

TARGETED PROTEOMIC ANALYSIS OF HUMAN PLASMA ON A DISCOVERY SCALE PQ500 & TSQ ALTIS MASS SPECTROMETER

In this technical note you will learn about:

- Set-up of selected/multiple reaction monitoring (SRM/MRM) workflow with PQ500 reference peptides and SpectroDive software.
- Technical capabilities of the Thermo Scientific™ TSQ Altis™ Triple-Stage Quadrupole Mass Spectrometer coupled to a capillary flow LC set-up.
- Anticipated results of quantifying the plasma proteome in 70 minutes per sample (throughput of ~ 20 samples per day).
- Experimentally determined lower limits of detection (LODs) in human plasma for > 500 proteins covered by PQ500 reference peptides with this technical set-up.

If you have any questions about this technical note or Biognosys' targeted proteomics platform, please contact us at support@biognosys.com

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

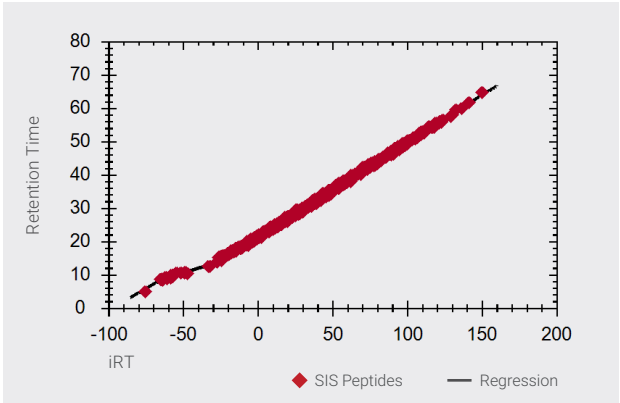
A pool of human plasma samples, as well as individual plasma samples, from a cohort of cancer patients (n = 21) and matched healthy donors (n = 15) were processed according to Biognosys' Sample Preparation Kit Pro for high-throughput proteomics. Injection-ready samples were spiked with PQ500 reference peptides before data acquisition. All samples were acquired on a TSQ Altis MS coupled to a Thermo Scientific Ultimate 3000 RSLCnano 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

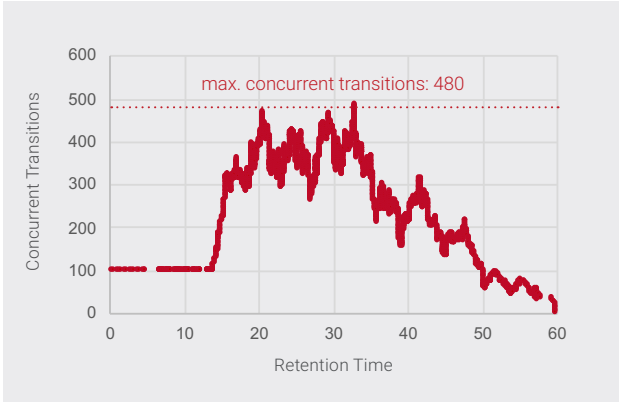
SpectroDive utilizes all SIS peptides from the PQ500 mix as anchor points for the scheduling of the SRM/MRM method (Figure 2a). This methodology accounts for short phases of non-linearity in the gradient, leading to a median offset of measured retention times compared to scheduled retention times of 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.

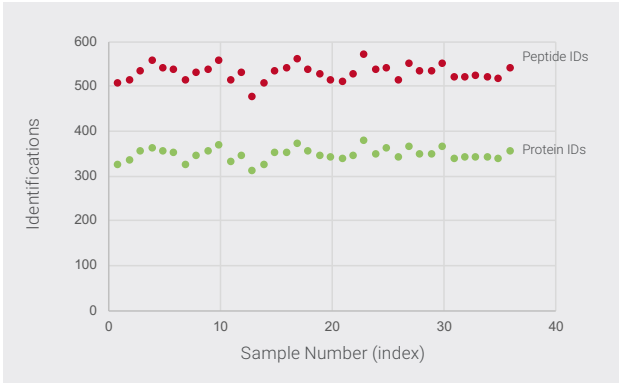
2a. RT Calibration Based on all SIS Peptides



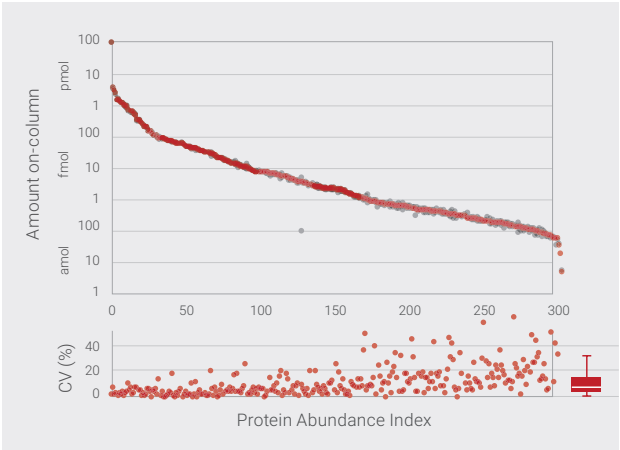
2b. PQ500 Scheduling with 2.5 min RT Windows



