

Validation data

Olink® Flex

Introduction

Olink® Flex is a reagent kit measuring 5 to 30 selected human protein biomarkers simultaneously. The biomarkers are selected from a mix-and-match library of around 200 inflammation-related human proteins with 99 % combinability. The analytical performance of the product has been carefully validated and the results are presented below.

Technology

The Olink reagents are based on the PEA™ (Proximity Extension Assay) technology^{1,2}, where oligonucleotide labeled antibody probe pairs are each allowed to bind to their respective target protein present in the sample. Following hybridization of the matched oligo sequences, a PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. The reporter sequence is then amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a multiplex format without any need for washing steps, and results can be reported in both standard concentration units (pg/mL, default) and in relative concentration units (NPX™, optional).

Quality controls

Internal and external controls have been developed by Olink for data normalization and quality control. These have been designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, providing information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include one Incubation control, one Extension control and one Detection control. The Incubation control (a non-human antigen) monitors all three laboratory steps

starting with the immuno reaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity that is independent of antigen binding) monitors the extension and read out steps and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded reporter sequence template) monitors the readout step. Samples that deviate from a pre-determined range for one or more of the internal control values will result in a QC (Quality Control) warning in the Data analysis software and results file.

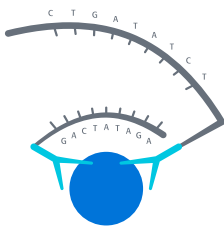
Each sample plate contains eight control samples. Triplicates of the Sample Control, duplicates of the Negative Control and triplicates of the Calibrator. The Calibrator allows for calculation of standard concentration units and is used in a second normalization step. It is designed to improve inter assay precision, enabling optimal comparison of data derived from multiple runs. The Sample Control is used to monitor and control the quality of reported output data by evaluating both accuracy and intra assay precision for all assays. Both the Sample Control and the Calibrator are composed of pooled plasma from healthy donors spiked with recombinant proteins known to have low endogenous levels in normal plasma. The Negative Control consists of buffer run as a normal sample. It sets the background levels for each protein assay and is used to calculate the limit of detection (LOD).

Data analysis and protein concentration calculation

Data analysis is performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Ct-value for the Extension control is subtracted, thus normalizing for technical variation within each sample. Normalization between runs is then performed for each assay by subtracting the corresponding dCt-value for the median of

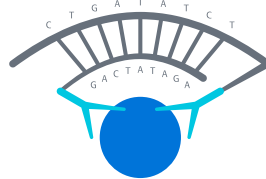
Immuno reaction

Allow the antibody probe pairs to bind to their respective proteins in your samples.



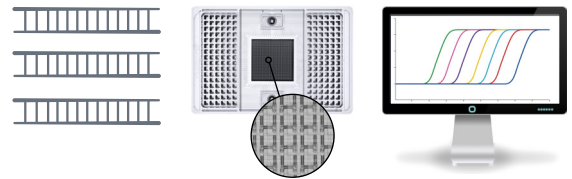
Extension and pre-amplification

Extend and pre-amplify the unique DNA reporter sequences by proximity extension.



Amplification and detection

Quantify each biomarker's DNA reporter using high throughput real-time qPCR.



Incubation control

Extension control

Detection control

Figure 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and are used for quality control and data normalization. Readout is performed by using Olink® Signature Q100.

the three Calibrator replicates from the dCt-values generated. The next step in the pre-processing procedure is to set the values relative to a bridging factor that bridges the data between different kit batches. The NPX unit generated is on a log₂ scale, where a larger number represents a higher protein level in the sample, typically with the background level at or close to zero. The protein concentration in standard concentration units (pg/mL) is obtained by fitting the NPX-value to a standard curve, describing the immunoassay shape, using four parameters in a non-linear logistic regression model. The standard curves are defined during the validation procedure and found via the panel product page (www.olink.com/flex). Three examples are shown in Figure 2.

Performance characteristics

Sample information

Olink Flex was validated using matched serum and plasma samples from 15 healthy, adult donors and 68 plasma samples from adult patients diagnosed with any of the following conditions: Asthma, Crohn's Disease, Atopic Dermatitis, Rheumatoid Arthritis, Ulcerative Colitis, Systemic Lupus Erythematosus, Cystic Fibrosis and Multiple Sclerosis.

Sample types

The ability to use different sample types was evaluated by collecting matched serum and EDTA plasma from the 15 healthy individuals. Table 1 summarizes the response values for 15 normal EDTA plasma samples expressed in pg/mL, as well as relative differences between the additional samples types compared to EDTA plasma. Acid citrate dextrose (ACD), and sodium heparin plasma samples have been evaluated and shown to work just as well as serum and EDTA plasma in the development of previous panels, and these tests have therefore not been repeated for Olink Flex.

Analytical measurement

Detection limit

Standard curves were determined for all biomarkers simultaneously in a multiplex format using recombinant proteins. LOD was defined as 3 standard deviations above background and reported in pg/mL (see Table 1 and Figure 2).

Measuring range

The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in order of log₁₀, see Table 1. To ensure accurate quantification from lot to lot Olink establish release specifications for the limits of quantification (LOQ) for every manufactured lot. The analytical measuring data shown in Table 1 is based on the validation results during product development. The ULOQ and LLOQ were calculated and reported in pg/mL. The values were back-calculated and compared to the pre-calculated 4PL-

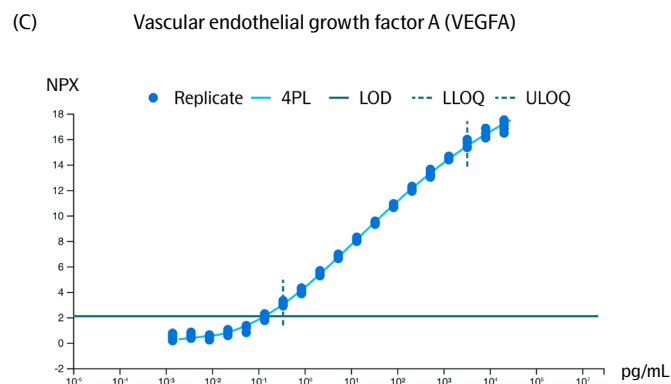
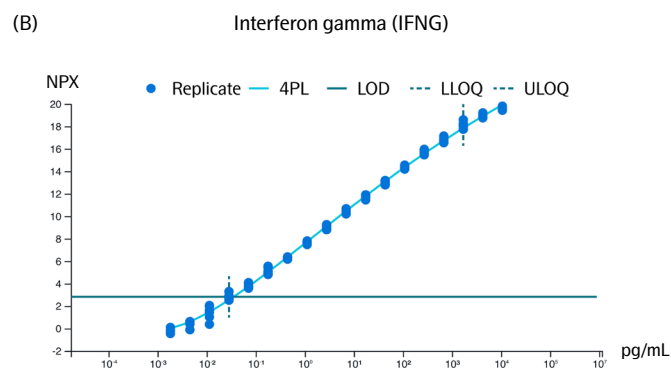
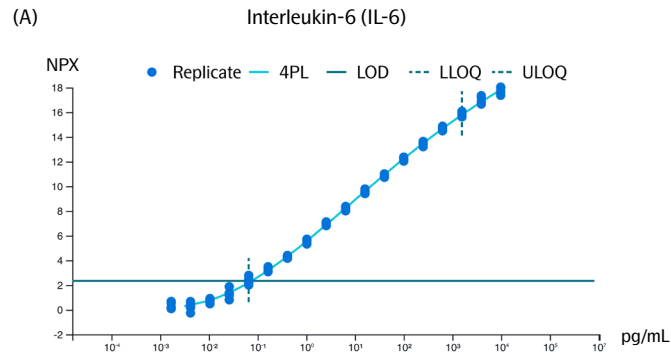


