

VALIDATION DATA

# 1. Introduction

Olink® Development is a reagent kit measuring 92 developmental related human protein biomarkers simultaneously. The assays on this panel have been selected to target high-abundance proteins, and 1 µL of a 1:100 dilution of sample is used. The analytical performance of the product has been carefully validated and the results are presented below.

## 1.1 TECHNOLOGY

The Olink reagents are based on the Proximity Extension Assay (PEA) technology<sup>1,2</sup>, where 92 oligonucleotide labeled antibody probe pairs are allowed to bind to their respective target protein present in the sample. A PCR reporter sequence is formed by a proximity dependent DNA polymerization event, amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format without any need for washing steps, see Figure 1.

## 1.2 QUALITY CONTROLS

Internal and external controls have been developed by Olink for data normalization and quality control purposes. These controls 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 two Immunoassay controls, one Extension control and one Detection control. The Immunoassay controls (two non-human proteins) monitor all three steps starting with the immunoreaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity independent of antigen binding) monitors the extension and readout steps and is used

for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) monitors the readout step. Samples for which one or more of the internal control values deviate from a pre-determined range will be flagged and may be removed before statistical analysis.

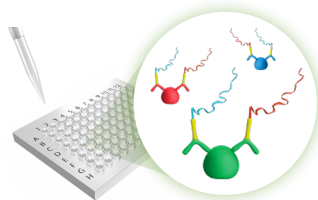
An external control, inter-plate control (IPC), is included on each plate and used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. Furthermore, the improves inter-assay precision and allows for optimal comparison of data derived from multiple runs. The term "Normalized Protein eXpression (NPX)" refers to normalized data as described above.

## 1.3 DATA ANALYSIS

Data analysis was performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control was subtracted, thus normalizing for technical variation within one run. Normalization between runs is then performed for each assay by subtracting the corresponding dCq-value for the Interplate Control (IPC) from the dCq-values generated. In the final step of the pre-processing procedure the values are set relative to a correction factor determined by Olink. The generated Normalized Protein eXpression (NPX) unit is on a log<sub>2</sub> scale where a larger number represents a higher protein level in the sample, typically with the background level at around zero. Linearization of data is performed by the mathematical operation 2<sup>NPX</sup>. Coefficient of variation (CV) calculations were performed on linearized values.

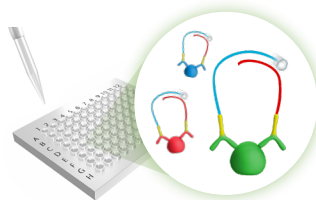
### IMMUNOASSAY

Allow the 92 antibody probe pairs to bind to their respective proteins in your samples.



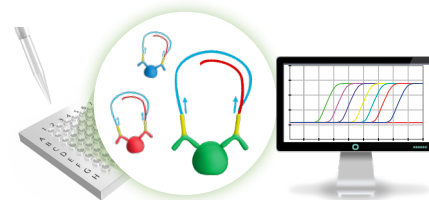
### EXTENSION

Extend and pre-amplify 92 unique DNA reporter sequences by proximity extension.



### DETECTION

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



Immunoassay control

Extension control

Detection control

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enables monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

## 2. Performance characteristics

### 2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Development by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Response values observed between heparin, citrate plasma or serum, are expressed as relative differences (%) compared to EDTA plasma and shown in Tabel 1 for each sample type. To evaluate the measuring range of endogenous protein levels, response values levels were assessed in 22 normal EDTA plasma samples and reported in NPX, Table 1.

### 2.2 ANALYTICAL MEASUREMENT

#### DETECTION LIMIT

Calibrator curves were determined for 92 biomarkers simultaneously in a multiplex format. Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL for all assays where recombinant protein antigen was available, see Table 1 and Figure 2. Please note that the Development panel uses a 1:100 dilution of sample, whereas our technical validation assays are performed *in vitro* using recombinant antigens. The data presented in this document are based on these *in vitro* assays and a multiplication factor of 100 should therefore be taken in consideration when comparing the addressable biological concentration to the *in vitro* validation data.

