Enhanced recovery of CD9-positive extracellular vesicles from human specimens by chelating reagent

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Abstract

Extracellular vesicles (EVs) have gained attention as potential targets of early diagnostics and prognosis in the field of liquid biopsy. Despite clinical potentials, the best method to isolate EVs from specimens remains controversial due to low purity, low specificity, and lack of reproducibility with current isolation methods. Here we show that a chelating reagent enhances the recovery efficiency of EVs from crude biological samples by immunoprecipitation using an anti-CD9 antibody. Proteomic and western blotting analyses show that the EVs isolated using the chelating reagent contain a wider variety of proteins than those isolated with PBS.

Introduction

EVs are secreted from all types of cells and found in most body fluids, including blood, urine, and saliva(1–3). Several functions of EVs have been reported e.g., intercellular interaction and cancer metastasis; however, the exact biological role of EVs is unclear.

In addition, EVs contain several biologically active molecules such as proteins, mRNA, miRNA, long non-coding RNA (lncRNA), DNA, and phospholipids, which reflect states of cells or organs (4,5). Such biomolecules found in EVs are expected to serve as diagnostic biomarkers or prognostic markers for various diseases in clinical fields.

Despite the high clinical need, EV-based liquid biopsy has not been widely used yet due to a lack of standardized isolation methods from specimens. The most commonly used techniques for EV isolation are ultracentrifugation (UC) and polymer-based precipitation. UC and polymer-based precipitation methods, however, cause aggregation of EVs (6). Furthermore, these techniques are compromised by low selectivity: EVs include microvesicles, apoptotic bodies, and exosomes (7), and such heterogeneity leads to the lack of reproducibility and reliability, hampering clinical utilization. Recent studies suggested that the surface of EVs is positively charged, and it attracts negatively charged molecules such as DNA- and RNA-binding proteins are found on surface of EVs (8).

Rapid and selective isolation of EVs is possible by immunoprecipitation (IP) methods using antibodies against surface marker proteins of interest. Hence, we employed IP method based on surface marker proteins for rapid and specific isolation of EVs. Though, the recovery rate of EVs by IP method is not enough for clinical use, here we report that IP method with a chelating reagent improves yields and purity of EVs from multiple human specimens. The isolated EVs could be used for quantitative and qualitative proteomic analyses with LC-MS/MS. We report that IP method with a chelating reagent improves yields and purity of EVs from multiple human specimens.

Materials and Methods

Materials

NIH-H1299 cell lines were obtained from ATCC. Minimum Essential Medium (MEM) Non-essential Amino Acids Solution and Penicillin-Streptomycin solution (x100) were purchased from FUJIFILM Wako Pure Chemical Corporation. Fetal Bovine Serum (FBS) was purchased from Gibco, Thermo Fisher Scientific Inc. Ethylenediaminetetraacetic acid (EDTA) and glycoletherdiamine-N,N,N',N'-tetraacetic acid (EGTA) were purchased from Dojindo Molecular Technologies, Inc. 1,4-Dithiothreitol (DTT) was purchased from FUJIFILM Wako Pure Chemical Corporation. Anti-CD81 antibody (SHI-EXO-MO3), and pooled human serum were purchased from Cosmo Bio Co., Ltd. A matched set of specimens was purchased from ProMedDx, LLC. MagCapture Exosome Isolation Kit PS was purchased from FUJIFILM Wako Pure Chemical Corporation. Total Exosome Isolation Reagent (from serum) was purchased from Thermo Fisher Scientific Inc. The following antibodies were used for immunostaining: Goat anti-human albumin antibody HRP Conjugated (A80-129P, Bethyl Laboratories, Inc.), goat anti-human IgG H&L (HRP) (ab6858, Abcam plc), anti-apolipoprotein E antibody (Biotin) (ab24274, Abcam plc). Volunteers for saliva and urine samples were recruited from Miraca Research Institute G.K., and the study was conducted with the approval (18-30) by the institutional review board of Miraca Holdings, Inc. Urine and saliva samples were diluted 3-fold with PBS or EDEG.

Preparation of serum EVs by UC

Serum (1 mL) was centrifuged at 15,000 x g for 15min at 4°C, and the supernatant was ultracentrifuged at 100,000 x g for 1 h at 4°C by himac (Koki Holdings Co., Ltd.) with S55A2 rotor (k-factor: 162). The pellet was resuspended in 1 mL of PBS or EDEG (50 mM EDTA and 50 mM EGTA in PBS), followed by ultracentrifugation at 100,000 x g for 1 h at 4°C to eliminate other contaminating molecules. Resulting EVs were resuspended in PBS and stored at 4°C until use.

EV isolation from culture media by UC

NIH-H1299 cells were grown in 10% FBS, MEM Non-essential Amino Acids Solution (x100), and Penicillin-Streptomycin solution (x100). Subconfluent cells were washed with PBS twice and grown for 72 h in FBS-free medium before EV isolation. The cell culture supernatants were collected and filtered through 0.22 µm pore membranes using the vacuum filter/storage bottle system (Corning Incorporated) to remove large contaminating vesicles. The obtained conditioned medium was centrifuged at 2,000 x g for 5 min at 4°C, followed by concentration using Amicon Ultra-15 Centrifugal Filter Unit (100 kDa cutoff, Merck KGaA). The concentrated medium (6 mL) was centrifuged at 15,000 x g for 15min at 4°C, and the supernatant was followed by ultracentrifugation at 100,000 x g for 1 h at 4°C by himac (Koki Holdings Co., Ltd.) with an S55A2 rotor (k-factor: 161.6). The pellet was resuspended in 100 µL of PBS or EDEG (50 mM EDTA and 50 mM EGTA in PBS), followed by ultracentrifugation at 100,000 x g for 1 h at 4°C. The resulting pellet that contains EVs was resuspended in PBS and stored at 4° C until use. The protein concentration of the purified EVs was determined by Qubit protein assay kit (Thermo Fisher Scientific Inc.).

Polymer-based precipitation

Total Exosome Isolation Reagent (from serum) was used according to the manufacturer's instruction. Frozen pooled serum was thawed at room temperature and centrifuged for 30 min at 2000 x g at 4°C. The supernatant (1 mL) was mixed with 60 µL of Total Exosome Isolation Reagent (from serum). The sample was incubated at 4°C for 30 min. The mixture was centrifuged at 10,000 x g at 4°C and the obtained pellet was resuspended in PBS. The pellet containing EVs was stored at 4°C until use.

Preparation of antibodies against CD9 and CD63

Antibodies against CD9 and CD63 were prepared as described previously (9) with the modification that 50 µg of EVs from NIH-H1299 obtained by ultracentrifugation method was used as a the source of antigen. Biotinylations of the anti-CD9 monoclonal antibody and anti-CD63 monoclonal antibody were performed using EZ-Link™ Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific Inc.) according to the manufacturer's protocol.

IP of EVs

Anti-CD9 antibody coupled with Dynabeads M-280 Tosylactivated (Thermo Fisher Scientific Inc.) was added to matched set specimens (serum and plasma), urine, and saliva that were diluted 1:3 with EDEG or PBS, followed by incubation on a rotator at 4°C for 18 h. The beads were washed three times with PBS and stored at 4°C until further analysis.

Western blotting analysis

The antibodies were diluted with Can Get Signal (TOYOBO Co., LTD.) to 1 µg/mL. Immunocaptured EVs on Dynabeads M-280 Tosylactivated were lysed by 4x Laemmli Sample Buffer (Bio-Rad Laboratories, Inc.) under nonreducing condition (without DTT) for CD9, CD63, and CD81 or reducing condition (supplemented with 50 mM DTT) for other proteins followed by boiling for 5 min at 96°C. The obtained protein samples were separated by SDS-PAGE and then transferred to PVDF membranes (ATTO Corporation.). After blocking with Blocking One (NACALAI TESQUE, INC.), the membranes were incubated with primary antibodies followed by incubation with secondary antibodies for 1 h at room temperature. The membranes were washed with PBS with Tween® 20 (TAKARA BIO INC.) three times and incubated with secondary antibodies for 1 h at room temperature. After washing, ECL™ Select Western Blotting Detection Reagent (GE Healthcare, GENERAL ELECRIC COMPANY) was added to the membrane. Protein was detected by using ImageQuant™ LAS 500 imager (GE Healthcare, GENERAL ELECTRIC COMPANY).

Preparation of peptide

Pelleted EVs were lysed with 40 µL of 1% *Rapi*Gest™ SF (Waters Corporation) in 50 mM ammonium bicarbonate (Honeywell Fluka™, Thermo Fisher Scientific, Inc.) supplemented with 50 mM DTT, followed by incubation at 60°C for 30 min. After being allowed to cool down to room temperature, the lysed samples were added by 4 µL of 150 mM 2-iodoacetamide and incubated at room temperature for 30 min in the dark. The lysates were incubated with 1 µg/mL of Trypsin/Lys-C Mix, Mass Spec Grade (Promega Corporation) at 37°C overnight. To break down the detergent, 4 µL of 10% of trifluoroacetic acid (Thermo Fisher Scientific, Inc.) was added to the digested mixture and incubated at 37 °C for 30min. After centrifugation at 13,000 x g for 10 min, the supernatant was collected and lyophilized with miVac system (Genevac Ltd) and desalted with Pierce C-18 Spin Columns according to the manufacturer's instruction. Obtained peptides were eluted with 70% acetonitrile, followed by lyophilization, and stored at -80 °C until use.