Figure 2 Calibrator curves from three assays and their corresponding analytical measurement data.

curve (see [Olink® Flex User Manual](#)), and the accuracy and precision criteria set to <30% (see Table 1). Separate calibrator curves were defined for each assay and can be accessed via the panel product page (www.olink.com/flex) together with the analytical data for the assay. Three examples of assays and their analytical data are shown in Figure 2. The sample distribution plots in Figure 3 show the levels of protein measured in commercial plasma samples.

Table 1. Sample types; Analytical measuring range; Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ), Range and Precision indicative of assay performance are shown for all protein biomarkers. Not available, NA ; <LLOQ, data could not be measured above LLOQ. *Measured in mU/mL.

Target		Sample types										Analytical measuring range		Precision		
Protein name (gene name)	UniProt No	Normal plasma levels (pg/mL)			Pathological plasma levels (pg/mL)			Relative to EDTA plasma		Healthy detectability (%)		(pg/mL)		log10 Range	% CV	
		10th %tile	Median	90th %tile	10th %tile	Median	90th %tile	Serum (%)	Correlation	Plasma	Serum	LLOQ	ULOQ		Intra	Inter
Agouti-related protein (AGRP)	O00253	51.2	70.8	79.5	38.8	62.5	100.8	27	0.56	100	100	1.61	2 455	3.2	6	8
DNA fragmentation factor subunit alpha (DFFA)	O00273	20.4	29.5	42.4	26.6	87.3	353.0	107	0.23	100	100	7.57	1 847	2.4	6	12
Tumor necrosis factor receptor superfamily member 11B (TNFRSF11B)	O00300	1 652.6	2 287.5	4 027.9	1 403.5	2 337.0	4 537.2	107	0.83	93	93	1.51	5 768	3.6	6	9
C-C motif chemokine 21 (CCL21)	O00585	633.6	756.2	1 005.7	383.1	716.1	1 024.0	55	0.82	100	100	4.19	2 560	2.8	4	8
C-X-C motif chemokine 11 (CXCL11)	O14625	24.4	38.1	65.6	24.5	73.0	270.6	186	0.34	100	100	0.52	1 976	3.6	6	7
Tumor necrosis factor ligand superfamily member 11 (TNFSF11)	O14788	21.6	37.5	72.2	20.6	41.5	75.1	103	0.96	100	100	3.86	5 890	3.2	5	8
Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B)	O14836	456.7	549.9	707.6	273.9	567.9	998.9	99	0.95	100	100	0.67	6 400	4.0	6	8
Angiotensin-2 (ANGPT2)	O15123	1 832.0	2 695.9	4 339.0	1 336.4	2 600.0	4 618.1	100	0.92	100	100	15.22	23 222	3.2	6	7
Axin-1 (AXIN1)	O15169	90.7	180.3	335.0	106.5	974.3	4 703.8	167	0.31	100	100	38.39	58 576	3.2	7	10
C-C motif chemokine 25 (CCL25)	O15444	61.2	93.3	146.9	56.8	103.2	177.6	114	0.96	100	100	0.27	1 024	3.6	5	6
Toll-like receptor 3 (TLR3)	O15455	82.4	111.5	148.5	64.7	113.5	180.2	107	0.97	100	100	1.60	6 107	3.6	5	7
Tumor necrosis factor ligand superfamily member 12 (TNFSF12)	O43508	273.9	355.5	478.5	277.7	423.0	613.8	163	0.66	100	100	1.76	2 683	3.2	6	6
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	O43557	47.3	72.1	92.7	42.8	101.7	352.7	350	0.28	100	100	4.84	7 392	3.2	5	6
Vascular endothelial growth factor D (VEGFD)	O43915	1 296.2	1 853.8	2 427.3	1 030.9	1 668.2	2 475.6	110	0.77	100	100	4.93	7 518	3.2	4	6
C-X-C motif chemokine 13 (CXCL13)	O43927	41.1	52.3	91.5	32.6	76.4	310.3	100	0.73	100	100	0.27	410	3.2	7	7
Lymphocyte antigen 75 (LY75)	O60449	201.9	238.2	311.4	160.2	230.9	343.6	105	0.85	100	100	26.21	6 400	2.4	7	9
Nephrin (NPHS1)	O60500	107.9	147.0	203.0	<LLOQ	116.5	180.7	113	1.00	100	100	58.47	14 274	2.4	6	8
ICOS ligand (ICOSLG)	O75144	1 122.3	1 286.2	1 681.0	1 221.6	1 558.5	2 404.4	143	0.13	100	87	10.49	2 560	2.4	9	14
Tumor necrosis factor ligand superfamily member 13 (TNFSF13)	O75888	14 927.0	18 771.1	27 703.1	11 181.7	18 459.0	32 706.7	108	0.31	100	100	46.91	71 572	3.2	5	7
Natural cytotoxicity triggering receptor 1 (NCR1)	O76036	95.2	135.4	217.6	107.6	238.2	416.8	113	0.94	100	100	59.90	14 623	2.4	6	9
Dickkopf-related protein 1 (DKK1)	O94907	784.7	1 204.7	2 810.2	873.9	2 203.1	4 244.4	375	0.13	100	80	0.67	6 400	4.0	6	9
Protein HEXIM1 (HEXIM1)	O94992	2.7	4.1	7.6	3.8	17.5	106.5	81	0.65	100	100	0.67	410	2.8	5	8
STAM-binding protein (STAMPB)	O95630	295.5	399.1	577.8	384.1	1 299.0	5 417.3	109	0.10	100	100	26.21	16 000	2.8	5	7
Interleukin-33 (IL33)	O95760	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	0.51	1 954	3.6	6	7
CD160 antigen (CD160)	O95971	58.3	78.2	119.4	51.6	84.2	142.6	107	0.93	100	100	0.67	2 560	3.6	5	6
Adenosine deaminase (ADA)	P00813	247.9	317.7	437.1	207.3	327.3	728.9	105	0.98	100	100	10.49	6 400	2.8	4	6
Pro-epidermal growth factor (EGF)	P01133	9.5	26.9	72.7	8.8	98.0	>ULOQ	1188	0.11	100	73	0.12	461	3.6	6	6
Protransforming growth factor alpha (TGFA)	P01135	3.2	4.8	7.4	3.0	5.8	16.1	233	0.77	100	100	1.60	974	2.8	5	6
Transforming growth factor beta-1.protein (TGFB1)	P01137	581.0	742.0	1 039.1	547.4	992.0	2 088.8	185	0.38	100	100	33.58	20 494	2.8	6	9
Pro-glucagon (GCG)	P01275	29.4	51.4	133.6	<LLOQ	47.6	102.7	48	0.83	87	33	23.02	5 620	2.4	6	11
Lymphotoxin-alpha (LTA)	P01374	4.0	6.6	9.8	5.3	7.3	11.5	115	1.00	100	100	0.58	892	3.2	6	7
Tumor necrosis factor (TNF)	P01375	11.8	14.4	19.3	10.5	18.8	98.1	103	0.99	100	100	4.79	2 922	2.8	6	8
Interferon alpha-2 (IFNA2)	P01563	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.4	NA	NA	0	0	0.25	2 338	4.0	8	10
Interferon beta (IFNB1)	P01574	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	4.19	6 400	3.2	6	10
Interferon gamma (IFNG)	P01579	0.1	0.1	0.3	0.1	0.2	0.9	106	1.00	100	100	0.03	1 699	4.8	9	11
Interleukin-1 alpha (IL1A)	P01583	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	1.68	2 560	3.2	4	12
Interleukin-1 beta (IL1B)	P01584	<LLOQ	<LLOQ	0.3	<LLOQ	0.1	4.2	74	0.96	20	67	0.09	866	4.0	9	10
Erythropoietin (EPO)*	P01588	4.2	6.0	16.5	4.7	8.6	19.2	109	0.99	93	93	3.01	735	2.4	6	10
T-cell surface glycoprotein CD4 (CD4)	P01730	155.7	234.3	261.7	101.5	172.4	255.0	97	0.53	100	87	92.07	22 479	2.4	5	6
HLA class II histocompatibility antigen. DR alpha chain (HLA-DRA)	P01903	<LLOQ	258.2	625.0	330.8	638.8	1 415.7	147	0.97	67	93	163.84	40 000	2.4	9	9
C-X-C motif chemokine 10 (CXCL10)	P02778	33.0	64.4	103.6	50.3	104.4	381.5	124	0.97	100	100	0.89	541	2.8	5	7
Interstitial collagenase (MMP1)	P03956	332.3	608.6	2 013.4	68.5	368.1	1 479.9	605	-0.37	100	47	0.91	3 476	3.6	5	6
Granulocyte-macrophage colony-stimulating factor (CSF2)	P04141	<LLOQ	<LLOQ	0.2	<LLOQ	<LLOQ	0.3	111	-1.00	13	33	0.18	1 692	4.0	7	7