#### HIGH DOSE HOOK EFFECT

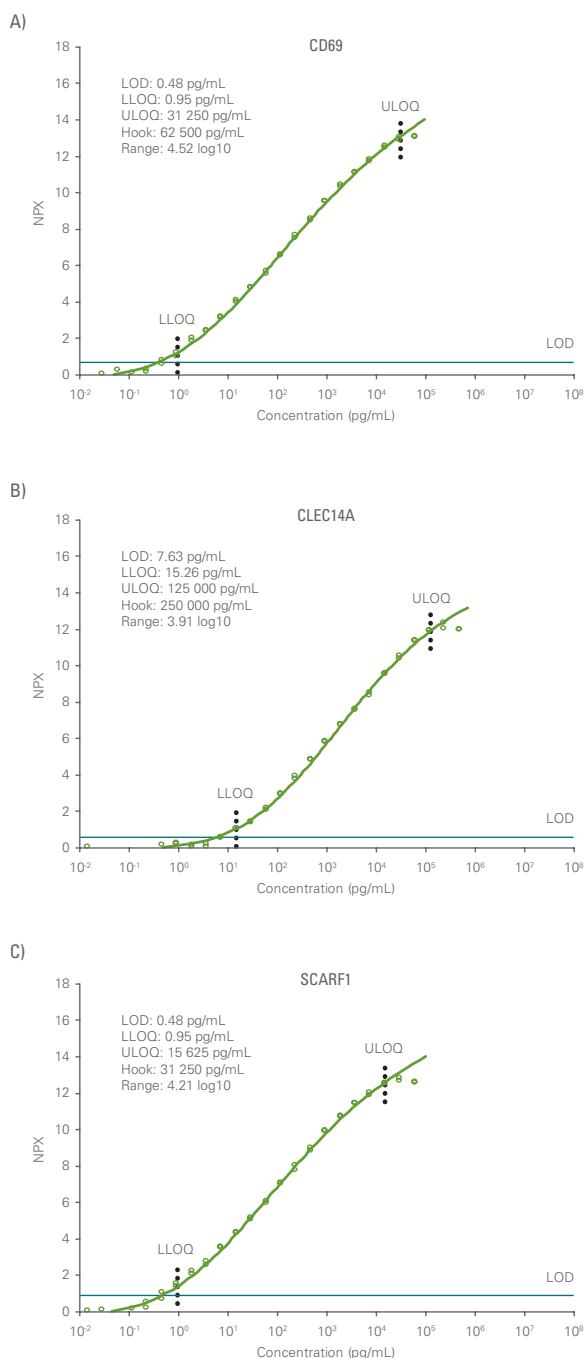
The high dose hook effect is a state of antigen excess relative to the reagent antibodies, resulting in falsely lower values. In such cases, a significantly lower value can be reported which leads to misinterpretation of results. Therefore, the hook effect was determined for each analyte, here reported in pg/mL for 92 assays, see Table 1.

