



VALIDATION DATA

# 1. Introduction

Olink® Cell Regulation is a reagent kit measuring 92 cell regulating related human protein biomarkers simultaneously. 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.

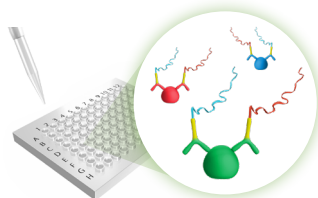
An external control, inter-plate control (IPC), is included on each plate and used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. Furthermore, the improves inter-assay precision 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 log2 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  $2^{NPX}$ . Coefficient of variation (CV) calculations were performed on linearized values.

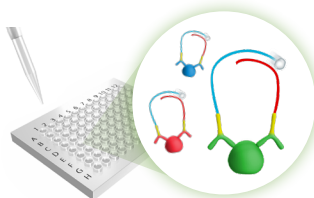
### IMMUNOASSAY

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



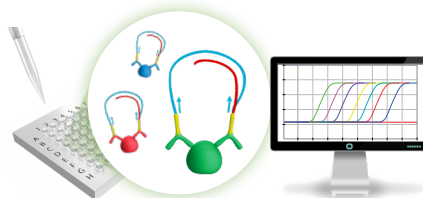
### EXTENSION

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 Cell Regulation 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 Table 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 80 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.

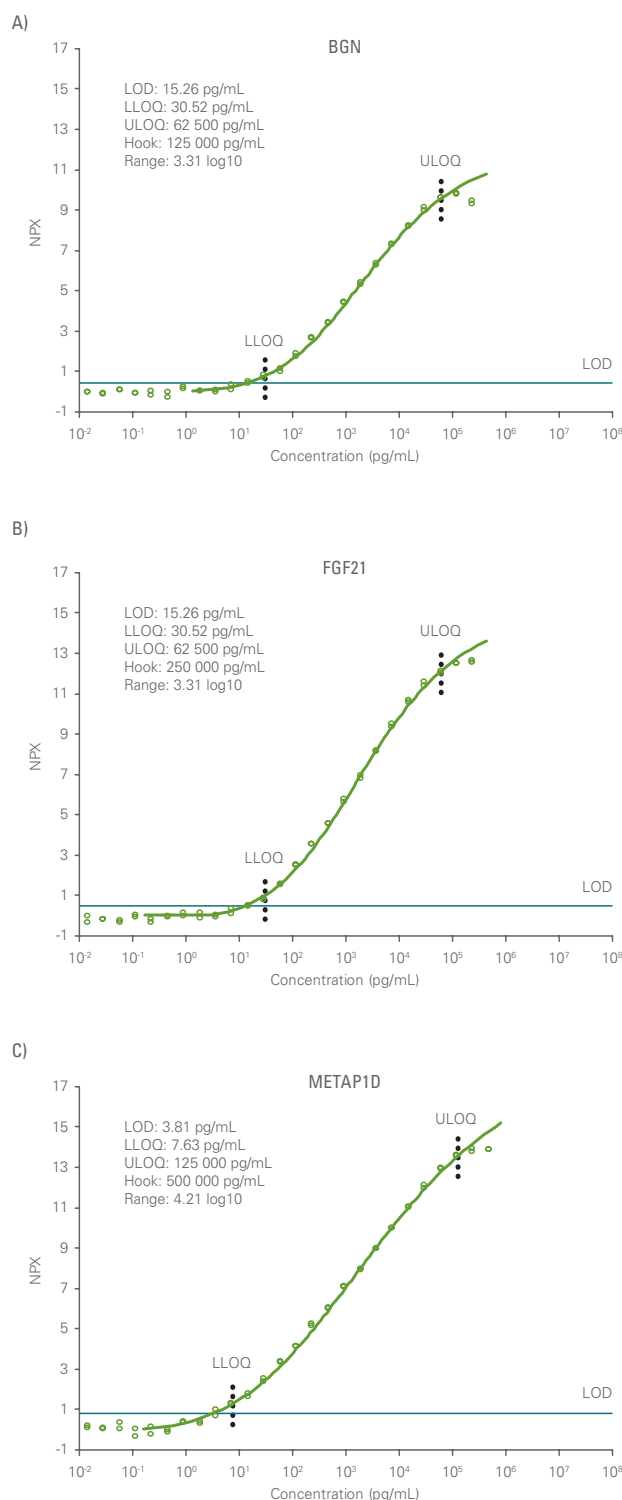
#### HIGH DOSE HOOK EFFECT

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 80 assays, see Table 1.