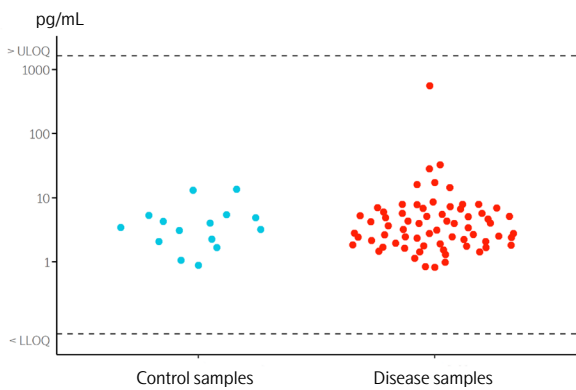
Target		Sample types										Analytical measuring range		Precision		
Protein name (gene name)	UniProt No	Normal plasma levels (pg/mL)			Pathological plasma levels (pg/mL)			Relative to EDTA plasma		Healthy detectability (%)		(pg/mL)		log10	% CV	
		10th %tile	Median	90th %tile	10th %tile	Median	90th %tile	Serum (%)	Correlation	Plasma	Serum	LLOQ	ULOQ	Range	Intra	Inter
Interleukin-4 (IL4)	P05112	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	0.05	1194	4.4	8	8
Interleukin-5 (IL5)	P05113	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	17.6	89	0.00	7	13	3.68	5 613	3.2	10	12
Interleukin-6 (IL6)	P05231	1.3	3.4	10.1	1.5	3.3	8.2	110	0.97	100	100	0.07	1 567	4.4	6	6
Transcription factor Jun (JUN)	P05412	<LLOQ	5.8	8.8	<LLOQ	7.8	22.8	92	0.99	73	73	4.19	6 400	3.2	5	7
Keratin, type I cytoskeletal 18 (KRT18)	P05783	25.6	49.0	507.2	40.5	76.0	223.4	95	0.98	100	100	1.68	6 400	3.6	9	11
Calbindin (CALB1)	P05937	21.4	26.8	36.4	22.8	38.9	65.7	104	0.88	100	100	11.39	6 950	2.8	6	7
Eukaryotic translation initiation factor 4E (EIF4E)	P06730	11.3	34.4	64.0	12.5	120.6	1 062.9	75	0.58	100	100	5.79	22 090	3.6	3	4
Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)	P06731	35.7	77.9	596.7	41.1	102.5	228.2	105	1.00	100	100	26.21	6 400	2.4	6	13
Interleukin-3 (IL3)	P08700	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	1.68	40 000	4.4	4	8
Fibroblast growth factor 2 (FGF2)	P09038	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	32.9	NA	NA	0	0	26.21	16 000	2.8	8	10
Matrilysin (MMP7)	P09237	499.3	1167.0	1867.5	83.2	213.9	672.4	470	-0.03	100	40	5.20	3 175	2.8	4	8
Stromelysin-2 (MMP10)	P09238	213.6	443.9	649.9	187.8	361.0	874.2	117	0.97	100	100	0.73	2 779	3.6	5	8
Growth-regulated alpha protein (CXCL1)	P09341	32.3	89.4	145.8	36.9	135.6	392.3	669	-0.03	100	100	0.67	2 560	3.6	6	8
Macrophage colony-stimulating factor 1 (CSF1)	P09603	101.6	125.7	154.9	113.8	154.5	216.3	117	0.94	100	100	0.31	1 166	3.6	4	5
Gastric inhibitory polypeptide (GIP)	P09681	<LLOQ	114.5	202.0	<LLOQ	93.7	164.9	61	0.84	87	60	41.94	10 240	2.4	4	4
Granulocyte colony-stimulating factor (CSF3)	P09919	70.2	105.8	159.7	171	95.6	199.9	118	0.86	100	100	3.44	2 102	2.8	4	5
Peptide YY (PYY)	P10082	29.2	89.4	196.3	47.5	90.8	241.7	92	0.95	93	93	26.21	6 400	2.4	3	4
Granzyme B (GZMB)	P10144	2.1	3.4	5.4	1.6	4.3	13.5	105	0.72	100	100	0.27	2 560	4.0	8	12
Interleukin-8 (CXCL8)	P10145	2.4	4.4	6.5	4.0	7.0	18.7	607	-0.16	100	100	0.19	1 818	4.0	6	6
C-C motif chemokine 3 (CCL3)	P10147	2.7	4.9	7.5	3.7	6.5	23.9	241	0.35	100	100	0.27	410	3.2	6	9
T-cell-specific surface glycoprotein CD28 (CD28)	P10747	334.4	525.5	1 022.1	406.3	658.6	926.2	115	1.00	100	100	104.86	25 600	2.4	6	9
Fibroblast growth factor 5 (FGF5)	P12034	10.9	15.4	20.9	9.3	15.7	24.0	86	0.89	100	100	4.19	6 400	3.2	5	7
Granzyme A (GZMA)	P12544	176.7	229.4	270.4	144.9	246.2	353.6	101	0.83	100	100	4.19	2 560	2.8	8	10
Interleukin-7 (IL7)	P13232	0.6	1.4	2.2	0.7	2.6	6.3	474	0.24	100	100	0.26	975	3.6	5	6
C-C motif chemokine 4 (CCL4)	P13236	39.7	48.8	85.4	37.8	72.0	189.0	213	0.50	100	100	0.11	1 086	4.0	5	8
C-C motif chemokine 2 (CCL2)	P13500	134.7	217.2	312.6	161.0	269.1	437.8	197	0.23	100	100	0.14	3 224	4.4	7	8
Oncostatin-M (OSM)	P13725	1.1	3.4	6.8	1.1	3.0	13.7	218	0.89	100	100	0.11	1 027	4.0	8	8
Hepatocyte growth factor (HGF)	P14210	209.9	318.6	473.4	196.4	339.6	638.9	159	0.77	100	100	0.63	2 417	3.6	6	6
Leukemia inhibitory factor (LIF)	P15018	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	0.67	1 024	3.2	7	9
Amphiregulin (AREG)	P15514	6.8	8.7	14.8	5.4	8.9	18.9	102	0.92	100	100	0.67	2 560	3.6	6	9
Vascular endothelial growth factor A (VEGFA)	P15692	173.0	276.0	522.0	194.8	320.9	811.9	218	0.68	100	100	0.34	3 264	4.0	7	8
Methylated-DNA--protein-cysteine methyltransferase (MGMT)	P16455	647.3	1 026.3	2 104.9	576.0	3 627.0	21 656.4	79	0.77	100	100	262.14	64 000	2.4	4	7
Platelet glycoprotein 4 (CD36)	P16671	<LLOQ	146.9	311.1	<LLOQ	141.7	288.2	222	0.16	73	100	100.34	24 497	2.4	6	11
Interleukin-1 receptor antagonist protein (IL1RN)	P18510	150.8	298.7	660.3	228.5	473.8	1 691.4	132	0.99	100	100	10.49	2 560	2.4	7	9
Integrin beta-6 (ITGB6)	P18564	9.0	13.0	17.7	7.8	12.4	19.8	103	0.96	100	100	4.19	6 400	3.2	5	8
Lymphocyte activation gene 3 protein (LAG3)	P18627	1 583.3	2 171.9	3 525.0	1 552.4	2 587.3	5 047.0	103	0.94	100	100	200.00	122 072	2.8	7	9
Follistatin (FST)	P19883	642.2	870.5	1 491.4	545.2	782.3	1 539.1	97	0.98	100	100	1.68	6 400	3.6	6	9
Neurotrophin-3 (NTF3)	P20783	2.3	3.0	4.6	2.8	4.7	7.6	124	0.75	93	100	1.68	2 560	3.2	6	9
Interleukin-11 (IL11)	P20809	<LLOQ	<LLOQ	6.4	<LLOQ	<LLOQ	7.4	109	1.00	27	27	4.19	16 000	3.6	5	10
Kit ligand (KITLG)	P21583	300.8	447.4	725.1	470.6	721.7	1 115.4	110	0.98	100	100	0.67	6 400	4.0	5	8
Cadherin-3 (CDH3)	P22223	2 626.7	4 136.4	4 889.3	2 330.2	4 091.1	8 771.2	119	0.89	100	100	41.94	25 600	2.8	5	12
Interleukin-10 (IL10)	P22301	3.7	4.8	8.8	0.8	5.0	14.1	115	0.95	100	100	0.36	8 698	4.4	9	11
Interleukin-32 (IL32)	P24001	21.7	27.7	48.1	17.7	28.6	60.7	102	1.00	100	100	1.68	6 400	3.6	6	8
Interleukin-4 receptor subunit alpha (IL4R)	P24394	316.1	442.3	499.7	88.3	171.5	310.9	105	0.86	100	100	9.03	34 453	3.6	5	7
Pentraxin-related protein PTX3 (PTX3)	P26022	293.6	472.6	658.5	278.4	484.3	893.6	72	0.77	100	100	4.19	6 400	3.2	4	8
Cystatin-D (CST5)	P28325	388.1	483.5	716.4	474.1	696.2	1 213.2	111	0.99	100	100	29.18	7 125	2.4	4	7
11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1)	P28845	35 226.5	46 354.4	83 896.0	32 357.6	49 321.0	67 873.7	106	0.99	100	100	4 096.00	400 000	2.0	3	4
Interleukin-12 p70 (IL12)	P29459, P29460	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	1.68	16 000	4.0	5	6

