receptor G6 (GPR126)	0.0455
Q86U17	Serpin A11	0.3777
Q86UD1	Out at first protein homolog	0.0682
Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	0.1715
Q86UX7	Fermitin family homolog 3 (Kindlin-3)	0.3399
Q86VB7	Scavenger receptor cysteine-rich type 1 protein M130	0.0839
Q86YZ3	Hornerin	15.1396
Q8IUX7	Adipocyte enhancer-binding protein 1	0.7476
Q8N4F0	BPI fold-containing family B member 2	0.1339
Q8N6C8	CD antigen CD85e	0.1878
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	0.1026
Q8NE71	ATP-binding cassette sub-family F member 1	0.0301
Q8TB96	T-cell immunomodulatory protein	0.0395
Q8WUJ3	CEMIP	0.1450
Q8WWA0	Intelectin-1 (Omentin)	0.7210
Q8WXD2	Secretogranin-3	0.2134
Q8WXI7	Mucin-16 (MUC-16; CA125)	0.3615
Q8WZ75	Roundabout homolog 4	0.0841
Q92496	Complement factor H-related protein 4 (FHR-4)	0.2030
Q92520	Protein FAM3C (Interleukin-like EMT inducer)	0.1157
Q92820	Gamma-glutamyl hydrolase	1.6103
Q92876	Kallikrein-6 (Neurosin)	0.0347
Q92954	Proteoglycan 4 (Lubricin)	0.0735
Q969H8	Myeloid-derived growth factor (MYDGF; IL-25)	0.1872
Q96IY4	Carboxypeptidase B2 (pCPB)	0.0850
Q96JB8	MAGUK p55 subfamily member 4	7.7415
Q96KN2	Beta-Ala-His dipeptidase (Serum carnosinase)	0.2184
Q96PD5	N-acetylmuramoyl-L-alanine amidase	0.8207
Q96QR1	Secretoglobin family 3A member 1	0.4734
Q96S96	Phosphatidylethanolamine-binding protein 4 (PEBP-4)	0.5215
Q99497	Protein/nucleic acid deglycase DJ-1	0.9656

Protein ID	Protein Name	LOD (fmol)
Q99784	Noelin (Olfactomedin-1)	0.1136
Q99969	Retinoic acid receptor responder protein 2 (Chemerin)	0.2194
Q99983	Osteomodulin (Osteoadherin)	0.1318
Q9BTY2	Plasma alpha-L-fucosidase	0.0545
Q9BWP8	Collectin-11 (CL-K1)	0.0994
Q9BXJ3	C1q/TNF-related protein 4	0.0642
Q9BXJ4	CORS26	0.1501
Q9BXP8	Pappalysin-2	0.1159
Q9BXR6	Complement factor H-related protein 5 (FHR-5)	0.1626
Q9BYH1	Seizure 6-like protein	1.3183
Q9BYJ0	Fibroblast growth factor-binding protein 2	0.0550
Q9GZX9	Twisted gastrulation protein homolog 1	0.0728
Q9H257	Caspase recruitment domain-containing protein 9	0.0724
Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	0.1620
Q9H4A9	Dipeptidase 2	0.0535
Q9H4B7	Tubulin beta-1 chain	0.8782
Q9H4G4	Golgi-associated PR-1 protein	0.0914
Q9H8L6	Multimerin-2 (EMILIN-3)	0.0914
Q9HBB8	Cadherin-related family member 5	0.4596
Q9HBI1	Beta-parvin (Affixin)	0.0667
Q9HCU0	Endosialin (Tumor endothelial marker 1; CD248)	0.1196
Q9HD89	Resistin (Adipose tissue-specific secretory factor)	0.0516
Q9HDC9	Adipocyte plasma membrane-associated protein	0.2339
Q9NP80	Calcium-independent phospholipase A2-gamma	0.0625
Q9NPH3	Interleukin-1 receptor accessory protein (IL-1RAcP)	0.0451
Q9NPY3	Complement component C1q receptor (CD93)	0.2401
Q9NQ79	Cartilage acidic protein 1	0.0931
Q9NQC3	Reticulon-4 (Nogo protein)	0.8217
Q9NY97	Beta-1,3-N-acetylglucosaminyltransferase 1	0.0225
Q9NZ08	Endoplasmic reticulum aminopeptidase 1 (ARTS-1)	0.1390
Q9NZP8	Complement C1r subcomponent-like protein (C1r-LP)	0.0999

Protein ID	Protein Name	LOD (fmol)
Q9UBR2	Cathepsin Z (Cathepsin X)	0.1241
Q9UBX5	Fibulin-5 (FIBL-5)	0.3187
Q9UEW3	Macrophage receptor MARCO	0.0127
Q9UGM5	Fetuin-B	0.0794
Q9UHG2	ProSAAS (Proprotein convertase 1 inhibitor)	0.0161
Q9UHG3	Prenylcysteine oxidase 1 (Prenylcysteine lyase)	1.3858
Q9UJJ9	GlcNAc-1-phosphotransferase subunit gamma	0.3967
Q9UK55	Protein Z-dependent protease inhibitor (Serpin A10)	0.1677
Q9ULI3	Protein HEG homolog 1	1.7909
Q9UNN8	Endothelial protein C receptor (CD antigen CD201)	0.0576
Q9UNW1	Multiple inositol polyphosphate phosphatase 1	0.1187
Q9Y490	Talin-1	0.0911
Q9Y4L1	Hypoxia up-regulated protein 1	0.2255
Q9Y5C1	Angiopoietin-related protein 3 (Angiopoietin-5)	4.8573
Q9Y5Y7	LYVE-1	0.0623
Q9Y646	Carboxypeptidase Q	0.1011
Q9Y6R7	IgGFc-binding protein	0.1309
Q9Y6Z7	Collectin-10 (CL-L1)	0.2703

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