3a. Overview Cancer Plasma Study



3b. Plasma Protein Quantities

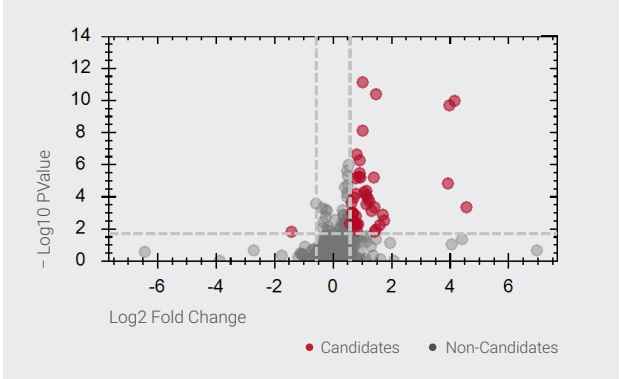


1. Human Plasma Analysis Workflow

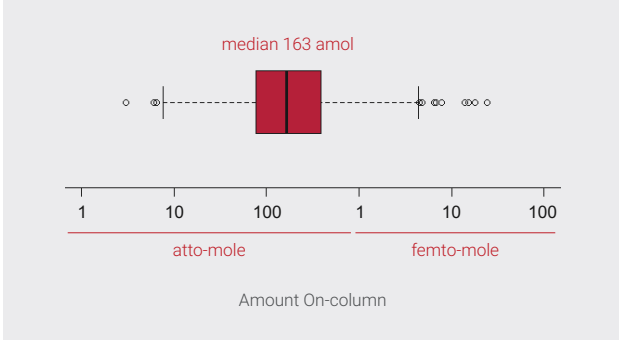


Assessment of the limit-of-detection (LOD) and quantification linearity of the whole PQ500 panel was performed. For this, a 3-fold serial dilution of pooled plasma spiked with PQ500 into native plasma matrix was carried out. To increase coverage, the complete PQ500 panel and associated transitions were 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 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 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).

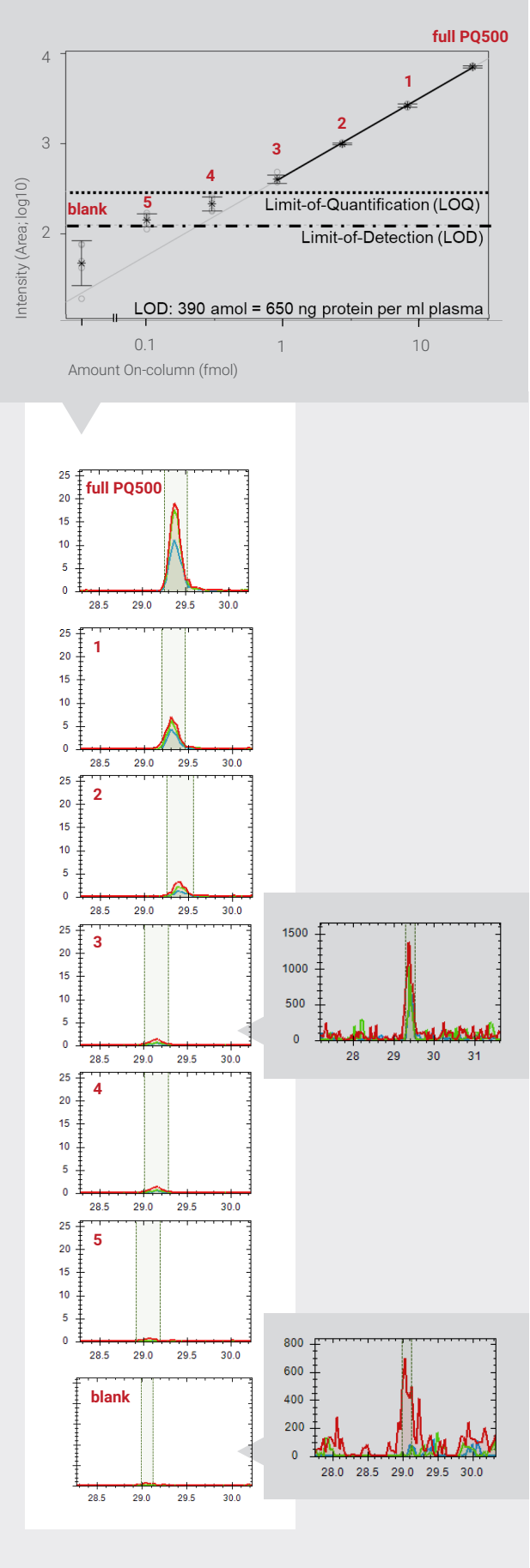
3c. Cancer vs Healthy



4b. PQ Assay LODs



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



CONCLUSION

The combination of the TSQ Altis 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

Disclaimer

! All mentioned products and procedures are meant for research use only. Not for use in diagnostic procedures.