Target		Sample types										Analytical measuring range		Precision		
Protein name (gene name)	UniProt No	Normal plasma levels (pg/mL)			Pathological plasma levels (pg/mL)			Relative to EDTA plasma		Healthy detectability (%)		(pg/mL)		log10	% CV	
		10th %tile	Median	90th %tile	10th %tile	Median	90th %tile	Serum (%)	Correlation	Plasma	Serum	LLOQ	ULOQ	Range	Intra	Inter
T-cell differentiation antigen CD6 (CD6)	P30203	135.0	207.7	274.3	131.7	221.2	341.3	100	0.90	100	100	4.19	6 400	3.2	5	9
Cytokine receptor common subunit beta (CSF2RB)	P32927	85.4	148.2	219.8	70.9	173.0	255.2	113	0.96	100	100	10.94	6 680	2.8	7	11
CD70 antigen (CD70)	P32970	769.5	1190.9	1548.7	635.3	1095.0	1775.0	129	0.91	100	100	50.73	77 408	3.2	6	5
T-lymphocyte activation antigen CD80 (CD80)	P33681	169.9	210.0	275.4	161.9	272.1	436.8	111	0.92	100	100	0.67	6 400	4.0	6	8
Interleukin-13 (IL13)	P35225	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	2.2	120	0.00	13	7	0.89	3 412	3.6	8	10
Pro-adrenomedullin (ADM)	P35318	776.2	1097.9	2043.9	512.2	1178.4	2464.5	15	0.78	100	100	4.19	6 400	3.2	5	7
Basigin (BSG)	P35613	46.6	54.1	65.6	44.7	60.1	74.0	108	0.88	100	100	1.68	6 400	3.6	6	8
Vascular endothelial growth factor receptor 2 (KDR)	P35968	1067.9	1421.9	1510.0	1040.3	1326.7	1666.3	104	0.89	100	100	1.41	13 407	4.0	5	5
Macrophage metalloelastase (MMP12)	P39900	94.7	156.6	267.1	74.8	167.3	412.5	173	0.88	100	100	1.48	14 101	4.0	5	7
Glial cell line-derived neurotrophic factor (GDNF)	P39905	0.1	0.2	0.2	<LLOQ	0.2	0.4	80	0.90	93	60	0.11	1 024	4.0	4	6
Thrombopoietin (THPO)	P40225	270.1	355.4	404.3	219.7	348.7	611.9	171	0.37	100	100	28.23	17 229	2.8	5	12
B-cell antigen receptor complex-associated protein beta chain (CD79B)	P40259	41.1	51.6	81.8	40.8	59.7	127.4	99	0.74	100	100	0.67	2 560	3.6	6	11
Interleukin-15 (IL15)	P40933	8.2	11.3	14.0	7.9	11.4	21.6	110	0.78	100	100	1.35	12 899	4.0	9	9
OX-2 membrane glycoprotein (CD200)	P41217	452.6	542.7	722.7	467.3	669.7	913.5	104	0.78	100	100	26.21	16 000	2.8	8	10
Leukemia inhibitory factor receptor (LIFR)	P42702	165.7	178.2	219.1	125.2	159.8	207.1	104	0.78	100	100	3.53	13 458	3.6	5	7
C-X-C motif chemokine 5 (CXCL5)	P42830	144.6	460.7	1258.9	109.1	883.0	>ULOQ	447	-0.26	100	60	3.93	2 399	2.8	6	9
Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)	P43489	55.3	72.7	95.6	59.8	93.7	176.3	105	0.96	100	100	0.67	6 400	4.0	7	7
Mitogen-activated protein kinase 9 (MAPK9)	P45984	<LLOQ	<LLOQ	358.4	<LLOQ	403.4	1622.5	112	0.59	40	53	262.14	160 000	2.8	4	6
Tumor necrosis factor ligand superfamily member 6 (FASLG)	P48023	97.7	139.2	170.5	107.6	178.8	280.2	103	0.92	100	100	0.67	6 400	4.0	5	8
Stromal cell-derived factor 1 (CXCL12)	P48061	111.9	145.8	173.0	77.3	124.9	176.9	64	0.24	100	100	37.83	3 695	2.0	5	8
Placenta growth factor (PGF)	P49763	28.8	36.7	48.9	28.8	42.0	66.7	97	0.87	100	100	0.11	6 400	4.8	7	11
Fms-related tyrosine kinase 3 ligand (FLT3LG)	P49771	69.6	92.2	159.0	67.5	126.4	200.0	111	0.98	100	100	0.57	873	3.2	5	8
Tumor necrosis factor ligand superfamily member 10 (TNFSF10)	P50591	304.5	351.4	454.8	284.8	384.8	483.6	107	0.87	100	100	1.18	1 797	3.2	4	7
Eotaxin (CCL11)	P51671	56.2	83.2	144.1	51.0	87.1	211.8	130	0.78	100	93	0.44	267	2.8	7	8
Period circadian protein homolog 3 (PER3)	P56645	<LLOQ	<LLOQ	7.6	<LLOQ	12.0	92.3	98	0.00	33	13	4.19	6 400	3.2	5	8
Prokineticin-1 (PROK1)	P58294	<LLOQ	12.7	18.4	<LLOQ	8.5	20.2	91	0.81	80	73	4.03	2 462	2.8	7	8
Interleukin-2 (IL2)	P60568	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	0.10	2 334	4.4	7	7
Visinin-like protein 1 (VSNL1)	P62760	26.4	38.6	50.8	27.3	38.1	55.3	112	0.94	100	100	10.49	6 400	2.8	7	10
Oxidized low-density lipoprotein receptor 1 (OLR1)	P78380	30.9	56.9	117.3	31.1	64.5	289.5	386	0.84	100	100	0.78	1 186	3.2	4	7
Butyrophilin subfamily 3 member A2 (BTN3A2)	P78410	164.1	175.8	232.6	195.4	312.5	489.3	125	0.45	100	100	16.97	25 893	3.2	6	10
C-C motif chemokine 20 (CCL20)	P78556	7.6	10.7	26.4	4.7	10.7	31.9	63	0.99	100	100	0.27	410	3.2	7	9
C-C motif chemokine 8 (CCL8)	P80075	15.1	25.4	39.7	18.5	38.1	92.3	215	0.56	100	100	0.05	486	4.0	6	8
C-C motif chemokine 7 (CCL7)	P80098	0.3	0.6	1.1	0.3	0.7	2.1	191	0.69	100	100	0.11	427	3.6	8	9
C-X-C motif chemokine 6 (CXCL6)	P80162	19.4	37.8	75.9	23.2	74.2	177.1	309	-0.04	100	87	0.11	410	3.6	4	9
E3 ubiquitin-protein ligase XIAP (XIAP)	P98170	62.8	108.7	185.7	102.8	335.3	1187.0	123	0.92	100	100	41.12	62 742	3.2	6	10
CD83 antigen (CD83)	Q01151	20.8	28.2	38.1	21.1	34.5	59.1	111	0.97	100	100	1.37	5 211	3.6	5	6
Interleukin-5 receptor subunit alpha (IL5RA)	Q01344	234.4	302.5	491.7	171.4	324.2	576.6	104	0.96	100	100	10.49	16 000	3.2	4	6
DNA topoisomerase 2-beta (TOP2B)	Q02880	48.1	74.7	124.7	42.9	96.6	335.3	192	0.56	100	100	10.49	2 560	2.4	4	8
Urokinase plasminogen activator surface receptor (PLAUR)	Q03405	1005.7	1438.7	1791.3	1170.0	1747.2	>ULOQ	118	0.70	100	100	0.27	2 560	4.0	6	9
Parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R)	Q03431	12.7	14.1	19.5	11.1	16.9	24.7	89	0.98	100	100	0.72	1 097	3.2	6	8
Glutamate carboxypeptidase 2 (FOLH1)	Q04609	<LLOQ	<LLOQ	1406.2	<LLOQ	<LLOQ	3 242.9	68	0.00	20	7	1158.09	113 095	2.0	15	35
Peroxiorexin-1 (PRDX1)	Q06830	17.6	21.8	43.4	26.5	106.3	387.0	117	0.70	100	100	10.49	6 400	2.8	3	3
Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)	Q07011	14.2	20.9	35.9	14.5	24.8	53.2	110	0.97	100	100	4.19	2 560	2.8	6	7
C-X-C motif chemokine 9 (CXCL9)	Q07325	26.9	44.2	64.9	32.2	75.3	286.8	101	0.95	100	100	0.23	862	3.6	6	9
CMRF35-like molecule 6 (CD300C)	Q08708	326.7	465.7	521.2	348.6	470.3	603.8	101	0.95	100	100	4.19	2 560	2.8	4	5
Natural killer cells antigen CD94 (KLRD1)	Q13241	164.1	202.7	303.2	144.4	270.4	461.3	106	0.98	100	100	4.69	7 157	3.2	4	6
Signaling lymphocytic activation molecule (SLAMF1)	Q13291	660.9	897.1	1202.5	684.2	1029.7	1418.5	113	0.91	100	100	104.86	64 000	2.8	5	9