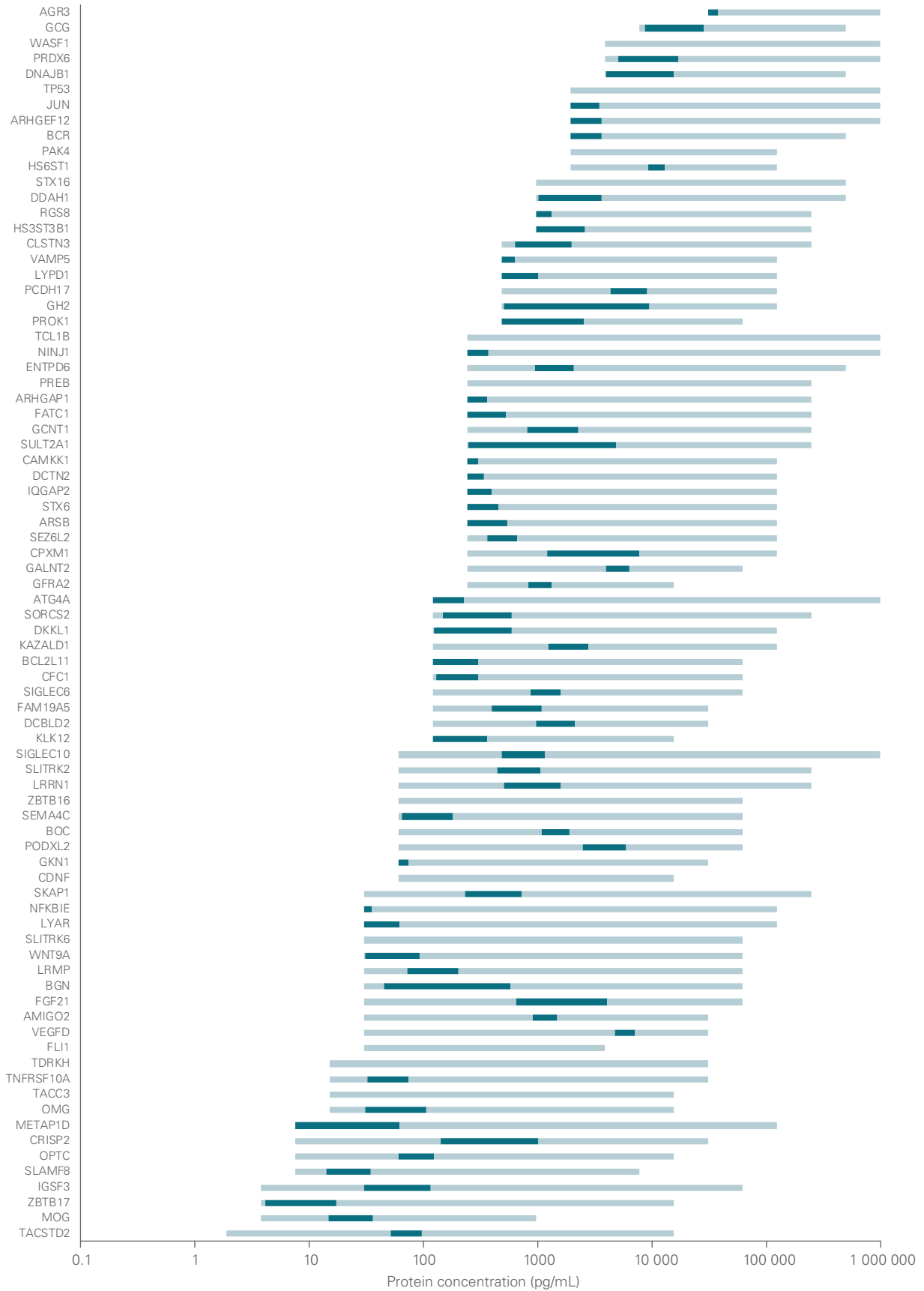
#### MEASURING RANGE

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  $\leq 30\%$  and CV  $\leq 30\%$ , 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 80 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 green bars) are denoted for 80 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.