Proteomic analysis with LC-MS

Obtained peptides were reconstituted by 20ul of water containing 0.1% formic acid (FA) (Fisher Chemical, Thermo Fisher Scientific, Inc.). The proteomic analysis of the peptides was carried out on Q Exactive (Thermo Fisher Scientific, Inc.) equipped with

UltiMate 3000 Nano LC Systems (Thermo Fisher Scientific, Inc.). Peptide sample (2 µL) was injected onto an Acclaim PepMap 1000 trap column (75 µm × 2 cm, nanoViper C18 3 µm, 100Å, Thermo Fisher Scientific) which was heated to 40 °C in a chamber which was connected to a C18 reverse-phase Aurora UHPLC Emitter Column with nano Zero & Captive Spray Insert (75 µm × 25 cm, Ion Opticks Pty Ltd) using Dreamspray interface (AMR INCORPORATED). Nano pump flow rate was set to 250 nL/min with 170 min gradient, where the mobile phases were A (0.1% FA in water, Fisher Chemical, Thermo Fisher Scientific, Inc.) and B (0.1% FA in acetonitrile, Fisher Chemical, Thermo Fisher Scientific, Inc.). The chromatography gradient was designed to provide a linear increase 0-8 min at 2% B, 8-15 min from 2% B to 15% B, 15-149 min from 15% B to 40% B, 149-150 min from 40% B to 95% B, wash, 8 min and 11 min equilibrium. The data-dependent acquisition was performed in positive ion mode. Mass spectrometer parameters were as follows: MS full scan from *m/z* 350–1500 at a resolution of 70,000, AGC target 3 x e^6, maximum injection time 100 ms, and dd-MS^2/dd-SIM parameters included AGC target: 1 x e^5, maximum injection time: 120 ms, TopN: 10, and isolation window 1.6 *m/z*. dd settings were Minimum AGC of 2.5 x e^3, Charge exclusion unassigned, 1, 7, 8, >8, and Dynamic exclusion was set to 30 s.

All MS/MS samples were analyzed using Proteome Discoverer 2.2.0.388, which was set up to search UniProt-human.fasta (downloaded January 2019). Proteins were identified using the following parameters: Precursor mass tolerance: 10ppm, Fragment mass tolerance: 0.02 Da, Max missed cleavage sites: 2, Dynamic modification: Oxidation / + 15.995 Da (M), Dynamic modifications (protein terminus): N-Terminal Modification: Acetyl/ +42.011 Da (N-Terminus), Static Modification: Carbamidomethyl / +57.021 Da (C). Target FDR (Strict): 0.01, Target FDR (Relaxed): 0.05.

Statistical analysis

For comparison between EVs that were CD9-immunoprecipitated with or without EDEG, proteins were identified in at least three runs and quantified with label-free quantification (LFQ) values using Proteome Discoverer. LFQ parameters were set to Normalization mode: Total peptide amount, Imputation mode: missing value: Low abundance resampling, Ratio calculation: Summed abundance based. Median of obtained normalized abundances of each identified protein, medians of proteins between two sample groups, log2, -log10, p-value, and fold change were calculated using Microsoft Excel and visualized by Volcano plots using R (version 3.5.3).

Results

Chelating reagent enhanced the efficiency of IP in serum, plasma, saliva, and urine samples using anti-CD9 antibody

We have compared the yields of immunocapture using the anti-CD9 antibody in matched set specimens. Western blot analysis showed that the CD9 signal of EDTA plasma was the strongest in comparison to serum and plasma containing other anticoagulants e.g., heparin, acid-citrate-dextrose, citrate phosphate dextrose, and sodium citrate (Fig.1 A). Based on the assumption that chelating reagent would enhance the efficiency of immunocapture using the anti-CD9 antibody, we performed immunoprecipitation of CD9 positive vesicles with EDEG reagent (50mM EDTA and 50mM EGTA in PBS). As we have expected, the yields of immunocaptured CD9 with EDEG reagent was improved compared with PBS dilution specimens (Fig.1 B). The yields of CD9 from urine or saliva were slightly improved (Fig.1 C). These differences might reflect the crudeness of specimens, that is, saliva and urine contained fewer contaminant proteins compared to serum and plasma(10).

The purity of immunocaptured CD9 positive vesicles was higher using EDEG reagent than immunocapture with PBS, ultracentrifugation method, and polymer-based precipitation

We carried out a series of western blot analyses for serum EVs isolated by Ultracentrifugation (UC), Total Exosome Isolation Reagent (polymer-based precipitation), and immunoprecipitation with an anti-CD9 antibody in EDEG-treated or PBS-treated specimens (EDEG-CD9IP and PBS-CD9IP, respectively). EVs isolated with UC or polymer-based precipitation method were shown to contain both EV-associated proteins (CD9, CD63, and CD81) and non-EV marker proteins (HSA, HIGG, and ApoE) (Fig.2). These results suggest that the EVs isolated using these methods contain a substantial amount of contaminant proteins, which is in line with the previous report (3). On the other hand, EVs in EDEG-CD9IP showed a dramatic reduction in the amount of non-EV marker proteins compared with UC and polymer-based purification. In addition, the amount of EV-associated proteins detected in EDEG-CD9IP was greater than that in PBS-CD9IP. These results indicate that EDEG reagent enables the isolation of highly pure EVs with increased efficiency.

Proteomic profiling of CD9 immunoprecipitated EVs from serum diluted with PBS or EDEG reagent

We have carried out proteomic analysis of CD9-immunoprecipitated EVs by LC-MS/MS. Proteomic analysis of EVs with and without EDEG reagent identified 159 proteins and 106 proteins, respectively. Here, EV markers such as CD9 and CD81 were identified only in EDEG diluted serum (Fig.3 A). LFQ analysis was performed to quantify each identified protein. Volcano plot analysis showed EVs immunoprecipitated without EDEG reagent (PBS) contained abundant complement proteins compared to those with EDEG reagent (Fig. 3B). Also, the EVs without EDEG reagent contained complement-related proteins such as properdin and complement C1r, and calcium-binding proteins (i.e. sorcin) (supplementary Table.1). These results suggested that EVs isolated by IP with the EDEG reagent give us access to more potential proteins for clinical diagnosis.

Discussion

Although a large number of EV purification methods and kits are now available, it has been demonstrated that non-EV associated proteins and molecules are co-isolated as contaminants (3). In this report, we have shown that IP method using a chelating reagent increases the purity and the yield of CD9 positive vesicles from multiple body fluids, such as serum, plasma, urine, and saliva.

In addition, EVs are heterogeneous vesicles composed of apoptotic bodies, microvesicles, and exosomes(7). The ratio of EV subpopulations and the recovery rate of each subpopulation depends on isolation methods and conditions of biofluids, both of which account for the low repeatability of EV separation.

Accordingly, we have developed a method to improve yields and purity of EVs isolated from multiple specimens for clinical use. In this study, we employed an immunocapture method to isolate EVs with high specificity. As shown in Fig.1A, the amount of recovered CD9 positive EVs varies depending on the added anticoagulants, and chelating reagent improves the reliability of IP isolation of EVs from biofluids. These results may reflect the fact that matrix proteins in specimens are associated with EV surface proteins (8), and these proteins inhibit interactions between antibodies and their targeted membrane proteins on EVs for IP assays.

Quantitative proteomic analysis revealed that IP using EDEG reduced the amount of

calcium-dependent adhesion proteins including complement family proteins and immunoglobulin families (Supplementary Table.1). It has been reported that membrane adhesion proteins such as integrin, albumin, and cadherin family need metallic ions for interaction with proteins on the surface of the membrane (11). Since the EV marker protein CD9 is known to interact with integrin families (α6β1 and α5β1), fibronectin, and immunoglobulins (12), chelating reagent enables the dissociation of extracellular contaminant proteins and enhance the interaction between EVs surface CD9 and anti-CD9 antibody.

Furthermore, the quantitative proteomic analysis showed that the reduction of contaminant proteins increased the number of identified proteins, and EV marker proteins such as CD9 and CD81 were only identified in EDEG-based IP (Fig.3A). It indicates that extracellular matrix proteins abound in EVs fraction and they mask the proteins of interest when carrying out proteomics analyses. UC method is time-consuming and it includes substantial amount of contaminant proteins, while polymer method is rapid but additives or ingredients contained in EV isolation reagents sometimes adversely affect subsequent analyses, e.g., mass spectrometry. Our study showed that the EDEG chelating reagent is applicable to proteomic analysis as well as other omics analyses.

Taken together, our results show that chelating reagents are capable of efficiently recovering CD9 positive EVs from serum, plasma, and urine, which could contribute to clinical application of EVs and biomarker detection for diagnosis. EDEG combined with IP using an antibody against specific protein may even enable the isolation of certain EVs population, e.g., ones containing organ-specific membrane protein of clinical interest. It will also provide a reliable tool in clinical labs with automated systems.

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Figure legends

Figure 1 Chelating reagent EDEG enhanced the recovery rate of CD9 positive EVs from serum, saliva, urine, and plasma.
(A) Immunoprecipitated CD9 positive EVs from matched set specimens, including serum and plasma containing different anticoagulants, heparin, acid-citrate-dextrose (ACD), citrate phosphate dextrose (CPD), sodium citrate (citrate), and EDTA were detected by anti-CD9 antibody.
(B) Western blotting analysis of immunoprecipitated CD9 positive EVs from matched specimen with or without EDEG were performed. (C) Saliva and urine with or without EDEG were subjected to western blot analysis by anti-CD9 antibody. Each specimen used for EV isolation was 300 µL.