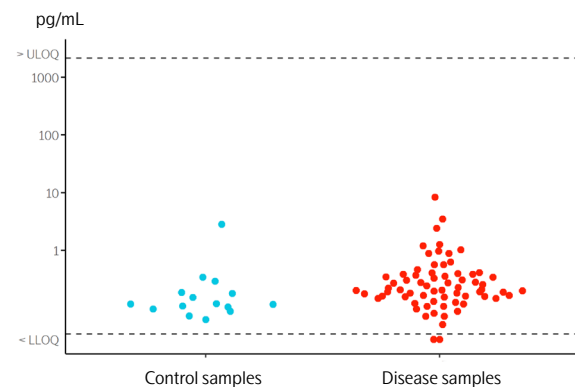
Target		Sample types										Analytical measuring range		Precision		
Protein name (gene name)	UniProt No	Normal plasma levels (pg/mL)			Pathological plasma levels (pg/mL)			Relative to EDTA plasma		Healthy detectability (%)		(pg/mL)		log10	% CV	
		10th %tile	Median	90th %tile	10th %tile	Median	90th %tile	Serum (%)	Correlation	Plasma	Serum	LLOQ	ULOQ	Range	Intra	Inter
Mesothelin (MSLN)	Q13421	3 268.5	6 690.6	18 282.1	4 070.5	7 432.1	25 608.3	107	0.99	100	100	946.23	231 012	2.4	4	6
Interleukin-18 receptor 1 (IL18R1)	Q13478	132.4	178.6	348.1	173.0	248.4	351.5	113	0.99	100	100	0.67	6 400	4.0	3	4
Pro-interleukin-16 (IL16)	Q14005	25.1	44.4	75.7	30.6	62.9	291.4	128	0.91	100	100	1.71	2 609	3.2	6	8
Interleukin-18 (IL18)	Q14116	186.6	311.7	451.8	185.0	338.7	2 818.8	106	0.93	100	100	0.23	5 464	4.4	6	7
Interleukin-27 (IL27)	Q14213, Q8NEV9	3.7	8.6	10.9	1.6	4.7	13.9	95	0.95	93	93	1.30	4 960	3.6	8	10
Caspase-8 (CASP8)	Q14790	4.3	6.2	10.7	11.6	48.3	277.7	181	0.60	100	100	0.75	2 871	3.6	6	11
Programmed cell death protein 1 (PDCD1)	Q15116	50.5	67.7	91.7	53.5	96.6	185.6	111	0.86	100	100	4.19	6 400	3.2	6	8
Angiotensinogen-converting enzyme 1 (ACE1)	Q15389	816.3	2 270.9	5 067.1	900.6	2 867.1	6 352.2	533	0.26	100	100	143.84	14 047	2.0	7	9
Toll-like receptor 1 (TLR1)	Q15399	5.2	9.0	17.2	6.3	9.0	18.1	115	0.98	100	100	1.68	6 400	3.6	4	5
CD226 antigen (CD226)	Q15762	3 127.0	4 829.2	7 576.6	3 336.6	7 640.8	22 998.4	303	0.23	100	73	655.36	25 600	1.6	10	13
Interleukin-17A (IL17A)	Q16552	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.7	128	0.00	7	20	1.30	4 972	3.6	7	8
Hypoxia-inducible factor 1-alpha (HIF1A)	Q16665	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.7	NA	NA	0	0	0.27	2 560	4.0	8	10
Kynureninase (KYNU)	Q16719	1 595.3	2 660.0	9 479.3	1 882.7	3 551.1	6 365.6	102	0.99	100	100	409.60	40 000	2.0	5	8
Carcinoembryonic antigen-related cell adhesion molecule 21 (CEACAM21)	Q3KPI0	1.4	2.3	8.2	1.6	4.3	12.9	113	0.92	100	100	0.27	1 022	3.6	5	8
CD276 antigen (CD276)	Q5ZPR3	23 389.1	28 425.8	38 966.7	18 616.9	27 942.5	43 205.0	85	0.86	100	100	40.90	156 031	3.6	4	8
Natural cytotoxicity triggering receptor 3 ligand 1 (NCR3LG1)	Q68D85	157.1	201.2	248.7	129.3	192.9	310.8	99	0.89	100	100	10.73	6 548	2.8	4	6
C-X-C motif chemokine 17 (CXCL17)	Q6UXB2	30.2	40.6	59.9	13.9	36.0	69.2	48	0.87	100	100	1.68	2 560	3.2	6	7
Interferon lambda-1 (IFNL1)	Q8IU54	<LLOQ	<LLOQ	1.9	<LLOQ	<LLOQ	3.0	92	-1.00	13	13	1.68	6 400	3.6	5	8
Interferon lambda receptor 1 (IFNLR1)	Q8IU57	7.8	10.2	16.3	7.5	10.6	16.0	104	0.88	100	100	1.68	6 400	3.6	6	11
NAD-dependent protein deacetylase sirtuin-2 (SIRT2)	Q8IXJ6	101.2	175.3	263.8	95.5	518.5	2 471.1	93	-0.34	100	100	16.78	10 240	2.8	7	12
Inactive dipeptidyl peptidase 10 (DPP10)	Q8N608	<LLOQ	46.6	85.0	31.5	51.4	136.9	105	0.93	93	100	26.21	16 000	2.8	6	8
Interleukin-31 receptor subunit alpha (IL31RA)	Q8NI17	16.5	24.2	43.3	13.2	25.1	48.1	108	0.90	100	100	5.05	7 699	3.2	5	6