DETERMINED PQ500 LODS

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
A0M8Q6	Immunoglobulin lambda constant 7	0.4802	P00740	Coagulation factor IX	0.0339
B1AJZ9	Forkhead-associated domain-containing protein 1	0.4253	P00742	Coagulation factor X	0.0171
O00151	PDZ and LIM domain protein 1 (Elfin)	0.0801	P00746	Complement factor D (Adipsin)	0.0480
O00187	Mannan-binding lectin serine protease 2	0.1470	P00747	Plasminogen	0.1681
O00299	Chloride intracellular channel protein 1	0.0385	P00748	Coagulation factor XII	0.0940
O00300	Osteoprotegerin	0.0757	P00751	Complement factor B (C3/C5 convertase)	0.7424
O00391	Sulfhydryl oxidase 1 (Quiescin Q6)	0.0878	P00915	Carbonic anhydrase 1 (CA-I)	0.1087
O00533	Neural cell adhesion molecule L1-like protein	0.0107	P00918	Carbonic anhydrase 2 (CA-II)	0.0661
O14498	Ig superfamily containing leucine-rich repeat protein	0.1979	P00966	Argininosuccinate synthase	1.9102
O14786	Neuropilin-1 (CD antigen CD304)	0.2438	P01008	Antithrombin-III (ATIII) (Serpins C1)	0.0179
O14791	Apolipoprotein L1 (Apo-L)	0.0504	P01009	Alpha-1-antitrypsin (Serpins A1)	0.0534
O15031	Plexin-B2	0.9922	P01011	Alpha-1-antichymotrypsin (Serpins A3)	0.1382
O15240	Neurosecretory protein VGF	0.1525	P01019	Angiotensinogen (Serpins A8)	0.0333
O15394	Neural cell adhesion molecule 2 (NCAM-2)	0.1997	P01023	Alpha-2-macroglobulin	0.0372
O43157	Plexin-B1 (Semaphorin receptor SEP)	0.0383	P01024	Complement C3	0.0913
O43301	Heat shock 70 kDa protein 12A	0.0836	P01031	Complement C5	0.0254
O43493	Trans-Golgi network integral membrane protein 2	0.0415	P01033	Metalloproteinase inhibitor 1 (TIMP-1)	0.0273
O43505	Beta-1,4-glucuronyltransferase 1	0.0077	P01034	Cystatin-C (Cystatin-3)	1.5553
O43653	Prostate stem cell antigen	0.0445	P01042	Kininogen-1	0.0871
O43866	CD5 antigen-like	0.0457	P01133	Pro-epidermal growth factor (EGF)	0.1907
O75015	CD antigen CD16b	4.4125	P01138	Beta-nerve growth factor (Beta-NGF)	0.0730
O75083	WD repeat-containing protein 1	1.2312	P01303	Pro-neuropeptide Y	0.0671
O75144	ICOS ligand (CD antigen CD275)	0.0426	P01344	Insulin-like growth factor II (IGF-II)	0.0266
O75173	ADAM-TS4	0.1223	P01591	Immunoglobulin J chain	0.0337
O75326	Semaphorin-7A (CD antigen CD108)	0.4532	P01602	Immunoglobulin kappa variable 1-5	0.3247
O75460	IRE1 (IRE1a)	0.3043	P01833	Polymeric immunoglobulin receptor	0.1951
O75636	Ficolin-3	0.0700	P01834	Immunoglobulin kappa constant	0.0323
O75874	Isocitrate dehydrogenase cytoplasmic (IDH)	0.2772	P01857	Immunoglobulin heavy constant gamma 1	0.3284
O75882	Attractin (DPPT-L)	0.1433	P01859	Immunoglobulin heavy constant gamma 2	0.0309
O94985	Calsyntenin-1	0.2607	P01860	Immunoglobulin heavy constant gamma 3	0.3283
O95445	Apolipoprotein M (Apo-M)	0.1058	P01861	Immunoglobulin heavy constant gamma 4	0.2342
O95479	GDH/6PGL endoplasmic bifunctional protein	0.7923	P01871	Immunoglobulin heavy constant mu	0.0846
O95497	Pantetheinase (Vanin-1)	0.3835	P01876	Immunoglobulin heavy constant alpha 1	0.1036
O95757	Heat shock 70 kDa protein 4L	0.1512	P01877	Immunoglobulin heavy constant alpha 2	0.0317
O95810	Caveolae-associated protein 2 (Cavin-2)	0.2050	P01880	Immunoglobulin heavy constant delta	0.0559
O95998	Interleukin-18-binding protein (IL-18BP)	0.3677	P02042	Hemoglobin subunit delta (Delta-globin)	0.3025
P00338	L-lactate dehydrogenase A chain (LDH-A)	2.9757	P02100	Hemoglobin subunit epsilon (Epsilon-globin)	0.2333
P00441	Superoxide dismutase (hSod1)	0.1846	P02144	Myoglobin	0.0506
P00450	Ceruloplasmin (Ferroxidase)	0.3040	P02549	Spectrin alpha chain, erythrocytic 1	0.1733
P00488	Coagulation factor XIII A chain	0.0279	P02647	Apolipoprotein A-I (Apo-AI)	0.0394
P00491	Purine nucleoside phosphorylase	0.3899	P02649	Apolipoprotein E (Apo-E)	0.0312
P00533	Epidermal growth factor receptor	0.1117	P02652	Apolipoprotein A-II (Apo-AII)	0.1940
P00558	Phosphoglycerate kinase 1	0.1500	P02654	Apolipoprotein C-I (Apo-CI)	0.2730
P00734	Prothrombin (Coagulation factor II)	0.0261	P02655	Apolipoprotein C-II (Apo-CII)	0.1455
P00736	Complement C1r subcomponent	0.1481	P02656	Apolipoprotein C-III (Apo-CIII)	0.0339
P00738	Haptoglobin	0.0146	P02671	Fibrinogen alpha chain	0.0865
P00739	Haptoglobin-related protein	0.0176	P02675	Fibrinogen beta chain	0.0677