#### MEASURING RANGE

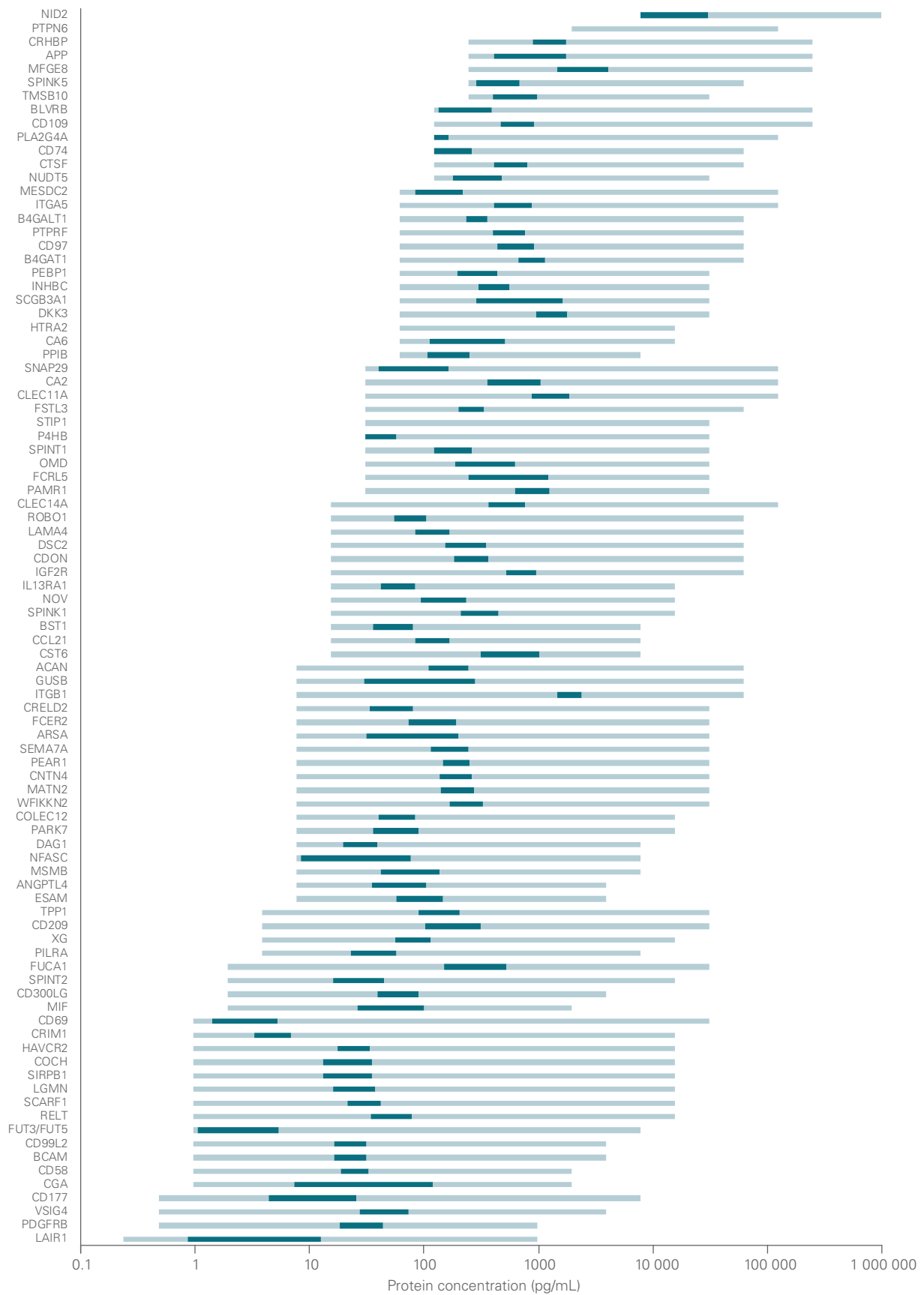
The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in order of log<sub>10</sub>, see Table 1. The upper and lower limits of quantification, ULOQ and LLOQ, respectively were calculated with the following trueness and precision criteria; relative error ≤ 30% and CV ≤ 30%, of back-calculated values, and reported in pg/mL, see Table 1.

Three assays with their analytical data are shown in

Figure 2 and the distribution of measuring ranges of 92 assays and endogenous plasma levels are shown in Figure 3. Separate calibrator curves established for each assay may be viewed at [www.olink.com](http://www.olink.com).



**Fig 2.** Calibrator curves from 3 assays and their corresponding analytical measurement data.



**Fig 3.** Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ) in pg/mL. Normal plasma levels (dark blue bars) are denoted for 92 analytes and here reported in pg/mL.

**Table 1.** Sample Types; Normalized Protein eXpression (NPX), Endogenous Interference, Analytical Measurement; Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for 92 analytes. Not available, NA. Please note: the Development panel uses a 1:100 dilution which should be taken in consideration when comparing biological concentrations to the *in vitro* validation data.