Interleukin-17D (IL17D)	Q8TAD2	1.9	2.9	4.4	1.7	2.6	4.8	82	0.97	100	100	0.67	1 024	3.2	5	8
Cell surface glycoprotein CD200 receptor 1 (CD200R1)	Q8TD46	444.5	617.5	711.4	395.0	559.0	822.4	95	0.92	100	100	10.49	16 000	3.2	4	5
C-type lectin domain family 4 member C (CLEC4C)	Q8WTT0	22.2	31.1	48.5	16.1	31.9	60.2	100	0.97	100	100	3.90	2 378	2.8	4	7
C-C motif chemokine 17 (CCL17)	Q92583	29.3	59.8	357.7	38.3	106.0	313.1	492	0.62	100	87	0.10	945	4.0	6	6
Thymic stromal lymphopoietin (TSLP)	Q969D9	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	1.34	2 043	3.2	6	8
Hepatitis A virus cellular receptor 1 (HAVCR1)	Q96D42	21.8	43.9	126.2	32.1	70.5	276.2	111	0.98	100	100	0.65	2 476	3.6	5	6
Fc receptor-like protein 2 (FCRL2)	Q96LA5	125.1	191.5	287.7	127.2	229.8	399.4	110	0.98	100	100	8.53	5 208	2.8	5	6
Nectin-4 (NECTIN4)	Q96NY8	293.6	342.2	485.5	281.1	370.2	615.1	99	0.89	100	100	10.49	2 560	2.4	6	9
Interleukin-17F (IL17F)	Q96PD4	<LLOQ	<LLOQ	1.7	<LLOQ	<LLOQ	3.5	112	0.98	33	47	1.25	1 908	3.2	7	7
Tumor necrosis factor receptor superfamily member 13C (TNFRSF13C)	Q96RJ3	9.2	12.6	23.8	8.1	14.4	30.5	87	0.98	100	100	4.30	2 622	2.8	5	8
Granulocyte colony-stimulating factor receptor (CSF3R)	Q99062	107.5	130.2	209.9	101.0	149.1	234.4	114	0.96	100	100	7.42	11 316	3.2	4	5
Proheparin-binding EGF-like growth factor (HBEGF)	Q99075	9.7	15.6	33.3	10.2	21.0	54.8	263	0.43	100	100	0.11	410	3.6	6	6
C-C motif chemokine 13 (CCL13)	Q99616	42.5	67.6	125.4	52.8	89.1	221.3	279	0.43	100	100	0.04	964	4.4	5	6
C-C motif chemokine 19 (CCL19)	Q99731	65.6	85.2	131.4	57.2	106.6	278.3	81	0.37	100	100	0.14	1 298	4.0	6	8
Programmed cell death 1 ligand 2 (PDCD1LG2)	Q9BQ51	4 789.3	6 432.5	7 870.1	4 990.4	7 266.2	10 422.5	109	0.91	100	100	921.99	90 038	2.0	9	9
C-type lectin domain family 7 member A (CLEC7A)	Q9BXN2	19.0	24.3	34.8	19.3	37.7	77.4	108	0.96	100	100	4.73	1 156	2.4	5	6
Egl nine homolog 1 (EGLN1)	Q9GZT9	23.2	32.4	73.8	27.4	48.4	337.3	141	0.32	100	100	4.19	6 400	3.2	4	7
Fibroblast growth factor 23 (FGF23)	Q9GZV9	24.7	48.6	185.6	27.4	38.4	101.1	31	0.96	100	100	1.68	2 560	3.2	5	8
Interleukin-25 (IL25)	Q9H293	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	NA	NA	0	0	0.27	2 560	4.0	5	9
CUB domain-containing protein 1 (CDCP1)	Q9H5V8	109.8	198.9	459.4	109.2	212.5	481.2	109	0.98	100	100	10.49	6 400	2.8	5	9
C-C motif chemokine 28 (CCL28)	Q9NRJ3	35.0	48.7	120.0	26.0	65.2	158.0	108	0.95	100	100	4.44	2 712	2.8	6	6
Fibroblast growth factor 21 (FGF21)	Q9NSA1	74.3	179.6	1 214.7	56.7	164.7	415.0	71	0.99	93	100	1.68	6 400	3.6	3	5
Interleukin-1 receptor-associated kinase 4 (IRAK4)	Q9NWZ3	115.9	223.6	433.2	174.9	1 337.6	10 217.6	152	0.07	100	100	41.94	25 600	2.8	6	10
Interleukin-20 (IL20)	Q9NYY1	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	117	0.00	7	7	4.19	6 400	3.2	5	8
Triggering receptor expressed on myeloid cells 2 (TREM2)	Q9NZC2	7 596.3	13 053.5	30 558.3	6 598.9	18 967.1	57 188.6	107	0.97	100	100	2.68	64 000	4.4	5	8
Interleukin-17C (IL17C)	Q9POM4	6.6	15.5	22.9	4.6	10.5	31.9	76	0.90	100	100	1.78	2 717	3.2	6	7