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
P02675	Fibrinogen beta chain	0.0677	P05062	Fructose-bisphosphate aldolase B	0.1561
P02679	Fibrinogen gamma chain	0.1319	P05067	Amyloid-beta A4 protein (ABPP; APP)	0.2231
P02730	Band 3 anion transport protein (CD antigen CD233)	0.7103	P05090	Apolipoprotein D (Apo-D)	0.1605
P02741	C-reactive protein (CRP)	0.0670	P05106	Integrin beta-3 (CD antigen CD61)	0.0158
P02743	Serum amyloid P-component (SAP)	0.1518	P05109	Protein S100-A8 (Calgranulin-A)	0.7201
P02745	Complement C1q subcomponent subunit A	0.0957	P05154	Plasma serine protease inhibitor (PAI-3; Serpin A5)	0.1051
P02746	Complement C1q subcomponent subunit B	0.0350	P05155	Plasma protease C1 inhibitor (Serpins G1)	0.4238
P02747	Complement C1q subcomponent subunit C	0.1156	P05156	Complement factor I (C3B/C4B inactivator)	0.0589
P02748	Complement component C9	0.0665	P05160	Coagulation factor XIII B chain	0.3055
P02749	Beta-2-glycoprotein 1	0.0431	P05164	Myeloperoxidase (MPO)	0.0120
P02750	Leucine-rich alpha-2-glycoprotein (LRG)	0.0562	P05362	Intercellular adhesion molecule 1 (ICAM-1)	0.0373
P02751	Fibronectin (FN)	0.0510	P05452	Tetranectin (TN)	0.0504
P02753	Retinol-binding protein 4	0.0818	P05543	Thyroxine-binding globulin (Serpins A7)	0.0533
P02760	Protein AMBP	0.0390	P05546	Heparin cofactor 2 (Serpins D1)	0.1276
P02763	Alpha-1-acid glycoprotein 1 (AGP 1)	0.1173	P05556	Integrin beta-1 (CD antigen CD29)	0.4921
P02765	Alpha-2-HS-glycoprotein (Fetuin-A)	0.1409	P06276	Cholinesterase	0.0724
P02766	Transthyretin (Prealbumin)	0.3864	P06396	Gelsolin (Brevin)	0.2397
P02768	Serum albumin	0.0962	P06681	Complement C2 (C3/C5 convertase)	0.0539
P02774	Vitamin D-binding protein	0.1907	P06702	Protein S100-A9 (Calgranulin-B)	0.0427
P02775	Platelet basic protein (PBP; C-X-C motif chemokine 7)	0.0739	P06727	Apolipoprotein A-IV (Apo-AIV)	0.0804
P02776	Platelet factor 4 (PF-4; C-X-C motif chemokine 4)	0.0189	P06731	CEA; CD antigen CD66e	0.0428
P02786	Transferrin receptor protein 1 (TfR1; CD antigen CD71)	0.0265	P06732	Creatine kinase M-type (M-CK)	0.6501
P02787	Serotransferrin (Transferrin)	0.0663	P06733	Alpha-enolase (Plasminogen-binding protein)	0.2538
P02788	Lactotransferrin (Lactoferrin)	0.1037	P06744	Glucose-6-phosphate isomerase (GPI)	0.1786
P02790	Hemopexin (Beta-1B-glycoprotein)	0.1021	P06753	Tropomyosin alpha-3 chain (Gamma-tropomyosin)	4.4424
P02792	Ferritin light chain (Ferritin L subunit)	0.0327	P06850	Corticoliberin	0.0556
P03950	Angiogenin	0.7583	P06870	Kallikrein-1 (Tissue kallikrein)	0.3480
P03951	Coagulation factor XI	0.1707	P07195	L-lactate dehydrogenase B chain (LDH-B)	0.0648
P03952	Plasma kallikrein (Kininogenin; PKK)	0.0232	P07225	Vitamin K-dependent protein S	0.0524
P04003	C4b-binding protein alpha chain (C4bp)	0.1123	P07237	Protein disulfide-isomerase (PDI)	0.0581
P04004	Vitronectin	0.1038	P07288	Prostate-specific antigen (PSA; Kallikrein-3)	0.0694
P04040	Catalase	0.0247	P07333	Macrophage colony-stimulating factor 1 receptor	0.0193
P04070	Vitamin K-dependent protein C	0.2074	P07339	Cathepsin D	0.0823
P04075	Fructose-bisphosphate aldolase A	0.0137	P07357	Complement component C8 alpha chain	0.1008
P04080	Cystatin-B (CPI-B; Stefin-B)	0.2742	P07358	Complement component C8 beta chain	0.0710
P04083	Annexin A1	1.1173	P07359	Platelet glycoprotein Ib alpha chain (CD42b)	0.0320
P04114	Apolipoprotein B-100 (Apo B-100)	0.0363	P07360	Complement component C8 gamma chain	0.1400
P04156	Major prion protein (PrP; CD antigen CD230)	0.1066	P07384	Calpain-1 catalytic subunit	2.0807
P04180	Phosphatidylcholine-sterol acyltransferase	0.0467	P07437	Tubulin beta chain (Tubulin beta-5 chain)	0.1477
P04196	Histidine-rich glycoprotein (HPRG)	0.1090	P07451	Carbonic anhydrase 3 (CA-III)	0.3644
P04217	Alpha-1B-glycoprotein	0.0157	P07585	Decorin (Bone proteoglycan II)	0.1634
P04275	von Willebrand factor (vWF)	0.0353	P07602	Prosaposin	0.0522
P04278	Sex hormone-binding globulin (SHBG)	0.0094	P07737	Profilin-1	0.1133
P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.1387	P07738	Bisphosphoglycerate mutase	0.3975
P04626	Receptor tyrosine-protein kinase erbB-2	0.3549	P07858	Cathepsin B (APP secretase)	0.5250
P04632	Calpain small subunit 1	0.0326	P07988	Pulmonary surfactant-associated protein B	0.0131
P04637	Cellular tumor antigen p53 (Tumor suppressor p53)	0.2223	P07996	Thrombospondin-1	0.0401
P04792	Heat shock protein beta-1 (HspB1)	0.2289	P07998	Ribonuclease pancreatic	0.2562
P05019	Insulin-like growth factor I (IGF-I)	0.9558	P08107	Heat shock 70 kDa protein 1	0.1292
P05060	Secretogranin-1 (Chromogranin-B)	0.1925	P08174	Complement decay-accelerating factor (CD55)	0.2944