Target	UniProt No	Sample types			Endogenous Interference			Analytical measurement					Precision		
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL			log10		% CV	
		10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2 (BST1)	Q10588	1.3	1.6	2.0	106	99	101	15	7.6	15	7812	31250	2.7	10	11
ADP-sugar pyrophosphatase (NUDT5)	Q9UUK9	1.4	3.2	3.9	63	62	110	0	122	122	31250	125000	2.4	11	13
Aggrecan core protein (ACAN)	P16112	2.7	3.1	3.7	94	89	94	15	7.6	7.6	62500	62500	3.9	8	10
Amyloid beta A4 protein (APP)	P05067	1.7	2.2	3.4	31	327	918	15	244	244	250000	250000	3.0	9	8
Angiotensin-related protein 4 (ANGPTL4)	Q9BY76	2.4	3.3	4.0	100	90	80	15	7.6	7.6	3906	62500	2.7	9	7
Arylsulfatase A (ASA)	P15289	1.8	2.9	3.8	87	97	155	15	7.6	7.6	31250	31250	3.6	9	14
Basal cell adhesion molecule (BCAM)	P50895	3.1	3.5	3.9	98	98	102	1.9	0.95	0.95	3906	7812	3.6	9	9
Beta-1,4-galactosyltransferase 1 (B4GALT1)	P15291	2.0	2.2	2.6	100	97	97	15	61	61	62500	250000	3.0	9	6
Beta-1,4-glucuronyltransferase 1 (B4GAT1)	O43505	4.4	4.8	5.3	96	98	103	15	31	61	62500	250000	3.0	10	10
Beta-glucuronidase (GUSB)	P08236	2.3	3.2	4.8	90	98	159	15	7.6	7.6	62500	125000	3.9	9	12
Beta-microseminoprotein (MSMB)	P08118	2.9	3.6	4.6	101	99	109	15	3.8	7.6	7812	31250	3.0	9	10
Carbonic anhydrase 2 (CA2)	P00918	3.1	4.1	4.7	23	17	95	0	31	31	125000	250000	3.6	10	9
Carbonic anhydrase 6 (CA6)	P23280	1.3	1.9	2.8	110	108	111	15	61	61	15625	125000	2.4	9	10
Cathepsin F (CTSF)	Q9UBX1	2.7	3.2	3.7	112	94	97	15	61	122	62500	250000	2.7	10	12
Cation-independent mannose-6-phosphate receptor (IGF2R)	P11717	5.5	5.9	6.4	99	99	109	15	15	15	62500	125000	3.6	9	13
C-C motif chemokine 21 (CCL21)	O00585	1.8	2.2	2.9	94	90	91	15	15	15	7812	250000	2.7	10	7
CD109 antigen (CD109)	Q6VHK3	2.7	3.0	3.5	101	102	115	15	61	122	250000	1000000	3.3	13	16
CD177 antigen (CD177)	Q8N6Q3	3.3	4.1	5.5	100	106	135	15	0.48	0.48	7812	7812	4.2	9	10
CD209 antigen (CD209)	Q9NNX6	4.3	4.9	5.7	99	99	103	15	1.9	3.8	31250	125000	3.9	8	8
CD97 antigen (CD97)	P48960	4.5	5.0	5.7	108	107	109	15	61	61	62500	250000	3.0	11	9
CD99 antigen-like protein 2 (CD99L2)	Q8TCZ2	2.8	3.2	3.5	103	100	104	15	0.95	0.95	3906	15625	3.6	9	9
Cell adhesion molecule-related/down-regulated by oncogenes (CDON)	Q4KMG0	3.1	3.7	4.2	101	96	93	15	15	15	62500	125000	3.6	9	10
CMRF35-like molecule 9 (CD300L6)	Q6UXG3	3.0	3.6	4.1	98	97	100	15	1.9	1.9	3906	62500	3.3	9	9
Cochlin (COCH)	O43405	2.9	3.4	4.1	76	126	146	15	0.95	0.95	15625	15625	4.2	10	7
Collectin-12 (COLECT12)	Q5KU26	3.7	4.2	4.8	98	99	102	15	3.8	7.6	15625	31250	3.3	12	14
Contactin-4 (CNTN4)	Q8IWW2	3.6	4.0	4.5	99	102	104	15	7.6	7.6	31250	250000	3.6	9	10
Corticotropin-releasing factor-binding protein (CRHBP)	P24387	2.3	2.7	3.2	97	96	113	15	122	244	250000	1000000	3.0	9	11
C-type lectin domain family 11 member A (CLEC11A)	Q9Y240	3.8	4.4	5.1	88	106	126	15	31	31	125000	125000	3.6	9	10
C-type lectin domain family 14 member A (CLEC14A)	Q86T13	4.3	4.8	5.3	99	99	103	15	7.6	15	125000	250000	3.9	8	8
Cystatin-M (CST6)	Q15828	4.9	5.7	6.6	98	80	85	15	7.6	15	7812	31250	2.7	9	7
Cysteine-rich motor neuron 1 protein (CRIM1)	Q9NZV1	1.6	1.9	2.2	94	97	104	15	0.95	0.95	15625	62500	4.2	9	8
Cysteine-rich with EGF-like domain protein 2 (CRELD2)	Q6UXH1	2.7	3.1	3.8	96	100	103	15	1.9	7.6	31250	250000	3.6	10	8
Cytosolic phospholipase A2 (cPLA2)	P47712	NA	NA	0.3	NA	NA	NA	15	122	122	125000	250000	3.0	12	12
Desmocollin-2 (DSC2)	Q02487	3.5	4.0	4.6	102	104	110	15	15	15	62500	125000	3.6	10	9
Dickkopf-related protein 3 (DKK3)	Q9UBP4	4.0	4.6	5.2	98	97	98	15	61	61	31250	125000	2.7	10	8
Dystroglycan (DAG1)	Q14118	2.0	2.4	2.8	97	97	169	15	3.8	7.6	7812	31250	3.0	9	9
Early activation antigen CD69 (CD69)	Q07108	1.5	2.2	2.7	123	116	112	15	0.48	0.95	31250	62500	4.5	9	9
Endothelial cell-selective adhesion molecule (ESAM)	Q96AP7	2.7	3.2	3.9	84	112	162	15	7.6	7.6	3906	15625	2.7	8	8
Fc receptor-like protein 5 (FCRL5)	Q96RD9	2.8	3.9	5.1	98	98	97	15	15	31	31250	250000	3.0	9	8
Flavin reductase (BLVRB)	P30043	0.3	0.8	1.3	76	NA	221	0	122	122	250000	250000	3.3	10	16
Follistatin-related protein 3 (FSTL3)	Q95633	2.1	2.5	2.9	98	102	103	15	31	31	62500	125000	3.3	10	11
Galactoside 3(4)-L-fucosyltransferase, Alpha-(1,3)-fucosyltransferase 5 (FUT3/FUT5)	Q11128, P21217	1.5	2.4	3.0	92	90	105	15	0.48	0.95	7812	31250	3.9	11	9
Glycoprotein hormones alpha chain (CGA)	P01215	3.2	4.1	6.6	95	97	101	15	0.95	0.95	1953	7812	3.3	15	10
Glycoprotein Xg (XG)	P55808	4.6	5.4	5.8	93	94	95	3.8	1.9	3.8	15625	31250	3.6	10	10
Hepatitis A virus cellular receptor 2 (HAVCR2)	Q8TDQ0	2.7	3.1	3.6	95	96	101	15	0.95	0.95	15625	31250	4.2	9	8