Target		Sample types								Analytical measuring range		Precision				
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		10th %tile	Median	90th %tile	10th %tile	Median	90th %tile	Serum (%)	Correlation	Plasma	Serum	LLOQ	ULOQ	Range	Intra	Inter
Appetite-regulating hormone (GHRL)	Q9UBU3	444.1	1 006.9	1 754.5	490.4	895.5	2 478.7	76	0.88	100	100	196	7 489	3.6	5	9
Interleukin-19 (IL19)	Q9UHD0	109.4	165.7	342.8	84.1	159.5	494.5	112	0.98	100	100	4.95	47 177	4.0	4	7
SLAM family member 5 (CD84)	Q9UIB8	1 822.8	2 500.7	3 120.9	1 573.2	2 947.3	4 634.9	249	0.36	100	100	38.27	9 343	2.4	5	7
Growth/differentiation factor 2 (GDF2)	Q9UK05	79.5	100.9	156.5	68.2	124.8	210.6	185	0.83	100	100	1.68	6 400	3.6	8	13
C-type lectin domain family 4 member A (CLEC4A)	Q9UMR7	13.0	16.5	24.8	9.6	14.7	22.2	106	0.92	100	100	1.68	2 560	3.2	6	6
Tumor necrosis factor receptor superfamily member EDAR (EDAR)	Q9UNE0	5.0	8.2	13.5	5.4	17.8	62.4	443	0.24	100	100	1.42	5 400	3.6	7	8
Lysosome-associated membrane glycoprotein 3 (LAMP3)	Q9UQV4	638.9	1 186.1	1 860.0	644.6	1 012.9	2 069.6	104	0.98	100	100	26.21	16 000	2.8	5	6
C-C motif chemokine 26 (CCL26)	Q9Y258	121.7	156.8	200.4	60.6	111.7	204.2	68	0.18	100	100	41.94	160 000	3.6	5	9
NEDD8 ultimate buster 1 (NUB1)	Q9Y5A7	<LLOQ	<LLOQ	3.2	<LLOQ	7.3	35.5	83	0.96	27	33	1.68	1 024	2.8	5	10
NF-kappa-B essential modulator (IKBKG)	Q9Y6K9	<LLOQ	<LLOQ	28.6	<LLOQ	39.9	288.4	146	1.00	13	60	20.66	12 609	2.8	6	8
Tumor necrosis factor receptor superfamily member 11A (TNFRSF11A)	Q9Y6Q6	30.6	45.1	69.7	40.7	72.6	150.6	138	0.94	100	100	0.67	2 560	3.6	5	7