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
P08185	Corticosteroid-binding globulin (SerpA6)	0.0838	P11279	Lysosome-associated membrane glycoprotein 1	0.1177
P08195	4F2 cell-surface antigen heavy chain (CD98)	0.0857	P11597	Cholesteryl ester transfer protein	0.2647
P08238	Heat shock protein HSP 90-beta (HSP 90)	0.1971	P11717	Cation-independent mannose-6-phosphate receptor	0.0260
P08253	72 kDa type IV collagenase (MMP-2)	0.3799	P12110	Collagen alpha-2(VI) chain	0.1335
P08294	Extracellular superoxide dismutase	0.0297	P12111	Collagen alpha-3(VI) chain	0.0836
P08319	Alcohol dehydrogenase 4	0.9411	P12259	Coagulation factor V	1.2074
P08493	Matrix Gla protein (MGP)	0.6761	P12318	Low affinity Ig gamma Fc region receptor II-a (CD32)	0.6545
P08514	Integrin alpha-IIb (CD antigen CD41)	0.0689	P12429	Annexin A3	0.0940
P08519	Apolipoprotein(a) (Lp(a))	0.0551	P12814	Alpha-actinin-1	0.6034
P08567	Pleckstrin	0.0948	P12830	Cadherin-1 (E-cadherin; CD antigen CD324)	0.0357
P08571	Monocyte differentiation antigen CD14	0.0527	P12955	Xaa-Pro dipeptidase (Peptidase D)	0.0305
P08575	Receptor-type tyrosine-protein phosphatase C (CD45)	0.8591	P13473	Lysosome-associated membrane glycoprotein 2	0.6787
P08603	Complement factor H	0.1653	P13521	Secretogranin-2 (Chromogranin-C)	0.0400
P08637	Low affinity Ig gamma Fc region receptor III-A (CD16a)	0.2893	P13591	Neural cell adhesion molecule 1 (NCAM-1; CD56)	0.0771
P08670	Vimentin	2.7130	P13598	Intercellular adhesion molecule 2 (ICAM-2; CD102)	0.4215
P08697	Alpha-2-antiplasmin (SerpA2)	0.3781	P13671	Complement component C6	0.1393
P08709	Coagulation factor VII	0.2250	P13716	Delta-aminolevulinic acid dehydratase	0.1028
P08887	Interleukin-6 receptor subunit alpha (CD126)	0.2600	P13727	Bone marrow proteoglycan	3.5091
P08949	Neuromedin-B	0.0853	P13796	Plastin-2 (L-plastin)	0.0400
P09104	Gamma-enolase	0.0401	P13987	CD59 glycoprotein (Protectin; CD antigen CD59)	0.0660
P09172	Dopamine beta-hydroxylase	0.0249	P14136	Glial fibrillary acidic protein (GFAP)	0.4149
P09486	SPARC (Osteonectin; BM-40)	0.5452	P14151	L-selectin (CD antigen CD62L)	0.0268
P09603	Macrophage colony-stimulating factor 1 (CSF-1)	0.0458	P14174	Macrophage migration inhibitory factor (MIF)	0.1239
P09681	Gastric inhibitory polypeptide (GIP; Incretin hormone)	0.1227	P14209	CD99 (T-cell surface glycoprotein E2)	0.0030
P09871	Complement C1s subcomponent	0.0307	P14543	Nidogen-1 (NID-1) (Entactin)	0.0663
P09972	Fructose-bisphosphate aldolase C	0.0309	P14618	Pyruvate kinase PKM	0.0722
P0C091	FRAS1-related extracellular matrix protein 3	0.1242	P14625	Endoplasmin (Tumor rejection antigen 1)	0.2391
P0C0L4	Complement C4-A	0.4389	P14780	Matrix metalloproteinase-9 (MMP-9)	0.0732
P0C0L5	Complement C4-B	0.2740	P15121	Aldose reductase (AR)	0.2030
P0C870	JmjC domain-containing protein 7	13.9457	P15144	Aminopeptidase N (CD antigen CD13)	0.1102
P0CF74	Immunoglobulin lambda constant 6	0.0972	P15151	Poliovirus receptor (CD antigen CD155)	0.9012
P0DJ18	Serum amyloid A-1 protein (SAA)	0.6686	P15169	Carboxypeptidase N catalytic chain (CPN)	0.0702
P0DJ19	Serum amyloid A-2 protein (SAA2)	0.0973	P15328	Folate receptor alpha (FR-alpha)	1.4187
P0DOX2	Immunoglobulin alpha-2 heavy chain	0.0826	P15692	Vascular endothelial growth factor A (VEGF-A)	0.0505
P0DOX3	Immunoglobulin delta heavy chain	0.1380	P15814	Immunoglobulin lambda-like polypeptide 1 (CD179b)	0.1610
P0DOX4	Immunoglobulin epsilon heavy chain	0.1245	P16070	CD44 antigen (Hyaluronate receptor; CD44)	0.0192
P0DOX5	Immunoglobulin gamma-1 heavy chain	0.3504	P16284	Platelet endothelial cell adhesion molecule (CD31)	0.1764
P0DOX6	Immunoglobulin mu heavy chain	0.0941	P16455	Methylated-DNA--protein-cysteine methyltransferase	0.0666
P0DOX7	Immunoglobulin kappa light chain	0.8218	P16870	Carboxypeptidase E (CPE)	0.2789
P0DOX8	Immunoglobulin lambda-1 light chain	0.3274	P16930	Fumarylacetoacetase (FAA)	3.0118
P0DOY3	Immunoglobulin lambda constant 3	n/a	P17066	Heat shock 70 kDa protein 6	0.0091
P10124	Serglycin	0.3874	P17174	Aspartate aminotransferase (Transaminase A)	0.1383
P10451	Osteopontin (Bone sialoprotein 1; SPP-1)	0.0643	P17813	Endoglin (CD antigen CD105)	0.0135
P10599	Thioredoxin (Trx)	0.2847	P17931	Galectin-3 (Gal-3)	1.2142
P10643	Complement component C7	0.0956	P17936	Insulin-like growth factor-binding protein 3 (IGFBP-3)	0.0753
P10645	Chromogranin-A (CgA; SP-I)	0.0061	P18065	Insulin-like growth factor-binding protein 2 (IGFBP-2)	0.0543
P10721	Mast/stem cell growth factor receptor Kit (CD117)	0.0093	P18206	Vinculin	1.1308
P10909	Clusterin (Apolipoprotein J; Apo-J)	0.0949	P18428	Lipopolysaccharide-binding protein (LBP)	0.0741
P11021	Endoplasmic reticulum chaperone BiP	0.0802	P18669	Phosphoglycerate mutase 1	0.5560
P11142	Heat shock cognate 71 kDa protein (LAP-1)	0.1795	P19022	Cadherin-2 (N-cadherin; CD antigen CD325)	0.1155
P11226	Mannose-binding protein C (Mannose-binding lectin)	0.1385	P19320	Vascular cell adhesion protein 1 (VCAM-1; CD106)	0.1341