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	Hemolytate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Amphoterin-induced protein 2 (AMIG02)	Q86SJ2	3.7	4	4.3	92	124	145	15	30	30	31250	1000000	3.0	5	18
Amyloid beta A4 precursor protein-binding family B member 1-interacting protein (APBB1IP)	Q7Z5R6	1	1.4	2.1	92	109	150	15	NA	NA	NA	NA	NA	5	16
Anterior gradient protein 3 (AGR3)	Q8TD06	NA	1.5	2.6	128	86	131	15	7812	31250	1000000	1000000	1.5	11	17
Arylsulfatase B (ARSB)	P15848	0.5	1	1.5	79	135	472	15	122	244	125000	1000000	2.7	7	16
ATP-dependent 6-phosphofructokinase, muscle type (PFKM)	P08237	0.9	1.5	2.4	70	77	112	0	NA	NA	NA	NA	NA	7	14
Bcl-2-like protein 11 isoform BimL (BCL2L11)	O43521	0.6	1.4	2.1	82	81	73	15	61	122	62500	125000	2.7	7	27
Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase (GCNT1)	Q02742	1.4	1.9	2.8	109	96	123	15	244	244	250000	1000000	3.0	5	21
Biglycan (BGN)	P21810	1	1.3	3.4	133	91	94	15	15	30	62500	125000	3.3	11	28
Bile salt sulfotransferase (SULT2A1)	Q06520	0.6	1.1	4.4	99	NA	127	15	122	244	250000	500000	3.0	11	21
Breakpoint cluster region protein (BCR)	P11274	0.7	1.2	1.7	40	32	44	15	1953	1953	500000	1000000	2.4	5	16
Brother of CDO (BOC)	Q9BWW1	4.5	5.1	5.4	92	98	113	15	30	61	62500	125000	3.0	6	25
Calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1)	Q8N5S9	NA	NA	0.6	NA	NA	NA	15	244	244	125000	250000	2.7	5	16
Calsyntenin-3 (CLSTN3)	Q98QT9	0.9	1.2	1.9	100	95	118	15	244	488	250000	500000	2.7	6	21
Cell growth-regulating nucleolar protein (LYAR)	Q9NX58	NA	NA	1.1	NA	NA	NA	15	30	30	125000	250000	3.6	5	18
Cellular tumor antigen p53 (TP53)	P04637	0.5	1.2	1.6	104	NA	104	15	976	1953	1000000	1000000	2.7	23	38
Cerebral dopamine neurotrophic factor (CDNF)	Q49AH0	0.3	0.6	1.1	107	NA	106	15	30	61	15625	62500	2.4	4	14
Collagen alpha-1(IV) chain (COL4A1)	P02462	5.5	6	6.4	79	102	107	15	NA	NA	NA	NA	NA	7	20
Cone-rod homeobox protein (CRX)	O43186	NA	NA	1.9	99	100	106	15	NA	NA	NA	NA	NA	10	20
Cryptic protein (CFC1)	P0CG37	0.9	1.3	1.7	100	78	98	15	122	122	62500	125000	2.7	4	19
C-type mannose receptor 2 (MRC2)	Q9UBG0	1.5	1.9	2.6	103	100	111	15	NA	NA	NA	NA	NA	8	15
Cysteine protease ATG4A (ATG4A)	Q8WYNO	0.1	1.2	2.2	48	45	118	0	61	122	1000000	1000000	3.9	8	23
Cysteine-rich secretory protein 2 (CRISP2)	P16562	5.1	6.9	8.2	94	109	117	15	3.8	7.6	31250	125000	3.6	6	23
Dickkopf-like protein 1 (DKKL1)	Q9UK85	0.8	1.4	2.2	100	102	115	15	122	122	125000	250000	3.0	6	23
Discoidin, CUB and LCCL domain-containing protein 2 (DCBLD2)	Q96PD2	2.7	3.2	3.8	94	109	121	15	122	122	31250	125000	2.4	6	17
DnaJ homolog subfamily B member 1 (DNAJB1)	P25685	0.7	1.8	2.7	29	24	76	0.5	3906	3906	500000	1000000	2.1	7	19
Dual specificity mitogen-activated protein kinase kinase 6 (MAP2K6)	P52564	1.1	1.6	2.4	88	78	113	0.9	NA	NA	NA	NA	NA	5	16