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		10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
HLA class II histocompatibility antigen gamma chain (CD74)	P04233	NA	0.4	1.4	NA	NA	NA	15	122	122	62500	62500	2.7	11	10
Inactive serine protease (PAMR1)	O6UXH9	4.2	4.6	5.3	93	93	94	15	15	31	31250	62500	3.0	10	10
Inhibin beta C chain (INHBC)	P55103	2.8	3.2	3.6	96	89	92	15	15	61	31250	62500	2.7	11	10
Integrin alpha-5 (ITGA5)	P08648	2.9	3.4	3.9	76	49	46	15	31	61	125000	250000	3.3	10	8
Integrin beta-1 (ITGB1)	P05556	6.4	6.8	7.1	90	80	82	15	7.6	7.6	62500	125000	3.9	8	7
Interleukin-13 receptor subunit alpha-1 (IL13RA1)	P78552	1.9	2.2	2.6	100	104	108	15	7.6	15	15625	31250	3.0	10	13
Kunitz-type protease inhibitor 1 (SPINT1)	O43278	1.8	2.1	2.7	102	97	107	15	31	31	31250	250000	3.0	9	12
Kunitz-type protease inhibitor 2 (SPINT2)	O43291	1.9	2.4	3.1	75	71	74	15	1.9	1.9	15625	31250	3.9	9	10
Lactadherin (MFGE8)	O08431	3.9	4.4	5.3	98	72	84	15	61	244	250000	1000000	3.0	14	15
Laminin subunit alpha-4 (LAMA4)	Q16363	2.2	2.6	3.1	75	66	106	15	15	15	62500	125000	3.6	8	8
LDLR chaperone MESD (MESDC2)	Q14696	1.1	1.5	2.0	78	53	51	3.8	61	61	125000	250000	3.3	9	12
Legumain (LGMN)	O99538	3.3	3.8	4.3	93	110	156	15	0.48	0.95	15625	31250	4.2	9	10
Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1)	O6GTX8	2.8	3.6	5.8	96	98	108	15	0.12	0.24	977	3906	3.6	10	8
Low affinity immunoglobulin epsilon Fc receptor (FCER2)	P06734	4.0	4.6	5.6	96	92	98	15	3.8	7.6	31250	62500	3.6	10	7
Lymphocyte function-associated antigen 3 (CD58)	P19256	2.6	2.9	3.3	99	96	103	3.8	0.95	0.95	1953	7812	3.3	10	12
Macrophage migration inhibitory factor (MIF)	P14174	3.9	5.0	6.0	44	40	67	0	1.9	1.9	1953	7812	3.0	10	9
Matrilin-2 (MATN2)	O00339	3.5	4.0	4.6	99	87	86	15	7.6	7.6	31250	62500	3.6	9	7
Mycilin (MYOC)	O99972	3.0	4.0	4.7	98	96	90	15	NA	NA	NA	NA	NA	11	11
Neurofascin (NFASC)	O94856	0.5	0.8	2.1	109	100	102	15	7.6	7.6	7812	15625	3.0	8	7
Nidogen-2 (NID2)	Q14112	0.3	1.1	2.3	66	124	215	15	7812	7812	1000000	1000000	2.1	8	7
Osteomodulin (OMD)	O99983	2.8	3.5	4.8	96	70	76	15	31	31	31250	125000	3.0	11	11
Paired immunoglobulin-like type 2 receptor alpha (PILRA)	Q9UJK1	2.6	3.1	3.7	95	96	100	15	0.95	3.8	7812	31250	3.3	9	8
Peptidyl-prolyl cis-trans isomerase B (PPIB)	P23284	1.4	2.1	2.7	71	94	144	3.8	31	61	7812	31250	2.1	10	12
Phosphatidylethanolamine-binding protein 1 (PEBP1)	P30086	2.8	3.4	4.0	50	45	102	0	61	61	31250	62500	2.7	13	16