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
P19367	Hexokinase-1 (HK I)	0.0381	P31949	Protein S100-A11 (Calgizzarin)	0.0862
P19429	Troponin I, cardiac muscle (Cardiac troponin I)	0.0952	P32004	Neural cell adhesion molecule L1 (N-CAM-L1; CD171)	0.0895
P19652	Alpha-1-acid glycoprotein 2 (AGP 2)	0.0712	P32119	Peroxiredoxin-2 (NKEF-B)	1.0247
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	0.5050	P32929	Cystathionine gamma-lyase	2.3504
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	0.0550	P33151	Cadherin-5 (VE-cadherin; CD antigen CD144)	0.0601
P20062	Transcobalamin-2	0.3486	P33908	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	0.0883
P20366	Protachykinin-1 (PPT)	0.1850	P34931	Heat shock 70 kDa protein 1-like	0.1416
P20742	Pregnancy zone protein	0.1587	P34932	Heat shock 70 kDa protein 4	0.3101
P20774	Mimecan (Osteoglycin)	0.7487	P35030	Trypsin-3	0.0810
P20851	C4b-binding protein beta chain	0.1061	P35232	Prohibitin	17.9476
P20929	Nebulin	0.3461	P35443	Thrombospondin-4	0.0572
P21246	Pleiotrophin (OSF-1)	0.1710	P35542	Serum amyloid A-4 protein (C-SAA)	0.1689
P21333	Filamin-A (FLN-A; Filamin-1)	0.7438	P35555	Fibrillin-1	0.4423
P21709	Ephrin type-A receptor 1 (hEpha1)	0.0447	P35579	Myosin-9 (Myosin heavy chain 9)	0.1983
P21926	CD9 antigen (Tetraspanin-29; CD antigen CD9)	0.4423	P35590	Tyrosine-protein kinase receptor Tie-1	0.0132
P22105	Tenascin-X (TN-X)	0.0593	P35858	ALS	0.0439
P22301	Interleukin-10 (IL-10)	0.2073	P35916	Vascular endothelial growth factor receptor 3 (FLT-4)	n/a
P22352	Glutathione peroxidase 3 (GPx-3)	0.1572	P36222	Chitinase-3-like protein 1	0.0738
P22692	Insulin-like growth factor-binding protein 4 (IGFBP-4)	0.8178	P36955	Pigment epithelium-derived factor (PEDF; Serpin F1)	0.3291
P22792	Carboxypeptidase N subunit 2	0.0394	P36980	Complement factor H-related protein 2 (FHR-2)	0.2603
P22891	Vitamin K-dependent protein Z	0.0342	P37840	Alpha-synuclein	0.2992
P22897	Macrophage mannose receptor 1 (MMR; CD206)	0.2195	P38646	Stress-70 protein (75 kDa glucose-regulated protein)	0.1391
P23142	Fibulin-1 (FIBL-1)	0.2111	P39060	Collagen alpha-1(XVIII) chain	0.0280
P23470	Receptor-type tyrosine-protein phosphatase gamma	0.1664	P40189	Interleukin-6 receptor subunit beta (IL-6R-beta; CD130)	0.6564
P23528	Cofilin-1	0.4214	P40197	Platelet glycoprotein V (CD antigen CD42d)	0.1947
P23560	Brain-derived neurotrophic factor (BDNF)	0.3563	P41159	Leptin (Obesity factor)	0.5014
P24387	Corticotropin-releasing factor-binding protein	0.2641	P41222	Prostaglandin-H2 D-isomerase (PGDS2)	0.2375
P24592	Insulin-like growth factor-binding protein 6 (IGFBP-6)	0.1232	P41271	Neuroblastoma suppressor of tumorigenicity 1	0.0720
P24593	Insulin-like growth factor-binding protein 5 (IGFBP-5)	0.1759	P42785	Lysosomal Pro-X carboxypeptidase	n/a
P24821	Tenascin (Tenascin-C; TN-C)	0.3237	P43121	Cell surface glycoprotein MUC18 (CD antigen CD146)	0.0709
P25311	Zinc-alpha-2-glycoprotein	0.0769	P43251	Biotinidase (Biotinase)	0.1001
P25774	Cathepsin S	1.9214	P43652	Afamin	0.0124
P25815	Protein S100-P (S100 calcium-binding protein P)	0.1956	P45379	Cardiac muscle troponin T	0.3522
P26038	Moesin	1.6651	P48059	LIMS1	2.4318
P26441	Ciliary neurotrophic factor (CNTF)	0.1360	P48061	Stromal cell-derived factor 1 (SDF-1)	0.9007
P26572	MGAT1	0.0836	P48681	Nestin	0.0967
P26927	Hepatocyte growth factor-like protein	1.2423	P48740	Mannan-binding lectin serine protease 1	0.2496
P27105	Erythrocyte band 7 integral membrane protein	0.3059	P49747	Cartilage oligomeric matrix protein	0.3168
P27169	Serum paraoxonase/arylesterase 1 (PON 1)	0.5451	P49908	Selenoprotein P (SeP)	0.1091
P27487	Dipeptidyl peptidase 4 (DPP IV; CD antigen CD26)	0.0715	P49913	Cathelicidin antimicrobial peptide	0.1976
P27797	Calreticulin	0.2913	P50552	Vasodilator-stimulated phosphoprotein (VASP)	0.4014
P27824	Calnexin	0.0916	P51610	Host cell factor 1 (HCF-1)	0.2194
P27918	Properdin (Complement factor P)	0.2008	P51884	Lumican	0.0357
P29401	Transketolase (TK)	0.4627	P52566	Rho GDP-dissociation inhibitor 2	0.1015
P29622	Kallistatin (Kallikrein inhibitor; Serpin A4)	0.1593	P54108	Cysteine-rich secretory protein 3	1.4589
P30041	Peroxiredoxin-6	0.0439	P54289	Voltage-dependent calcium channel alpha-2/delta-1	0.0596
P30043	Flavin reductase (NADPH)	0.1399	P54802	Alpha-N-acetylglucosaminidase (NAG)	0.1530
P30101	Protein disulfide-isomerase A3	0.5113	P55056	Apolipoprotein C-IV (Apo-CIV)	0.0453
P30990	Neurotensin/neuromedin N	6.5641	P55058	Phospholipid transfer protein (Lipid transfer protein II)	0.0552
P31151	Protein S100-A7 (Psoriasin)	0.1713	P55072	Transitional endoplasmic reticulum ATPase	0.0622
P31944	Caspase-14 (CASP-14)	0.3485	P55103	Inhibin beta C chain	2.3424