Figure 2 Western blot analysis of serum EVs isolated by CD9 immunoaffinity. PBS-CD9IP, EDEG-CD9IP, UC, and Polymer represent EVs isolated by IP method with CD9 antibody using PBS, using EDEG, ultracentrifugation method, and polymer-based precipitation, respoctively. Abbreviations: HSA, Human serum albumin; HIGG, human immunoglobulin; ApoE, apolipoprotein E.

Figure 3 LC-MS analysis of immunocaptured EVs using anti-CD9 antibody from serum diluted with PBS or EDEG reagent. A. Venn diagram displaying proteins immunocaptured CD9 positive EVs from serum diluted with PBS or EDEG. B. Volcano plot analysis. Complement proteins and tetraspanins of interest are highlighted in red.

Supplementary Table.1

List of proteins identified in volcano plot analysis.

Author contributions

Conceptualization: Ayako Kurimoto, Tatsutoshi Inuzuka.
Data curation: Ayako Kurimoto,
Investigation: Tatsutoshi Inuzuka, Yuki Kawasaki, Ayako Kurimoto, Toshiki Ueda.
Methodology: Tatsutoshi Inuzuka, Yuki Kawasaki, Ayako Kurimoto
Visualization: Ayako Kurimoto
Writing – original draft: Ayako Kurimoto
Writing – review & editing: Ayako Kurimoto, Tatsutoshi Inuzuka, Yuki Kawasaki, Fumi Asai, Koichiro Murashima, Kazuya Omi.

AK and TI conceived the project and designed the research. TI, YK, TU, and AK performed the experiments for isolation and characterization of EVs. AK wrote the paper. All authors read and approved the final manuscript.

Fig.1

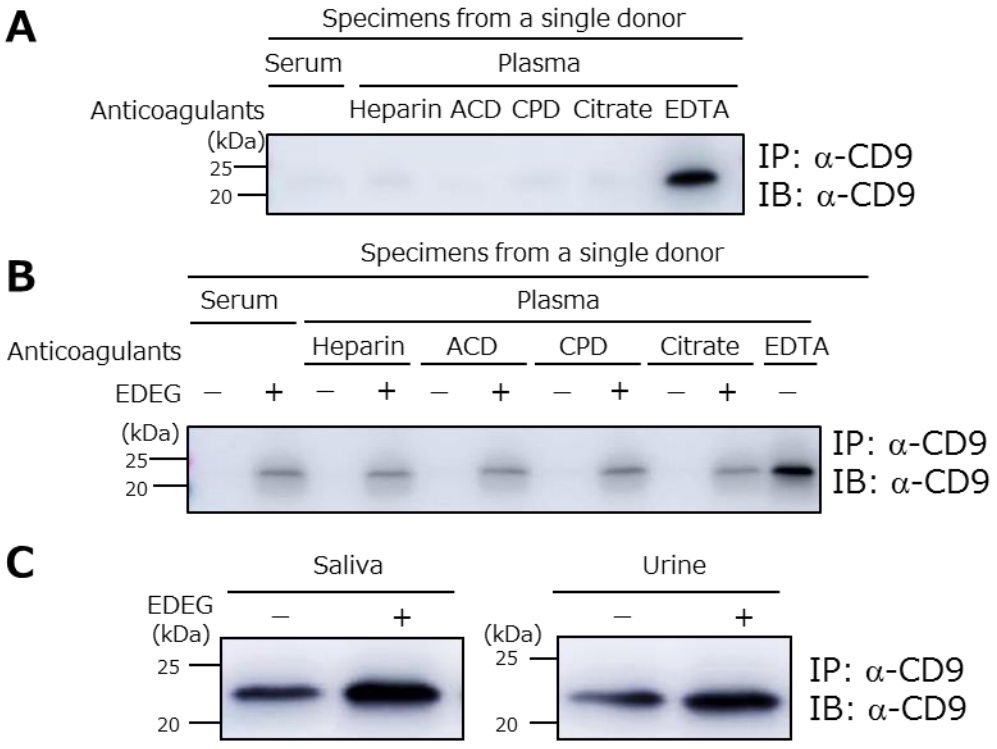


Fig.2

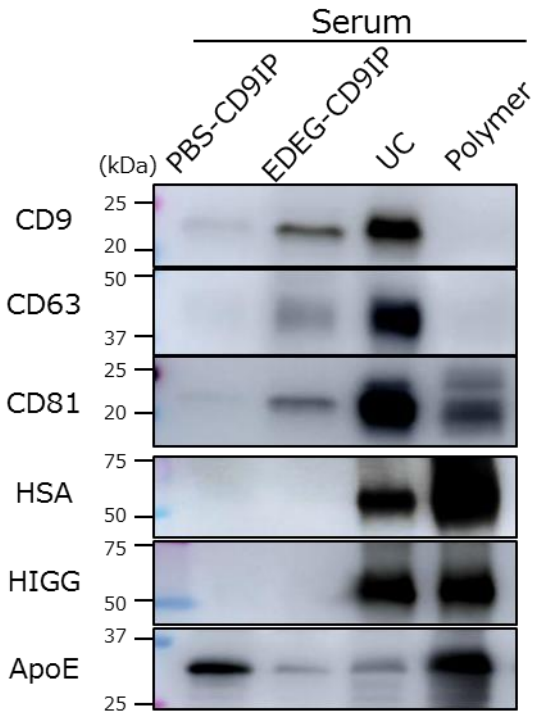
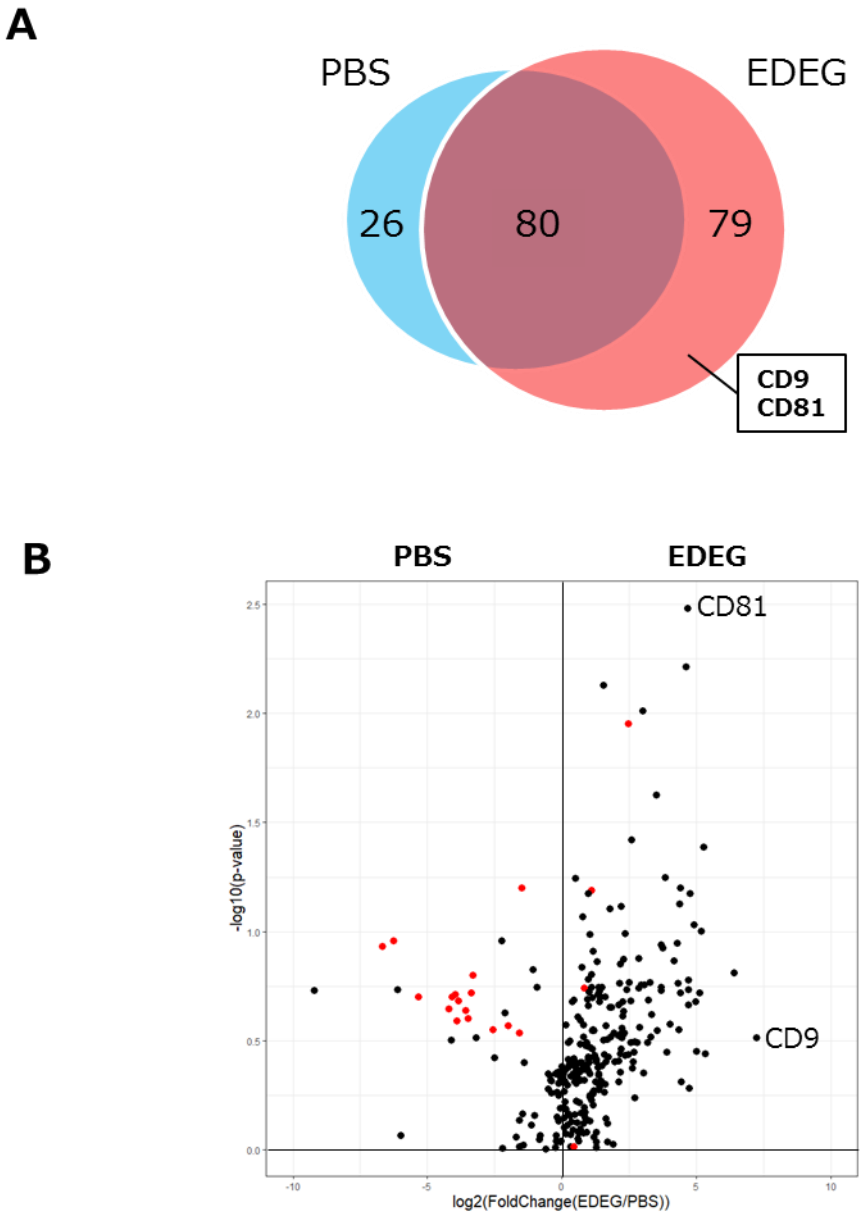