Platelet endothelial aggregation receptor 1 (PEAR1)	O5VY43	4.8	5.1	5.6	94	100	109	15	1.9	7.6	31250	125000	3.6	10	10
Platelet-derived growth factor receptor beta (PDGFRB)	P09619	3.8	4.3	4.8	99	97	97	15	0.48	0.48	977	3906	3.3	8	9
Protein deglycase DJ-1 (PARK7)	O99497	1.8	2.4	2.9	55	47	81	0	7.6	7.6	15625	250000	3.3	10	9
Protein disulfide-isomerase (P4HB)	P07237	0.7	1.1	1.5	98	91	97	15	15	31	31250	125000	3.0	8	6
Protein NOV homolog (NOV)	P48745	2.7	3.3	4.0	93	97	114	15	15	15	15625	31250	3.0	10	11
Receptor-type tyrosine-protein phosphatase F (PTPRF)	P10586	3.1	3.6	4.0	101	98	99	15	61	61	62500	500000	3.0	9	8
Roundabout homolog 1 (ROBO1)	Q9Y6N7	2.1	2.5	2.8	99	100	105	15	7.6	15	62500	250000	3.6	10	11
Scavenger receptor class F member 1 (SCARF1)	Q14162	4.7	5.2	5.6	94	121	174	15	0.48	0.95	15625	31250	4.2	9	10
Secretoglobin family 3A member 1 (SCGB2A1)	Q96QR1	2.5	4.0	4.7	96	100	104	15	31	61	31250	125000	2.7	10	13
Semaphorin-7A (SEMA7A)	O75326	3.9	4.4	4.8	97	100	112	1.9	1.9	7.6	31250	125000	3.6	8	10
Serine protease HTRA2, mitochondrial (HTRA2)	O43464	0.2	0.8	1.2	100	NA	93	15	31	61	15625	31250	2.4	9	8
Serine protease inhibitor Kazal-type 1 (SPINK1)	P00995	2.9	3.5	4.1	93	93	96	15	15	15	15625	62500	3.0	9	9
Serine protease inhibitor Kazal-type 5 (SPINK5)	Q9NQ38	0.6	0.9	1.5	110	101	108	15	244	244	62500	125000	2.4	10	11
Signal-regulatory protein beta-1 (SIRPB1)	O00241	2.7	3.2	3.8	98	99	105	15	0.95	0.95	15625	62500	4.2	10	7
Stress-induced-phosphoprotein 1 (STIP1)	P31948	NA	NA	0.9	NA	NA	NA	0.5	15	31	31250	31250	3.0	10	16
Synaptosomal-associated protein 29 (SNAP29)	O95721	1.8	2.6	3.4	88	76	90	7.5	31	31	125000	250000	3.6	15	10
Thymosin beta-10 (TMSB10)	P63313	1.6	2.4	3.4	72	72	73	7.5	244	244	31250	62500	2.1	13	9
Tissue alpha-L-fucosidase (FUCA1)	P04066	5.4	6.4	7.1	90	90	114	15	0.95	1.9	31250	250000	4.2	11	13
Tripeptidyl-peptidase 1 (TPP1)	O14773	4.0	4.5	5.1	89	114	148	15	3.8	3.8	31250	125000	3.9	9	9
Tumor necrosis factor receptor superfamily member 19L (RELT)	Q96924	3.8	4.4	4.9	92	125	155	15	0.95	0.95	15625	31250	4.2	10	9
Tyrosine-protein phosphatase non-receptor type 6 (PTPNE6)	P29350	NA	NA	0.5	NA	NA	NA	15	976	1953	125000	1000000	1.8	11	9
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2 (WFKKN2)	Q8TEU8	3.9	4.4	5.0	98	94	94	15	7.6	7.6	31250	31250	3.6	10	6
V-set and immunoglobulin domain-containing protein 4 (VSI4)	Q9Y279	4.3	5.0	5.6	91	96	97	15	0.48	0.48	3906	15625	3.9	10	10