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
P55290	Cadherin-13	0.0522	Q13228	Methanethiol oxidase (Selenium-binding protein 1)	0.0650
P58335	Anthrax toxin receptor 2 (CMG-2)	0.2602	Q13231	Chitotriosidase-1 (Chitinase-1)	0.2433
P60174	Triosephosphate isomerase	0.0330	Q13332	Receptor-type tyrosine-protein phosphatase S	0.5877
P60484	PTEN	0.1020	Q13418	Integrin-linked protein kinase (ILK-1; ILK-2)	0.6104
P60660	Myosin light polypeptide 6 (Myosin light chain 3)	0.1812	Q13421	Mesothelin (CAK1 antigen)	0.6963
P60985	Keratinocyte differentiation-associated protein	6.7672	Q13449	Limbic system-associated membrane protein	0.3120
P61626	Lysozyme C	0.2710	Q13740	CD166 antigen	0.0895
P61769	Beta-2-microglobulin	0.3994	Q13790	Apolipoprotein F (Apo-F; LTIP)	0.0566
P61981	14-3-3 protein gamma	0.5181	Q13822	E-NPP 2; Autotaxin	0.1194
P62258	14-3-3 protein epsilon	0.7076	Q14118	Dystroglycan (Dystrophin-associated glycoprotein 1)	0.2632
P62328	Thymosin beta-4	0.1603	Q14126	Desmoglein-2	0.1112
P63104	14-3-3 protein zeta/delta	2.0270	Q14247	Src substrate cortactin (Amplaxin; Oncogene EMS1)	n/a
P67936	Tropomyosin alpha-4 chain (Tropomyosin-4)	0.3050	Q14393	Growth arrest-specific protein 6 (GAS-6)	0.2512
P68871	Hemoglobin subunit beta (Beta-globin)	0.0709	Q14508	WAP four-disulfide core domain protein 2	0.0110
P69905	Hemoglobin subunit alpha (Alpha-globin)	0.0521	Q14515	SPARC-like protein 1	0.1519
P78417	Glutathione S-transferase omega-1	0.0464	Q14520	Hyaluronan-binding protein 2	0.0725
P78509	Reelin	0.0064	Q14623	Indian hedgehog protein (IHH)	0.1837
P78563	Double-stranded RNA-specific editase 1	0.3069	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	0.3453
P80108	Phosphatidylinositol-glycan-specific phospholipase D	0.0227	Q14643	Inositol 1,4,5-trisphosphate receptor type 1	0.1965
P80188	Neutrophil gelatinase-associated lipocalin	0.0916	Q14697	Neutral alpha-glucosidase AB	0.2819
P80370	Protein delta homolog 1 (DLK-1)	0.7403	Q14766	LTBP-1; TGF-beta1-BP-1	0.1517
P80511	Protein S100-A12(Calgranulin-C)	3.8371	Q15063	Periostin (PN; OSF-2)	0.6659
P80723	Brain acid soluble protein 1 (NAP-22)	0.1549	Q15113	Procollagen C-endopeptidase enhancer 1	0.0617
P81605	Dermcidin	0.1256	Q15166	Serum paraoxonase/lactonase 3	0.1951
P98160	Perlecan	0.0205	Q15465	Sonic hedgehog protein (SHH)	0.2928
P98164	Low-density lipoprotein receptor-related protein 2	0.2689	Q15485	Ficolin-2	0.1813
Q01459	Di-N-acetylchitobiase	0.1566	Q15582	Beta ig-h3	0.0104
Q01518	Adenylyl cyclase-associated protein 1 (CAP 1)	0.0991	Q15848	Adiponectin	0.0898
Q02985	Complement factor H-related protein 3 (FHR-3)	0.0242	Q15942	Zyxin (Zyxin-2)	0.1274
Q03167	Transforming growth factor beta receptor type 3	0.0355	Q16270	Insulin-like growth factor-binding protein 7 (IGFBP-7)	0.2505
Q03591	Complement factor H-related protein 1 (FHR-1)	0.1224	Q16563	Synaptophysin-like protein 1 (Pantophysin)	0.1083
Q04721	Neurogenic locus notch homolog protein 2 (Notch 2)	0.2506	Q16610	Extracellular matrix protein 1	0.0613
Q04756	Hepatocyte growth factor activator (HGFA)	0.1154	Q16627	C-C motif chemokine 14	0.3806
Q04760	Lactoylglutathione lyase	0.0915	Q16706	Alpha-mannosidase 2	1.4220
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	0.1400	Q16853	Membrane primary amine oxidase (VAP-1)	0.0718
Q06830	Peroxiredoxin-1 (NKEF-A)	1.3683	Q2KHT4	Germ cell-specific gene 1 protein	n/a
Q07954	Prolow-density lipoprotein receptor-related protein 1	0.4915	Q53RD9	Fibulin-7 (FIBL-7)	0.1634
Q08380	Galectin-3-binding protein	0.0341	Q5SRH9	Tetratricopeptide repeat protein 39A	0.8524
Q08629	Testican-1	0.0314	Q5T5C0	Syntaxin-binding protein 5 (Tomosyn-1)	0.1625
Q08830	Fibrinogen-like protein 1	0.7219	Q6B0K9	Hemoglobin subunit mu (Mu-globin)	0.0124
Q0VDF9	Heat shock 70 kDa protein 14 (HSP60)	0.1171	Q6EMK4	Vasorin (Protein slit-like 2)	0.1397
Q10588	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2	0.2465	Q6GTX8	LAIR-1; CD antigen CD305	0.0466
Q12794	Hyaluronidase-1 (Lung carcinoma protein 1)	0.1710	Q6Q788	Apolipoprotein A-V (Apo-AV)	0.0317
Q12805	Fibulin-3	0.1188	Q6UX71	Plexin domain-containing protein 2	0.0566
Q12860	Contactin-1 (Glycoprotein gp135)	0.0862	Q6UXB8	Peptidase inhibitor 16	0.0867
Q12884	Prolyl endopeptidase FAP (Sepsase)	0.4573	Q6UXD5	Seizure 6-like protein 2	0.3949
Q12912	Lymphoid-restricted membrane protein	0.4817	Q6UY14	ADAMTS-like protein 4 (ADAMTSL-4)	0.1159
Q12913	Receptor-type tyrosine-protein phosphatase eta	0.0650	Q6YHK3	CD109 antigen (CD antigen CD109)	0.1679
Q13093	Platelet-activating factor acetylhydrolase	0.1624	Q76LX8	ADAM-TS13	0.1030
Q13103	Secreted phosphoprotein 24 (Spp-24)	0.0423	Q7L4I2	Arginine/serine-rich coiled-coil protein 2	1.2032
Q13201	Multimerin-1 (EMILIN-4)	0.1337	Q7Z7D3	V-set domain-containing T-cell activation inhibitor 1	1.2438