Fig.3



Supplementary Table.1

Accession	Description	Log2 Ratio	-Log10 Pvalue
P27918	Properdin OS=Homo sapiens OX=9606 GN=CFP PE=1 SV=2	-9.21E+00	0.732022915
P00736	Complement C1r subcomponent OS=Homo sapiens OX=9606 GN=C1R PE=1 SV=2	-6.67E+00	0.932703645
P09871	Complement C1s subcomponent OS=Homo sapiens OX=9606 GN=C1S PE=1 SV=1	-6.27E+00	0.958798169
P04003	C4b-binding protein alpha chain OS=Homo sapiens OX=9606 GN=C4BPA PE=1 SV=2	-6.12E+00	0.736169924
P30626	Sorcin OS=Homo sapiens OX=9606 GN=SRI PE=1 SV=1	-6.00E+00	0.066304008
P0C0L5	Complement C4-B OS=Homo sapiens OX=9606 GN=C4B PE=1 SV=2	-5.33E+00	0.701717383
P13671	Complement component C6 OS=Homo sapiens OX=9606 GN=C6 PE=1 SV=3	-4.20E+00	0.645828021
P07225	Vitamin K-dependent protein S OS=Homo sapiens OX=9606 GN=PROS1 PE=1 SV=1	-4.12E+00	0.50545492
P01024	Complement C3 OS=Homo sapiens OX=9606 GN=C3 PE=1 SV=2	-4.09E+00	0.702957103
P08603	Complement factor H OS=Homo sapiens OX=9606 GN=CFH PE=1 SV=4	-3.96E+00	0.711196913
P07360	Complement component C8 gamma chain OS=Homo sapiens OX=9606 GN=C8G PE=1 SV=3	-3.90E+00	0.590594893
Q9BXR6	Complement factor H-related protein 5 OS=Homo sapiens OX=9606 GN=CFHR5 PE=1 SV=1	-3.84E+00	0.682086128
P01031	Complement C5 OS=Homo sapiens OX=9606 GN=C5 PE=1 SV=4	-3.57E+00	0.641050121
P0C0L4	Complement C4-A OS=Homo sapiens OX=9606 GN=C4A PE=1 SV=2	-3.50E+00	0.604352541
P07357	Complement component C8 alpha chain OS=Homo sapiens OX=9606 GN=C8A PE=1 SV=2	-3.38E+00	0.719130797
P06681	Complement C2 OS=Homo sapiens OX=9606 GN=C2 PE=1 SV=2	-3.30E+00	0.802126212
P20851	C4b-binding protein beta chain OS=Homo sapiens OX=9606 GN=C4BPB PE=1 SV=1	-3.20E+00	0.51566606
B4E1Z4	cDNA FLJ55673, highly similar to Complement factor B OS=Homo sapiens OX=9606 PE=1 SV=1	-2.56E+00	0.552723029
P05155	Plasma protease C1 inhibitor OS=Homo sapiens OX=9606 GN=SERPING1 PE=1 SV=2	-2.52E+00	0.422465227
Q8N4Y2	EF-hand calcium-binding domain-containing protein 4A OS=Homo sapiens OX=9606 GN=CRACR2B PE=1 SV=3	-2.24E+00	0.959703387
A0A0C4DH35	Immunoglobulin heavy variable 3-35 (non-functional) (Fragment) OS=Homo sapiens OX=9606 GN=IGHV3-35 PE=1 SV=1	-2.21E+00	0.00798
A0A2R8Y3M9	Uncharacterized protein OS=Homo sapiens OX=9606 PE=4 SV=1	-2.13E+00	0.628116708
P07358	Complement component C8 beta chain OS=Homo sapiens OX=9606 GN=C8B PE=1 SV=3	-1.99E+00	0.570594253
P01701	Immunoglobulin lambda variable 1-51 OS=Homo sapiens OX=9606 GN=IGLV1-51 PE=1 SV=2	-1.70E+00	0.05916425
A0A0B4J1Y8	Immunoglobulin lambda variable 9-49 OS=Homo sapiens OX=9606 GN=IGLV9-49 PE=1 SV=1	-1.59E+00	0.014684573
P23142	Fibulin-1 OS=Homo sapiens OX=9606 GN=FBLN1 PE=1 SV=4	-1.57E+00	0.137267383
P02748	Complement component C9 OS=Homo sapiens OX=9606 GN=C9 PE=1 SV=2	-1.57E+00	0.53668074
P10643	Complement component C7 OS=Homo sapiens OX=9606 GN=C7 PE=1 SV=2	-1.50E+00	1.201645461
P01602	Immunoglobulin kappa variable 1-5 OS=Homo sapiens OX=9606 GN=IGKV1-5 PE=1 SV=2	-1.47E+00	0.166642098
Q96IY4	Carboxypeptidase B2 OS=Homo sapiens OX=9606 GN=CPB2 PE=1 SV=2	-1.42E+00	0.02285973
Q8WWA0	Intelectin-1 OS=Homo sapiens OX=9606 GN=ITLN1 PE=1 SV=1	-1.41E+00	0.400892024
Q16610	Extracellular matrix protein 1 OS=Homo sapiens OX=9606 GN=ECM1 PE=1 SV=2	-1.15E+00	0.113122544
Q9H4M9	EH domain-containing protein 1 OS=Homo sapiens OX=9606 GN=EHD1 PE=1 SV=2	-1.09E+00	0.827058267
Q9Y251	Heparanase OS=Homo sapiens OX=9606 GN=HPSE PE=1 SV=2	-1.01E+00	0.15999596
Q6UXH0	Angiopoietin-like protein 8 OS=Homo sapiens OX=9606 GN=ANGPTL8 PE=1 SV=1	-9.26E-01	0.7470323
P02776	Platelet factor 4 OS=Homo sapiens OX=9606 GN=PF4 PE=1 SV=2	-8.29E-01	0.049963814
P02751	Fibronectin OS=Homo sapiens OX=9606 GN=FN1 PE=1 SV=4	-8.15E-01	0.067937752
A0A0C4DH69	Immunoglobulin kappa variable 1-9 OS=Homo sapiens OX=9606 GN=IGKV1-9 PE=3 SV=1	-6.11E-01	0.00420412
P02654	Apolipoprotein C-I OS=Homo sapiens OX=9606 GN=APOC1 PE=1 SV=1	-5.15E-01	0.348040007
O75915	PRA1 family protein 3 OS=Homo sapiens OX=9606 GN=ARL6IP5 PE=1 SV=1	-5.04E-01	0.280049575
P68371	Tubulin beta-4B chain OS=Homo sapiens OX=9606 GN=TUBB4B PE=1 SV=1	-4.36E-01	0.342736137
Q93084	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3 OS=Homo sapiens OX=9606 GN=ATP2A3 PE=1 SV=2	-4.16E-01	0.319053436
P01860	Immunoglobulin heavy constant gamma 3 OS=Homo sapiens OX=9606 GN=IGHG3 PE=1 SV=2	-4.00E-01	0.261859785
A0A0J9YY99	Uncharacterized protein (Fragment) OS=Homo sapiens OX=9606 PE=1 SV=1	-3.93E-01	0.315021209
P03952	Plasma kallikrein OS=Homo sapiens OX=9606 GN=KLKB1 PE=1 SV=1	-2.45E-01	0.010823604
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens OX=9606 GN=ATP5F1A PE=1 SV=1	-2.41E-01	0.348834496
P06732	Creatine kinase M-type OS=Homo sapiens OX=9606 GN=CKM PE=1 SV=2	-2.24E-01	0.039870339
P08519	Apolipoprotein(a) OS=Homo sapiens OX=9606 GN=LPA PE=1 SV=1	-2.23E-01	0.065477187
Q9HC84	Mucin-5B OS=Homo sapiens OX=9606 GN=MUC5B PE=1 SV=3	-1.80E-01	0.153130918
P51148	Ras-related protein Rab-5C OS=Homo sapiens OX=9606 GN=RAB5C PE=1 SV=2	-1.68E-01	0.265744495
Q9NQC3	Reticulon-4 OS=Homo sapiens OX=9606 GN=RTN4 PE=1 SV=2	-1.58E-01	0.355278863
P61026	Ras-related protein Rab-10 OS=Homo sapiens OX=9606 GN=RAB10 PE=1 SV=1	-1.29E-01	0.30668235
Q71U36	Tubulin alpha-1A chain OS=Homo sapiens OX=9606 GN=TUBA1A PE=1 SV=1	-1.25E-01	0.355947417
P35030	Trypsin-3 OS=Homo sapiens OX=9606 GN=PRSS3 PE=1 SV=2	-1.22E-01	0.13414886
Q99829	Copine-1 OS=Homo sapiens OX=9606 GN=CPNE1 PE=1 SV=1	-1.18E-01	0.250353965
P01703	Immunoglobulin lambda variable 1-40 OS=Homo sapiens OX=9606 GN=IGLV1-40 PE=1 SV=2	-7.55E-02	0.193278561
P48426	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha OS=Homo sapiens OX=9606 GN=PIP4K2A PE=1 SV=2	-5.88E-02	0.33608603
O14787	Transportin-2 OS=Homo sapiens OX=9606 GN=TNPO2 PE=1 SV=3	-5.54E-02	0.037911024
P48740	Mannan-binding lectin serine protease 1 OS=Homo sapiens OX=9606 GN=MASP1 PE=1 SV=3	-5.21E-02	0.316418091
P00747	Plasminogen OS=Homo sapiens OX=9606 GN=PLG PE=1 SV=2	-3.29E-02	0.04221068
P02787	Serotransferrin OS=Homo sapiens OX=9606 GN=TF PE=1 SV=3	-1.19E-02	0.383400825