\*U/μl

## 2.3 PRECISION

### REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 individual samples run in triplicates within each of 10 separate runs during the validation studies. Inter-assay variation (between runs) was calculated between experiments with the same operator. The reported inter-assay %CV is the average of three operators' %CV. Variation calculations were performed on linearized values for 92 analytes for which response levels could be measured in serum and normal plasma, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 9.8% and 9.9%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

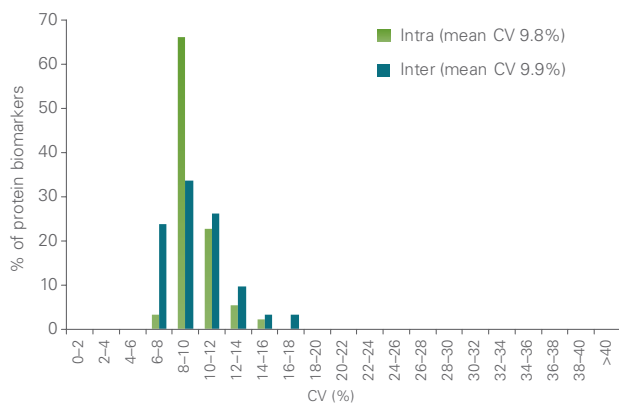


Fig 4. Distribution of intra-assay and inter-assay variations of Olink Development.

### REPRODUCIBILITY

Inter-site variations (between-site) have been investigated during validation of previous panels in a beta-site study to estimate the expected increase in values between different laboratories, with different operators and using different equipment. The beta-site studies have previously shown reproducibility and repeatability in line with Olink Proteomics, and therefore not performed for Olink Development. For more information, please download our Data Validation documents at [www.olink.com](http://www.olink.com)

## 2.4 ANALYTICAL SPECIFICITY

### ASSAY SPECIFICITY

The antibodies used in Olink Development were all specific for their respective targets. In principle, the specificity is tested by creating a test sample, consisting of a pool of antigens, which is then incubated with all 92 antibody probe pairs from the panel. Only if there is a correct match will a reporter sequence be created and serve as a template for subsequent real-time qPCR. Ten sub-pools of antigen are evaluated to cover the 92 assays in Olink, see Figure 5.