Protein ID	Protein Name	LOD (fmol)	Protein ID	Protein Name	LOD (fmol)
Q7Z7G0	Target of Nesh-SH3	0.0867	Q9HBB8	Cadherin-related family member 5	0.4596
Q7Z7M0	EGF-like protein 4	0.2609	Q9HBI1	Beta-parvin (Affixin)	0.0667
Q86SQ4	Adhesion G-protein coupled receptor G6 (GPR126)	0.0455	Q9HCU0	Endosialin (Tumor endothelial marker 1; CD248)	0.1196
Q86U17	Serpin A11	0.3777	Q9HD89	Resistin (Adipose tissue-specific secretory factor)	0.0516
Q86UD1	Out at first protein homolog	0.0682	Q9HDC9	Adipocyte plasma membrane-associated protein	0.2339
Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	0.1715	Q9NP80	Calcium-independent phospholipase A2-gamma	0.0625
Q86UX7	Fermitin family homolog 3 (Kindlin-3)	0.3399	Q9NPH3	Interleukin-1 receptor accessory protein (IL-1RAcP)	0.0451
Q86VB7	Scavenger receptor cysteine-rich type 1 protein M130	0.0839	Q9NPY3	Complement component C1q receptor (CD93)	0.2401
Q86YZ3	Hornerin	15.1396	Q9NQ79	Cartilage acidic protein 1	0.0931
Q8IUX7	Adipocyte enhancer-binding protein 1	0.7476	Q9NQC3	Reticulon-4 (Nogo protein)	0.8217
Q8N4F0	BPI fold-containing family B member 2	0.1339	Q9NY97	Beta-1,3-N-acetylglucosaminyltransferase 1	0.0225
Q8N6C8	CD antigen CD85e	0.1878	Q9NZ08	Endoplasmic reticulum aminopeptidase 1 (ARTS-1)	0.1390
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	0.1026	Q9NZP8	Complement C1r subcomponent-like protein (C1r-LP)	0.0999
Q8NE71	ATP-binding cassette sub-family F member 1	0.0301	Q9UBR2	Cathepsin Z (Cathepsin X)	0.1241
Q8TB96	T-cell immunomodulatory protein	0.0395	Q9UBX5	Fibulin-5 (FIBL-5)	0.3187
Q8WUJ3	CEMP	0.1450	Q9UEW3	Macrophage receptor MARCO	0.0127
Q8WWA0	Intellectin-1 (Omentin)	0.7210	Q9UGM5	Fetuin-B	0.0794
Q8WXD2	Secretogranin-3	0.2134	Q9UHG2	ProSAAS (Proprotein convertase 1 inhibitor)	0.0161
Q8WXI7	Mucin-16 (MUC-16; CA125)	0.3615	Q9UHG3	Prenylcysteine oxidase 1 (Prenylcysteine lyase)	1.3858
Q8WZ75	Roundabout homolog 4	0.0841	Q9UJJ9	GlcNAc-1-phosphotransferase subunit gamma	0.3967
Q92496	Complement factor H-related protein 4 (FHR-4)	0.2030	Q9UK55	Protein Z-dependent protease inhibitor (Serpin A10)	0.1677
Q92520	Protein FAM3C (Interleukin-like EMT inducer)	0.1157	Q9ULI3	Protein HEG homolog 1	1.7909
Q92820	Gamma-glutamyl hydrolase	1.6103	Q9UNN8	Endothelial protein C receptor (CD antigen CD201)	0.0576
Q92876	Kallikrein-6 (Neurosin)	0.0347	Q9UNW1	Multiple inositol polyphosphate phosphatase 1	0.1187
Q92954	Proteoglycan 4 (Lubricin)	0.0735	Q9Y490	Talin-1	0.0911
Q969H8	Myeloid-derived growth factor (MYDGF; IL-25)	0.1872	Q9Y4L1	Hypoxia up-regulated protein 1	0.2255
Q96IY4	Carboxypeptidase B2 (pCPB)	0.0850	Q9Y5C1	Angiopoietin-related protein 3 (Angiopoietin-5)	4.8573
Q96JB8	MAGUK p55 subfamily member 4	7.7415	Q9Y5Y7	LYVE-1	0.0623
Q96KN2	Beta-Ala-His dipeptidase (Serum carnosinase)	0.2184	Q9Y646	Carboxypeptidase Q	0.1011
Q96PD5	N-acetylmuramoyl-L-alanine amidase	0.8207	Q9Y6R7	IgGfc-binding protein	0.1309
Q96QR1	Secretoglobin family 3A member 1	0.4734	Q9Y6Z7	Collectin-10 (CL-L1)	0.2703
Q96S96	Phosphatidylethanolamine-binding protein 4 (PEBP-4)	0.5215			
Q99497	Protein/nucleic acid deglycase DJ-1	0.9656			
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			