Accession	Description	Log2 Ratio	-Log10 Pvalue
P04275	von Willebrand factor OS=Homo sapiens OX=9606 GN=VWF PE=1 SV=4	5.88E-02	0.348791815
P02766	Transthyretin OS=Homo sapiens OX=9606 GN=TTR PE=1 SV=1	5.93E-02	0.196454504
P61158	Actin-related protein 3 OS=Homo sapiens OX=9606 GN=ACTR3 PE=1 SV=3	6.93E-02	0.323351268
A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11 OS=Homo sapiens OX=9606 GN=IGKV3D-11 PE=3 SV=6	7.73E-02	0.307939121
P69905	Hemoglobin subunit alpha OS=Homo sapiens OX=9606 GN=HBA1 PE=1 SV=2	8.50E-02	0.147784901
P04114	Apolipoprotein B-100 OS=Homo sapiens OX=9606 GN=APOB PE=1 SV=2	9.09E-02	0.103008407
P19823	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens OX=9606 GN=ITIH2 PE=1 SV=2	9.75E-02	0.267318015
Q9NTJ5	Phosphatidylinositide phosphatase SAC1 OS=Homo sapiens OX=9606 GN=SACM1L PE=1 SV=2	1.03E-01	0.1878762
P01011	Alpha-1-antichymotrypsin OS=Homo sapiens OX=9606 GN=SERPINA3 PE=1 SV=2	1.12E-01	0.219837346
P01700	Immunoglobulin lambda variable 1-47 OS=Homo sapiens OX=9606 GN=IGLV1-47 PE=1 SV=2	1.26E-01	0.368834522
Q13790	Apolipoprotein F OS=Homo sapiens OX=9606 GN=APOF PE=1 SV=2	1.29E-01	0.313723345
P08319	Alcohol dehydrogenase 4 OS=Homo sapiens OX=9606 GN=ADH4 PE=1 SV=5	1.34E-01	0.18989947
P01709	Immunoglobulin lambda variable 2-8 OS=Homo sapiens OX=9606 GN=IGLV2-8 PE=1 SV=2	1.38E-01	0.372456184
P01743	Immunoglobulin heavy variable 1-46 OS=Homo sapiens OX=9606 GN=IGHV1-46 PE=1 SV=2	1.57E-01	0.075058586
P02649	Apolipoprotein E OS=Homo sapiens OX=9606 GN=APOE PE=1 SV=1	1.58E-01	0.573947181
Q9P1Z9	Coiled-coil domain-containing protein 180 OS=Homo sapiens OX=9606 GN=CCDC180 PE=2 SV=3	2.03E-01	0.113890218
Q14141	Septin-6 OS=Homo sapiens OX=9606 GN=SEPT6 PE=1 SV=4	2.16E-01	0.299053235
A0A0A0MS15	Immunoglobulin heavy variable 3-49 OS=Homo sapiens OX=9606 GN=IGHV3-49 PE=3 SV=1	2.32E-01	0.166589487
P19652	Alpha-1-acid glycoprotein 2 OS=Homo sapiens OX=9606 GN=ORM2 PE=1 SV=2	2.33E-01	0.41734336
P15153	Ras-related C3 botulinum toxin substrate 2 OS=Homo sapiens OX=9606 GN=RAC2 PE=1 SV=1	2.34E-01	0.380713027
A0A096LPE2	SAA2-SAA4 readthrough OS=Homo sapiens OX=9606 GN=SAA2-SAA4 PE=4 SV=1	2.36E-01	0.493858432
P68366	Tubulin alpha-4A chain OS=Homo sapiens OX=9606 GN=TUBA4A PE=1 SV=1	2.51E-01	0.388559309
P00450	Ceruloplasmin OS=Homo sapiens OX=9606 GN=CP PE=1 SV=1	3.03E-01	0.500317117
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Homo sapiens OX=9606 GN=ITIH3 PE=1 SV=2	3.04E-01	0.160757905
P20340	Ras-related protein Rab-6A OS=Homo sapiens OX=9606 GN=RAB6A PE=1 SV=3	3.27E-01	0.339965087
P01782	Immunoglobulin heavy variable 3-9 OS=Homo sapiens OX=9606 GN=IGHV3-9 PE=1 SV=2	3.29E-01	0.07264338
Q8WUA8	Tsukushin OS=Homo sapiens OX=9606 GN=TSKU PE=2 SV=3	3.32E-01	0.01463832
A0A075B6K4	Immunoglobulin lambda variable 3-10 OS=Homo sapiens OX=9606 GN=IGLV3-10 PE=3 SV=2	3.52E-01	0.13001641
P02768	Serum albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 SV=2	3.82E-01	0.678114607
Q9Y277	Voltage-dependent anion-selective channel protein 3 OS=Homo sapiens OX=9606 GN=VDAC3 PE=1 SV=1	3.92E-01	0.412709926
P35527	Keratin, type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN=KRT9 PE=1 SV=3	3.99E-01	0.349880943
P12259	Coagulation factor V OS=Homo sapiens OX=9606 GN=F5 PE=1 SV=4	4.07E-01	0.094021819
P01824	Immunoglobulin heavy variable 4-39 OS=Homo sapiens OX=9606 GN=IGHV4-39 PE=1 SV=2	4.43E-01	0.418424153
P36980	Complement factor H-related protein 2 OS=Homo sapiens OX=9606 GN=CFHR2 PE=1 SV=1	4.46E-01	0.01712067
P55209	Nucleosome assembly protein 1-like 1 OS=Homo sapiens OX=9606 GN=NAP1L1 PE=1 SV=1	4.55E-01	0.40280576
P13646	Keratin, type I cytoskeletal 13 OS=Homo sapiens OX=9606 GN=KRT13 PE=1 SV=4	4.57E-01	0.688957995
P07477	Trypsin-1 OS=Homo sapiens OX=9606 GN=PRSS1 PE=1 SV=1	4.61E-01	0.161887604
P02774	Vitamin D-binding protein OS=Homo sapiens OX=9606 GN=GC PE=1 SV=2	4.78E-01	0.405490358
B9A064	Immunoglobulin lambda-like polypeptide 5 OS=Homo sapiens OX=9606 GN=IGLL5 PE=2 SV=2	4.80E-01	0.311743345
P05090	Apolipoprotein D OS=Homo sapiens OX=9606 GN=APOD PE=1 SV=1	4.94E-01	1.246121451
Q9C0H2	Protein tweety homolog 3 OS=Homo sapiens OX=9606 GN=TTYH3 PE=1 SV=3	5.14E-01	0.378099969
P00738	Haptoglobin OS=Homo sapiens OX=9606 GN=HP PE=1 SV=1	5.22E-01	0.303984798
P55058	Phospholipid transfer protein OS=Homo sapiens OX=9606 GN=PLTP PE=1 SV=1	5.35E-01	0.374877886