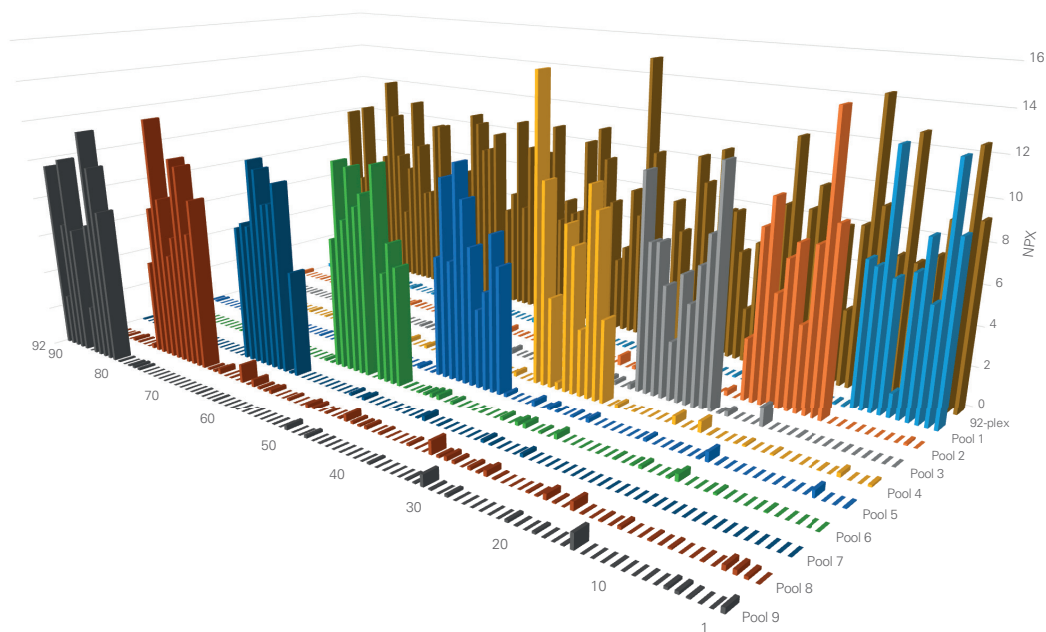
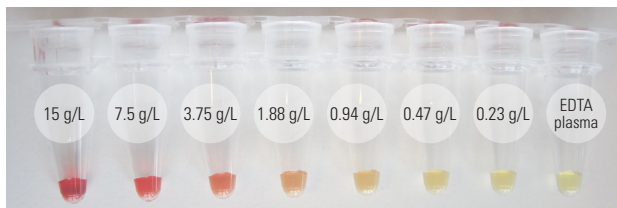


Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

## ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are 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 could be detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown).

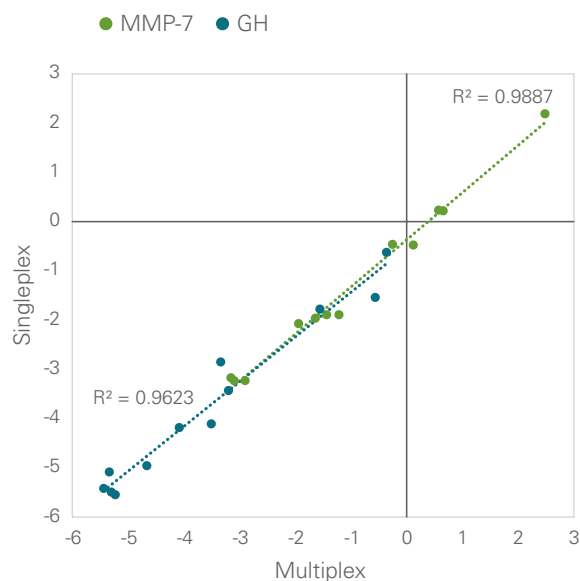


**Fig 6.** Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of bilirubin, lipids and hemolysate, known interfering plasma and serum components, were evaluated at different added concentrations. An example of hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. Interferens by bilirubin and lipids has previously been evaluated, and disturbance has only been observed at extrem levels corresponding to 8 or 10 times normal<sup>3, 4</sup> values and therefore not performed for Olink Development. In 15 out of 92 assays, altered signal was observed by the addition of hemolysate. The reason is most likely due to actual analyte leaking out of the disrupted blood cells. A concentration of 15 g/L of hemolysate represents 10% hemolysis of a sample. Table 1 reports the highest concentration of hemolysate that does not have an impact on assay performance.

## 2.5 SCALABILITY

Assay performance was further evaluated with regard to scalability, meaning the capability of the Olink technology to maintain the same quality of performance irrespective of multiplex level. Previously, we have shown that a step-wise increase of multiplex grade (8, 24, 48, 72 and 96) does not compromise assay performance (data not shown). To further strengthen that Olink provides consistent results, single assays for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) were compared when run in a full 96-plex reaction. The results for each assay and their observed dCq-values were plotted against the entire 96-plex reaction. The square of the correlation coefficient ( $R^2$ ) value was generated by linear regression.



**Fig 7.** Scalability of the Olink technology platform. The experiment was performed using the Olink CVD II <sup>96x96</sup> panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The observed dCq (log2) values were plotted, and the correlation coefficient  $R^2$  value was generated by linear regression.



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### TECHNICAL SUPPORT

For technical support, please contact us at [support@olink.com](mailto:support@olink.com) or +46 18 444 3970

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