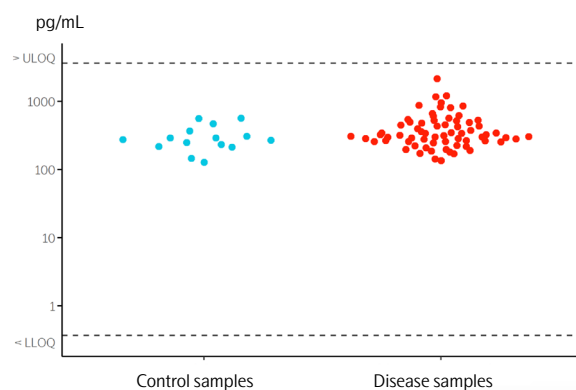
Interleukin-6 (IL-6)



Interferon gamma (IFNG)



Vascular endothelial growth factor A (VEGFA)



C-C motif chemokine 25 (CCL25)

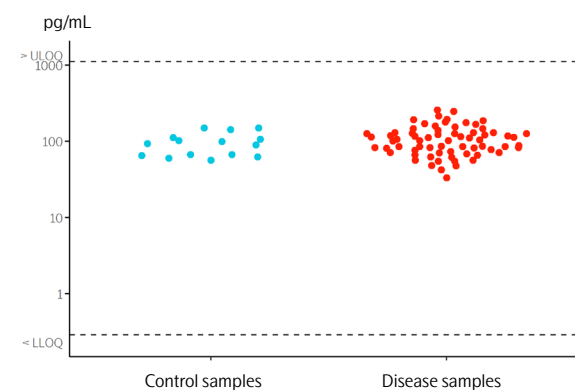


Figure 3. Sample distribution plots for four Olink® Flex assays. The plots show the levels of protein measured in a number of commercial plasma samples. LLOQ (lower limit of quantification) and ULOQ (upper limit of quantification) are indicated by the dotted lines. The y-axis is on a logarithmic scale. Healthy subjects are shown in blue and samples obtained from patients with a range of diseases are shown in red. The diseases include for example Asthma, Crohn's Disease, Atopic Dermatitis, Rheumatoid Arthritis, Ulcerative Colitis, Systemic Lupus Erythematosus, Cystic Fibrosis and Multiple Sclerosis.

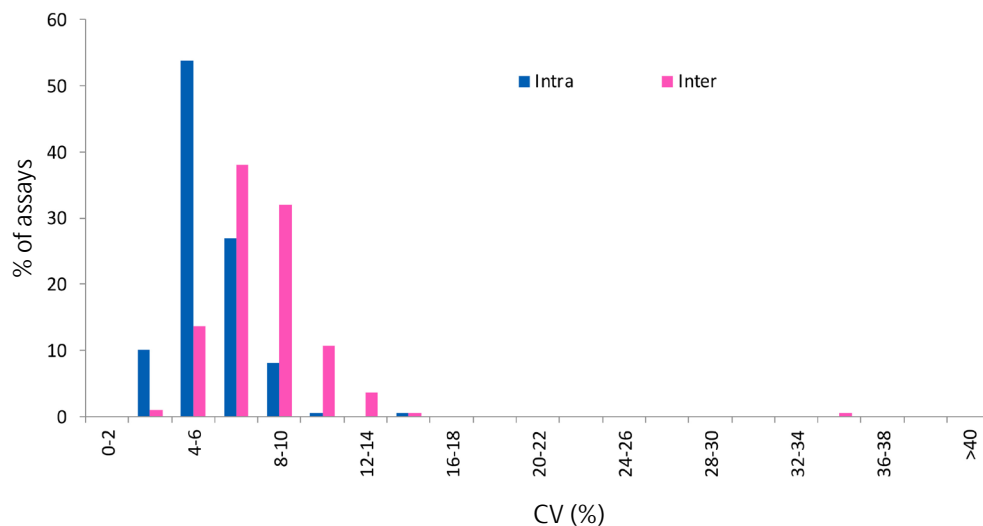


Figure 4. Distribution of intra-assay and inter-assay variations of Olink® Flex.

Precision

Repeatability

Inter (between run) and intra (within run) CV were assessed by evaluating triplicate measurements of the Sample Control on each plate, based on 6 plate runs performed by multiple operators.

Inter assay variation (between runs) was calculated between experiments performed by multiple operators. CV calculations were performed on data in pg/mL for the analytes for which response levels within LOQ were detected, see Table 1. Across all Olink® Flex assays, the mean intra assay and inter assay variations observed were 6 % and 8 %, respectively. The distribution of both intra assay and inter assay variations are shown in Figure 4.

Reproducibility

Inter-site (between sites) variation was investigated during the validation in a beta-site study. Sample plates were distributed to six laboratories together with 20-plex Olink® Flex reagent kits. The sample plates contained triplicates of 10 samples and a duplicate of a pooled plasma sample. Selected samples were run in 1:4 dilutions. The total number of assays were 20 per site, with an overlap of 14 assays that were run on all sites.

Two operators per site performed the analysis of the samples according to instructions. The intra and inter assay CVs are listed in Table 2.

Table 2. Inter-site variation seen during beta-site study.

	Pooled sample (plasma)	Sample control (spiked plasma)
Intra CV	5.8 %	7.1 %
Inter CV	10.9 %	9.7 %
Inter-site CV	10.9 %	5.6 %

There are many laboratories around the world trained by Olink to run panels (see www.olink.com/service for details). Our experience over several years is that inter-site reproducibility is very good provided that operators are properly trained, although technical

variation between sites must be considered in experimental designs. For more information please contact support@olink.com.

Analytical Specificity

Assay specificity

To test the target-protein specificity of the PEA probes used in the Flex library, all of the antibodies used were tested for cross-reactivity against all of the recombinant proteins used during assay development.

This was carried out by creating test samples consisting of pools of antigens, which were then incubated with all 197 antibody probe pairs from the panel. To optimize this analysis, 29 sub-pools of antigen were evaluated to cover the 197 assays.

The lack of significant signal from these tests (defined as less than 10% at endogenous level) indicates that each probe pair is specific for its target antigen, demonstrating the readout specificity of the PEA method.

The probes were also checked for cross-reactivity to homologous proteins with known sequence similarity to some of the Olink Flex target proteins.

Endogenous interference

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor is known to cause problems in some immunoassays. Evaluation of the potential impact of this specific interference was investigated during the validation of previous panels. No interference due to HAMA or RF was detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown).

Bilirubin, lipids and hemolysate, are plasma and serum components that are known to interfere with some analytical assays. Interference by bilirubin and lipids has previously been evaluated, and disturbance was only observed at extreme levels corresponding to 8 or 10 times normal values^{3,4}. This test was therefore not repeated for Olink Flex.

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Technical support

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1322, v1.2, 2024-09-30