O00391	Sulfhydryl oxidase 1 OS=Homo sapiens OX=9606 GN=QSOX1 PE=1 SV=3	5.43E-01	0.223394396
P01857	Immunoglobulin heavy constant gamma 1 OS=Homo sapiens OX=9606 GN=IGHG1 PE=1 SV=1	5.50E-01	0.124243038
A0A0U1RRH7	Histone H2A OS=Homo sapiens OX=9606 PE=3 SV=1	5.71E-01	0.336193279
Q9UBV8	Peflin OS=Homo sapiens OX=9606 GN=PEF1 PE=1 SV=1	5.86E-01	0.087304802
P06312	Immunoglobulin kappa variable 4-1 OS=Homo sapiens OX=9606 GN=IGKV4-1 PE=1 SV=1	5.91E-01	0.05269245
P02790	Hemopexin OS=Homo sapiens OX=9606 GN=HPX PE=1 SV=2	6.05E-01	0.610684724
P02656	Apolipoprotein C-III OS=Homo sapiens OX=9606 GN=APOC3 PE=1 SV=1	6.08E-01	0.0787766
A0A075B7D0	Immunoglobulin heavy variable 1/OR15-1 (non-functional) (Fragment) OS=Homo sapiens OX=9606 GN=IGHV1OR15-1 PE=1 SV=1	6.37E-01	0.041992049
P00488	Coagulation factor XIII A chain OS=Homo sapiens OX=9606 GN=F13A1 PE=1 SV=4	6.51E-01	0.356359047
P02763	Alpha-1-acid glycoprotein 1 OS=Homo sapiens OX=9606 GN=ORM1 PE=1 SV=1	6.55E-01	0.361439579
P02679	Fibrinogen gamma chain OS=Homo sapiens OX=9606 GN=FGG PE=1 SV=3	6.57E-01	0.217862997
P07384	Calpain-1 catalytic subunit OS=Homo sapiens OX=9606 GN=CAPN1 PE=1 SV=1	6.70E-01	0.594538723
O00299	Chloride intracellular channel protein 1 OS=Homo sapiens OX=9606 GN=CLIC1 PE=1 SV=4	6.81E-01	0.400944884
P11142	Heat shock cognate 71 kDa protein OS=Homo sapiens OX=9606 GN=HSPA8 PE=1 SV=1	6.84E-01	0.484300133
P02671	Fibrinogen alpha chain OS=Homo sapiens OX=9606 GN=FGA PE=1 SV=2	6.96E-01	0.095256409
P23083	Immunoglobulin heavy variable 1-2 OS=Homo sapiens OX=9606 GN=IGHV1-2 PE=1 SV=2	7.13E-01	0.168375197
P12429	Annexin A3 OS=Homo sapiens OX=9606 GN=ANXA3 PE=1 SV=3	7.20E-01	0.319517789
P09972	Fructose-bisphosphate aldolase C OS=Homo sapiens OX=9606 GN=ALDOC PE=1 SV=2	7.27E-01	0.400708539
P01009	Alpha-1-antitrypsin OS=Homo sapiens OX=9606 GN=SERPINA1 PE=1 SV=3	7.33E-01	0.838712625
O75083	WD repeat-containing protein 1 OS=Homo sapiens OX=9606 GN=WDR1 PE=1 SV=4	7.62E-01	0.389503844
P10909	Clusterin OS=Homo sapiens OX=9606 GN=CLU PE=1 SV=1	7.66E-01	1.06756596
O60814	Histone H2B type 1-K OS=Homo sapiens OX=9606 GN=HIST1H2BK PE=1 SV=3	7.74E-01	0.368557306
P06702	Protein S100-A9 OS=Homo sapiens OX=9606 GN=S100A9 PE=1 SV=1	7.96E-01	0.123238064
P22352	Glutathione peroxidase 3 OS=Homo sapiens OX=9606 GN=GPX3 PE=1 SV=2	8.08E-01	0.57343368
A0A1B0GTC6	Uncharacterized protein C3orf85 OS=Homo sapiens OX=9606 GN=C3orf85 PE=3 SV=1	8.09E-01	0.079920304
Q8TDL5	BPI fold-containing family B member 1 OS=Homo sapiens OX=9606 GN=BPIFB1 PE=1 SV=1	8.11E-01	0.516638234
P02746	Complement C1q subcomponent subunit B OS=Homo sapiens OX=9606 GN=C1QB PE=1 SV=3	8.29E-01	0.743244149
P02675	Fibrinogen beta chain OS=Homo sapiens OX=9606 GN=FGB PE=1 SV=2	8.34E-01	0.195935414
P02652	Apolipoprotein A-II OS=Homo sapiens OX=9606 GN=APOA2 PE=1 SV=1	8.38E-01	0.321925905
P21333	Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4	8.47E-01	0.173786138
A0A0B4J2D9	Immunoglobulin kappa variable 1D-13 OS=Homo sapiens OX=9606 GN=IGKV1D-13 PE=3 SV=1	8.64E-01	0.146461291
P05106	Integrin beta-3 OS=Homo sapiens OX=9606 GN=ITGB3 PE=1 SV=2	8.82E-01	0.47986135
Q9H3U1	Protein unc-45 homolog A OS=Homo sapiens OX=9606 GN=UNC45A PE=1 SV=1	8.89E-01	0.374106181
Q8IWA5	Choline transporter-like protein 2 OS=Homo sapiens OX=9606 GN=SLC44A2 PE=1 SV=3	8.95E-01	0.36912325
P04792	Heat shock protein beta-1 OS=Homo sapiens OX=9606 GN=HSPB1 PE=1 SV=2	9.08E-01	0.491654414
P24844	Myosin regulatory light polypeptide 9 OS=Homo sapiens OX=9606 GN=MYL9 PE=1 SV=4	9.09E-01	0.382828144
P01817	Immunoglobulin heavy variable 2-5 OS=Homo sapiens OX=9606 GN=IGHV2-5 PE=1 SV=2	9.21E-01	0.37239784
F5H423	Uncharacterized protein OS=Homo sapiens OX=9606 PE=3 SV=1	9.25E-01	0.389293578
Q04771	Activin receptor type-1 OS=Homo sapiens OX=9606 GN=ACVR1 PE=1 SV=1	9.26E-01	0.378025773
P04430	Immunoglobulin kappa variable 1-16 OS=Homo sapiens OX=9606 GN=IGKV1-16 PE=1 SV=2	9.34E-01	0.380172367
P68871	Hemoglobin subunit beta OS=Homo sapiens OX=9606 GN=HBB PE=1 SV=2	9.34E-01	0.083278562
P08697	Alpha-2-antiplasmin OS=Homo sapiens OX=9606 GN=SERPINF2 PE=1 SV=3	9.61E-01	0.692634659
P07911	Uromodulin OS=Homo sapiens OX=9606 GN=UMOD PE=1 SV=1	9.67E-01	0.365936489
Q9H4B7	Tubulin beta-1 chain OS=Homo sapiens OX=9606 GN=TUBB1 PE=1 SV=1	9.67E-01	0.426890221
P61769	Beta-2-microglobulin OS=Homo sapiens OX=9606 GN=B2M PE=1 SV=1	9.79E-01	0.414421024

Q08380	Galectin-3-binding protein OS=Homo sapiens OX=9606 GN=LGALS3BP PE=1 SV=1	9.82E-01	0.6607924
P48059	LIM and senescent cell antigen-like-containing domain protein 1 OS=Homo sapiens OX=9606 GN=LIMS1 PE=1 SV=4	9.87E-01	1.174749508
P30041	Peroxiredoxin-6 OS=Homo sapiens OX=9606 GN=PRDX6 PE=1 SV=3	9.89E-01	0.781281616
P02760	Protein AMBP OS=Homo sapiens OX=9606 GN=AMBP PE=1 SV=1	9.91E-01	0.136668614
P13645	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6	1.00E+00	0.419183873
P11597	Cholesteryl ester transfer protein OS=Homo sapiens OX=9606 GN=CETP PE=1 SV=2	1.01E+00	0.399620449
P02749	Beta-2-glycoprotein 1 OS=Homo sapiens OX=9606 GN=APOH PE=1 SV=3	1.01E+00	0.722385828
P06727	Apolipoprotein A-IV OS=Homo sapiens OX=9606 GN=APOA4 PE=1 SV=3	1.03E+00	0.131697744
O75340	Programmed cell death protein 6 OS=Homo sapiens OX=9606 GN=PDCD6 PE=1 SV=1	1.03E+00	0.350029289
P0CF74	Immunoglobulin lambda constant 6 OS=Homo sapiens OX=9606 GN=IGLC6 PE=1 SV=1	1.03E+00	0.24489747
P35579	Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4	1.04E+00	0.471620926
P04004	Vitronectin OS=Homo sapiens OX=9606 GN=VTN PE=1 SV=1	1.04E+00	0.986828903
O95445	Apolipoprotein M OS=Homo sapiens OX=9606 GN=APOM PE=1 SV=2	1.05E+00	0.251100095
P04899	Guanine nucleotide-binding protein G(i) subunit alpha-2 OS=Homo sapiens OX=9606 GN=GNAI2 PE=1 SV=3	1.08E+00	0.423772089
P02747	Complement C1q subcomponent subunit C OS=Homo sapiens OX=9606 GN=C1QC PE=1 SV=3	1.09E+00	1.189303679
P60953	Cell division control protein 42 homolog OS=Homo sapiens OX=9606 GN=CDC42 PE=1 SV=2	1.10E+00	0.804881366
K7ER74	APOC4-APOC2 readthrough (NMD candidate) OS=Homo sapiens OX=9606 GN=APOC4-APOC2 PE=1 SV=1	1.10E+00	0.229184598
P23528	Cofilin-1 OS=Homo sapiens OX=9606 GN=CFL1 PE=1 SV=3	1.11E+00	0.747469635
P60709	Actin, cytoplasmic 1 OS=Homo sapiens OX=9606 GN=ACTB PE=1 SV=1	1.12E+00	0.464957052
P55056	Apolipoprotein C-IV OS=Homo sapiens OX=9606 GN=APOC4 PE=1 SV=1	1.14E+00	0.262994256
P02647	Apolipoprotein A-I OS=Homo sapiens OX=9606 GN=APOA1 PE=1 SV=1	1.14E+00	0.696953247
P01023	Alpha-2-macroglobulin OS=Homo sapiens OX=9606 GN=A2M PE=1 SV=3	1.14E+00	0.548774908
P34931	Heat shock 70 kDa protein 1-like OS=Homo sapiens OX=9606 GN=HSPA1L PE=1 SV=2	1.15E+00	0.910671974
O43866	CD5 antigen-like OS=Homo sapiens OX=9606 GN=CD5L PE=1 SV=1	1.16E+00	0.206937143
P08133	Annexin A6 OS=Homo sapiens OX=9606 GN=ANXA6 PE=1 SV=3	1.16E+00	0.550072984
P01871	Immunoglobulin heavy constant mu OS=Homo sapiens OX=9606 GN=IGHM PE=1 SV=4	1.17E+00	0.257113883
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens OX=9606 GN=LDHA PE=1 SV=2	1.18E+00	0.547256721
Q9UHG3	Prenylcysteine oxidase 1 OS=Homo sapiens OX=9606 GN=PCYOX1 PE=1 SV=3	1.20E+00	0.272129129
Q9P2E9	Ribosome-binding protein 1 OS=Homo sapiens OX=9606 GN=RRBP1 PE=1 SV=5	1.20E+00	0.059138873
P01042	Kininogen-1 OS=Homo sapiens OX=9606 GN=KNG1 PE=1 SV=2	1.23E+00	0.433330114
P09525	Annexin A4 OS=Homo sapiens OX=9606 GN=ANXA4 PE=1 SV=4	1.24E+00	0.313538481
P01780	Immunoglobulin heavy variable 3-7 OS=Homo sapiens OX=9606 GN=IGHV3-7 PE=1 SV=2	1.27E+00	0.415154041
P01593	Immunoglobulin kappa variable 1D-33 OS=Homo sapiens OX=9606 GN=IGKV1D-33 PE=1 SV=2	1.27E+00	0.037738077
O00194	Ras-related protein Rab-27B OS=Homo sapiens OX=9606 GN=RAB27B PE=1 SV=4	1.28E+00	0.404917715
P62805	Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2	1.29E+00	0.080138918
S4R460	Immunoglobulin heavy variable 3/OR16-9 (non-functional) OS=Homo sapiens OX=9606 GN=IGHV3OR16-9 PE=1 SV=2	1.29E+00	0.011437451
P00734	Prothrombin OS=Homo sapiens OX=9606 GN=F2 PE=1 SV=2	1.32E+00	0.862640142
A0A0C4DH43	Immunoglobulin heavy variable 2-70D OS=Homo sapiens OX=9606 GN=IGHV2-70D PE=3 SV=1	1.34E+00	0.378075535
P01008	Antithrombin-III OS=Homo sapiens OX=9606 GN=SERPINC1 PE=1 SV=1	1.34E+00	0.712451556
Q9Y210	Short transient receptor potential channel 6 OS=Homo sapiens OX=9606 GN=TRPC6 PE=1 SV=1	1.36E+00	0.343122432
O95810	Caveolae-associated protein 2 OS=Homo sapiens OX=9606 GN=CAVIN2 PE=1 SV=3	1.37E+00	0.351502311
P01704	Immunoglobulin lambda variable 2-14 OS=Homo sapiens OX=9606 GN=IGLV2-14 PE=1 SV=2	1.37E+00	0.745079552
P28676	Grancalcin OS=Homo sapiens OX=9606 GN=GCA PE=1 SV=2	1.38E+00	0.278029461
P16284	Platelet endothelial cell adhesion molecule OS=Homo sapiens OX=9606 GN=PECAM1 PE=1 SV=2	1.39E+00	0.6802527
Q6Q788	Apolipoprotein A-V OS=Homo sapiens OX=9606 GN=APOA5 PE=1 SV=1	1.39E+00	0.72920102
P01591	Immunoglobulin J chain OS=Homo sapiens OX=9606 GN=JCHAIN PE=1 SV=4	1.40E+00	0.288969133
P01019	Angiotensinogen OS=Homo sapiens OX=9606 GN=AGT PE=1 SV=1	1.41E+00	0.706067153
P06733	Alpha-enolase OS=Homo sapiens OX=9606 GN=ENO1 PE=1 SV=2	1.41E+00	0.299423638
Q15485	Ficolin-2 OS=Homo sapiens OX=9606 GN=FCN2 PE=1 SV=2	1.44E+00	0.296232385
A0A087WW87	Immunoglobulin kappa variable 2-40 OS=Homo sapiens OX=9606 GN=IGKV2-40 PE=3 SV=2	1.45E+00	0.31838071
P00739	Haptoglobin-related protein OS=Homo sapiens OX=9606 GN=HPR PE=2 SV=2	1.49E+00	0.744053633
A0A075B6H7	Immunoglobulin kappa variable 3-7 (non-functional) (Fragment) OS=Homo sapiens OX=9606 GN=IGKV3-7 PE=1 SV=1	1.49E+00	0.385967577
Q9BXN1	Asporin OS=Homo sapiens OX=9606 GN=ASPN PE=1 SV=2	1.54E+00	2.129773143
P15311	Ezrin OS=Homo sapiens OX=9606 GN=EZR PE=1 SV=4	1.55E+00	0.502695696
P20073	Annexin A7 OS=Homo sapiens OX=9606 GN=ANXA7 PE=1 SV=3	1.58E+00	0.264334651
A0A0C4DH38	Immunoglobulin heavy variable 5-51 OS=Homo sapiens OX=9606 GN=IGHV5-51 PE=3 SV=1	1.59E+00	0.437648502
P01619	Immunoglobulin kappa variable 3-20 OS=Homo sapiens OX=9606 GN=IGKV3-20 PE=1 SV=2	1.62E+00	0.701428794
P01859	Immunoglobulin heavy constant gamma 2 OS=Homo sapiens OX=9606 GN=IGHG2 PE=1 SV=2	1.62E+00	0.301539727
A0A0C4DH72	Immunoglobulin kappa variable 1-6 OS=Homo sapiens OX=9606 GN=IGKV1-6 PE=3 SV=1	1.63E+00	0.568650152
P01876	Immunoglobulin heavy constant alpha 1 OS=Homo sapiens OX=9606 GN=IGHA1 PE=1 SV=2	1.65E+00	0.406552526
P28039	Acyloxyacyl hydrolase OS=Homo sapiens OX=9606 GN=AOAH PE=1 SV=1	1.65E+00	0.143645721
P00558	Phosphoglycerate kinase 1 OS=Homo sapiens OX=9606 GN=PGK1 PE=1 SV=3	1.69E+00	0.038560384
P35908	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens OX=9606 GN=KRT2 PE=1 SV=2	1.71E+00	0.509175908
P01834	Immunoglobulin kappa constant OS=Homo sapiens OX=9606 GN=IGKC PE=1 SV=2	1.71E+00	0.123026497
O14950	Myosin regulatory light chain 12B OS=Homo sapiens OX=9606 GN=MYL12B PE=1 SV=2	1.73E+00	0.427333916
P12931	Proto-oncogene tyrosine-protein kinase Src OS=Homo sapiens OX=9606 GN=SRC PE=1 SV=3	1.78E+00	1.105165113
O14791	Apolipoprotein L1 OS=Homo sapiens OX=9606 GN=APOL1 PE=1 SV=5	1.79E+00	0.654978306
P43304	Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Homo sapiens OX=9606 GN=GPD2 PE=1 SV=3	1.80E+00	0.517304542
P13598	Intercellular adhesion molecule 2 OS=Homo sapiens OX=9606 GN=ICAM2 PE=1 SV=2	1.83E+00	0.655981535
A0A0J9YX35	Immunoglobulin heavy variable 3-64D OS=Homo sapiens OX=9606 GN=IGHV3-64D PE=3 SV=1	1.89E+00	0.026757005
Q01518	Adenylyl cyclase-associated protein 1 OS=Homo sapiens OX=9606 GN=CAP1 PE=1 SV=5	1.93E+00	0.53513779
P50995	Annexin A11 OS=Homo sapiens OX=9606 GN=ANXA11 PE=1 SV=1	1.95E+00	0.405416257
P62979	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens OX=9606 GN=RPS27A PE=1 SV=2	2.09E+00	0.526883504
P08758	Annexin A5 OS=Homo sapiens OX=9606 GN=ANXA5 PE=1 SV=2	2.11E+00	0.409123639
A0A075B6Q5	Immunoglobulin heavy variable 3-64 OS=Homo sapiens OX=9606 GN=IGHV3-64 PE=3 SV=1	2.12E+00	0.312871229
P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	2.12E+00	0.665466929
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens OX=9606 GN=GAPDH PE=1 SV=3	2.15E+00	0.763380793
O75131	Copine-3 OS=Homo sapiens OX=9606 GN=CPNE3 PE=1 SV=1	2.17E+00	0.441430348
O14672	Disintegrin and metalloproteinase domain-containing protein 10 OS=Homo sapiens OX=9606 GN=ADAM10 PE=1 SV=1	2.17E+00	0.358563926
Q31612	HLA class I histocompatibility antigen, B-73 alpha chain OS=Homo sapiens OX=9606 GN=HLA-B PE=1 SV=1	2.18E+00	0.853828502
P80108	Phosphatidylinositol-glycan-specific phospholipase D OS=Homo sapiens OX=9606 GN=GPLD1 PE=1 SV=3	2.19E+00	0.534435615
Q9NR31	GTP-binding protein SAR1a OS=Homo sapiens OX=9606 GN=SAR1A PE=1 SV=1	2.20E+00	0.51678583
P12814	Alpha-actinin-1 OS=Homo sapiens OX=9606 GN=ACTN1 PE=1 SV=2	2.21E+00	0.464403778
Q13418	Integrin-linked protein kinase OS=Homo sapiens OX=9606 GN=ILK PE=1 SV=2	2.21E+00	1.114687748
P47756	F-actin-capping protein subunit beta OS=Homo sapiens OX=9606 GN=CAPZB PE=1 SV=4	2.23E+00	0.514881382
Q9Y490	Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3	2.24E+00	0.559138282
P06396	Gelsolin OS=Homo sapiens OX=9606 GN=GSN PE=1 SV=1	2.25E+00	0.530033356
P0DP02	Immunoglobulin heavy variable 3-30-3 OS=Homo sapiens OX=9606 GN=IGHV3-30-3 PE=3 SV=1	2.26E+00	0.679429238
P61160	Actin-related protein 2 OS=Homo sapiens OX=9606 GN=ACTR2 PE=1 SV=1	2.27E+00	0.615639626

P51149	Ras-related protein Rab-7a OS=Homo sapiens OX=9606 GN=RAB7A PE=1 SV=1	2.28E+00	0.873401382
P08567	Pleckstrin OS=Homo sapiens OX=9606 GN=PLEK PE=1 SV=3	2.29E+00	0.635389335
P14618	Pyruvate kinase PKM OS=Homo sapiens OX=9606 GN=PKM PE=1 SV=4	2.29E+00	0.53390118
P37802	Transgelin-2 OS=Homo sapiens OX=9606 GN=TAGLN2 PE=1 SV=3	2.31E+00	0.535294743
P01877	Immunoglobulin heavy constant alpha 2 OS=Homo sapiens OX=9606 GN=IGHA2 PE=1 SV=4	2.34E+00	0.432809214
P19827	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Homo sapiens OX=9606 GN=ITIH1 PE=1 SV=3	2.36E+00	0.992170994
P02743	Serum amyloid P-component OS=Homo sapiens OX=9606 GN=APCS PE=1 SV=2	2.42E+00	0.735933194
P13647	Keratin, type II cytoskeletal 5 OS=Homo sapiens OX=9606 GN=KRT5 PE=1 SV=3	2.47E+00	0.436609313
P02745	Complement C1q subcomponent subunit A OS=Homo sapiens OX=9606 GN=C1QA PE=1 SV=2	2.48E+00	1.951448407
P08571	Monocyte differentiation antigen CD14 OS=Homo sapiens OX=9606 GN=CD14 PE=1 SV=2	2.51E+00	0.769293691
P27701	CD82 antigen OS=Homo sapiens OX=9606 GN=CD82 PE=1 SV=1	2.56E+00	0.49184045
P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 OS=Homo sapiens OX=9606 GN=GNB1 PE=1 SV=3	2.57E+00	0.682530119
P10114	Ras-related protein Rap-2a OS=Homo sapiens OX=9606 GN=RAP2A PE=1 SV=1	2.60E+00	1.421701331
P18428	Lipopolysaccharide-binding protein OS=Homo sapiens OX=9606 GN=LBP PE=1 SV=3	2.62E+00	0.374808189
Q8WWI5	Choline transporter-like protein 1 OS=Homo sapiens OX=9606 GN=SLC44A1 PE=1 SV=1	2.64E+00	0.404110843
P07996	Thrombospondin-1 OS=Homo sapiens OX=9606 GN=THBS1 PE=1 SV=2	2.67E+00	0.448870766
P01861	Immunoglobulin heavy constant gamma 4 OS=Homo sapiens OX=9606 GN=IGHG4 PE=1 SV=1	2.71E+00	0.238474113
P18206	Vinculin OS=Homo sapiens OX=9606 GN=VCL PE=1 SV=4	2.72E+00	0.496092381
P04075	Fructose-bisphosphate aldolase A OS=Homo sapiens OX=9606 GN=ALDOA PE=1 SV=2	2.79E+00	0.49304897
P13224	Platelet glycoprotein Ib beta chain OS=Homo sapiens OX=9606 GN=GP1BB PE=1 SV=1	2.86E+00	0.878211167
P01833	Polymeric immunoglobulin receptor OS=Homo sapiens OX=9606 GN=PIGR PE=1 SV=4	2.87E+00	0.743485138
Q9HBI1	Beta-parvin OS=Homo sapiens OX=9606 GN=PARVB PE=1 SV=1	2.89E+00	0.563525142
P05546	Heparin cofactor 2 OS=Homo sapiens OX=9606 GN=SERPIND1 PE=1 SV=3	2.96E+00	0.756724119
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens OX=9606 GN=PPIA PE=1 SV=2	3.00E+00	2.010982207
Q8NG11	Tetraspanin-14 OS=Homo sapiens OX=9606 GN=TSPAN14 PE=1 SV=1	3.04E+00	0.353311491
P63104	14-3-3 protein zeta/delta OS=Homo sapiens OX=9606 GN=YWHAZ PE=1 SV=1	3.08E+00	0.758125226
O00501	Claudin-5 OS=Homo sapiens OX=9606 GN=CLDN5 PE=2 SV=1	3.18E+00	0.494005709
P67936	Tropomyosin alpha-4 chain OS=Homo sapiens OX=9606 GN=TPM4 PE=1 SV=3	3.21E+00	0.687740383
P23229	Integrin alpha-6 OS=Homo sapiens OX=9606 GN=ITGA6 PE=1 SV=5	3.27E+00	0.766723146
P60660	Myosin light polypeptide 6 OS=Homo sapiens OX=9606 GN=MYL6 PE=1 SV=2	3.29E+00	0.517286157
P29992	Guanine nucleotide-binding protein subunit alpha-11 OS=Homo sapiens OX=9606 GN=GNA11 PE=1 SV=2	3.32E+00	0.622948241
P27169	Serum paraoxonase/arylesterase 1 OS=Homo sapiens OX=9606 GN=PON1 PE=1 SV=3	3.52E+00	1.626537414
O75954	Tetraspanin-9 OS=Homo sapiens OX=9606 GN=TSPAN9 PE=1 SV=1	3.54E+00	0.548778203
O00560	Syntenin-1 OS=Homo sapiens OX=9606 GN=SDCBP PE=1 SV=1	3.70E+00	0.732101061
Q15404	Ras suppressor protein 1 OS=Homo sapiens OX=9606 GN=RSU1 PE=1 SV=3	3.70E+00	0.94030846
P07737	Profilin-1 OS=Homo sapiens OX=9606 GN=PFN1 PE=1 SV=2	3.70E+00	0.746589315
P05556	Integrin beta-1 OS=Homo sapiens OX=9606 GN=ITGB1 PE=1 SV=2	3.74E+00	0.925079212
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens OX=9606 GN=HSP90AA1 PE=1 SV=5	3.85E+00	1.249920841
Q8N699	Myc target protein 1 OS=Homo sapiens OX=9606 GN=MYCT1 PE=1 SV=1	3.89E+00	0.448147657
P01721	Immunoglobulin lambda variable 6-57 OS=Homo sapiens OX=9606 GN=IGLV6-57 PE=1 SV=2	4.03E+00	0.578331422
P62820	Ras-related protein Rab-1A OS=Homo sapiens OX=9606 GN=RAB1A PE=1 SV=3	4.17E+00	0.866219377
P01891	HLA class I histocompatibility antigen, A-68 alpha chain OS=Homo sapiens OX=9606 GN=HLA-A PE=1 SV=4	4.28E+00	0.947627644
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens OX=9606 GN=AHSG PE=1 SV=2	4.32E+00	0.76288846
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens OX=9606 GN=ITIH4 PE=1 SV=4	4.35E+00	0.549963775
P61224	Ras-related protein Rap-1b OS=Homo sapiens OX=9606 GN=RAP1B PE=1 SV=1	4.39E+00	1.127007157
P63000	Ras-related C3 botulinum toxin substrate 1 OS=Homo sapiens OX=9606 GN=RAC1 PE=1 SV=1	4.40E+00	1.201202214
P01893	Putative HLA class I histocompatibility antigen, alpha chain H OS=Homo sapiens OX=9606 GN=HLA-H PE=5 SV=3	4.40E+00	0.719110965
P53801	Pituitary tumor-transforming gene 1 protein-interacting protein OS=Homo sapiens OX=9606 GN=PTTG1IP PE=1 SV=1	4.45E+00	0.312547647
Q9Y624	Junctional adhesion molecule A OS=Homo sapiens OX=9606 GN=F11R PE=1 SV=1	4.61E+00	2.213381992
P60033	CD81 antigen OS=Homo sapiens OX=9606 GN=CD81 PE=1 SV=1	4.68E+00	2.482436129
P11169	Solute carrier family 2, facilitated glucose transporter member 3 OS=Homo sapiens OX=9606 GN=SLC2A3 PE=1 SV=1	4.70E+00	0.778908576
P55083	Microfibril-associated glycoprotein 4 OS=Homo sapiens OX=9606 GN=MFAP4 PE=1 SV=2	4.72E+00	0.734394639
P14770	Platelet glycoprotein IX OS=Homo sapiens OX=9606 GN=GP9 PE=1 SV=3	4.72E+00	0.664627063
A0A0B4J1V0	Immunoglobulin heavy variable 3-15 OS=Homo sapiens OX=9606 GN=IGHV3-15 PE=3 SV=1	4.74E+00	0.283161543
P08514	Integrin alpha-IIb OS=Homo sapiens OX=9606 GN=ITGA2B PE=1 SV=3	4.77E+00	1.174897104
Q86UX7	Fermitin family homolog 3 OS=Homo sapiens OX=9606 GN=FERMT3 PE=1 SV=1	4.92E+00	1.031727479
P16671	Platelet glycoprotein 4 OS=Homo sapiens OX=9606 GN=CD36 PE=1 SV=2	4.96E+00	0.678010559
P62736	Actin, aortic smooth muscle OS=Homo sapiens OX=9606 GN=ACTA2 PE=1 SV=1	5.01E+00	0.452723403
P07359	Platelet glycoprotein Ib alpha chain OS=Homo sapiens OX=9606 GN=GP1BA PE=1 SV=2	5.12E+00	0.720287173
Q13103	Secreted phosphoprotein 24 OS=Homo sapiens OX=9606 GN=SPP2 PE=1 SV=1	5.17E+00	1.00144857
Q08722	Leukocyte surface antigen CD47 OS=Homo sapiens OX=9606 GN=CD47 PE=1 SV=1	5.27E+00	1.386630692
P27105	Erythrocyte band 7 integral membrane protein OS=Homo sapiens OX=9606 GN=STOM PE=1 SV=3	5.32E+00	0.442537231
A0A075B7B8	Immunoglobulin heavy variable 3/OR16-12 (non-functional) (Fragment) OS=Homo sapiens OX=9606 GN=IGHV3OR16-12 PE=1 SV=1	6.40E+00	0.812079004
P21926	CD9 antigen OS=Homo sapiens OX=9606 GN=CD9 PE=1 SV=4	7.23E+00	0.514149099