Dynactin subunit 2 (DCTN2)	Q13561	0.3	0.7	1	119	105	107	0.2	244	244	125000	500000	2.7	7	21
E3 ubiquitin-protein ligase CBL (CBL)	P22681	NA	0.6	1	80	60	80	15	NA	NA	NA	NA	NA	5	16
Ectonucleoside triphosphate diphosphohydrolase 6 (ENTPD6)	Q75354	1.8	2.3	2.7	95	104	113	15	244	244	500000	1000000	3.3	5	20
Fibroblast growth factor 21 (FGF21)	Q9NSA1	4.9	5.9	8.3	91	99	93	15	15	30	62500	250000	3.3	6	17
Friend leukemia integration 1 transcription factor (FLI1)	Q01543	NA	0.2	0.8	NA	NA	86	15	15	30	3906	7812	2.1	4	18
Galectin-7 (LGALS7)	P47929	2.7	3.3	3.7	120	92	89	15	NA	NA	NA	NA	NA	13	22
Gamma-secretase-activating protein (GSAP)	A4D1B5	NA	0.2	1.8	NA	NA	NA	15	NA	NA	NA	NA	NA	11	19
Gastrokine (GKN1)	Q9NS71	0.6	0.9	1.2	112	NA	100	15	61	61	31250	1000000	2.7	6	14
GDNF family receptor alpha-2 (GFRA2)	O00451	2.1	2.4	2.7	94	87	115	15	122	244	15625	15625	1.8	3	18
Glucagon (GCG)	P01275	1.5	2.6	4.5	91	93	68	15	7812	7812	500000	500000	1.8	10	16
Growth hormone variant (GH2)	P01242	0.6	2.1	4	91	92	114	15	488	488	125000	125000	2.4	7	13
Heparan sulfate glucosamine 3-O-sulfotransferase 3B1 (HS3ST3B1)	Q9Y662	1.3	2.3	2.9	86	71	98	15	244	976	250000	500000	2.4	7	19
Heparan-sulfate 6-O-sulfotransferase 1 (HS6ST1)	O60243	3.7	4.1	4.5	88	11	110	15	976	1953	125000	125000	1.8	7	17
Immunoglobulin superfamily member 3 (IGSF3)	Q75054	3.1	3.8	4.8	99	108	113	15	0.95	3.8	62500	125000	4.2	8	28
Interleukin-17 receptor B (IL17RB)	Q9NRM6	3.6	4.4	5.1	95	110	116	15	NA	NA	NA	NA	NA	6	21
Kallikrein-12 (KLK12)	Q9UKR0	NA	1.6	3	104	94	106	15	61	122	15625	31250	2.1	8	20
Kazal-type serine protease inhibitor domain-containing protein 1 (KAZALD1)	Q96I82	2.9	3.5	4	88	103	97	15	122	122	125000	250000	3.0	5	19
Leucine-rich repeat neuronal protein 1 (LRN1)	Q6UXK5	3.6	4.5	5.3	96	109	109	15	61	61	250000	500000	3.6	7	22
Ly6/PLAUR domain-containing protein 1 (LYPD1)	Q8N2G4	NA	0.4	1.2	NA	NA	NA	15	388	488	125000	1000000	2.4	6	13

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		10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Lymphoid-restricted membrane protein (LRMP)	Q12912	1.1	1.5	2.3	133	113	70	15	30	30	62500	125000	3.3	5	19
Methionine aminopeptidase 1D, mitochondrial (METAP1D)	Q6UB28	0.4	1.4	3.2	82	61	52	0.9	3.8	7.6	125000	500000	4.2	6	17
Myelin-oligodendrocyte glycoprotein (MOG)	Q16653	2.3	3	3.5	93	101	114	15	1.9	3.8	977	3906	2.4	5	16
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (DDAH1)	Q94760	0.9	1.4	2.3	101	99	110	0.5	976	976	500000	1000000	2.7	7	20
NF-kappa-B inhibitor epsilon (NFKBIE)	Q00221	0.3	0.8	1.1	89	81	100	3.8	15	30	125000	250000	3.6	6	19
Nicalin (NCLN)	Q969V3	NA	NA	0.3	NA	NA	NA	15	NA	NA	NA	NA	NA	2	NA
Ninjurin-1 (NINJ1)	Q92982	0.6	0.9	1.2	102	77	87	15	244	244	1000000	1000000	3.6	8	17
Nuclear factor of activated T-cells, cytoplasmic 1 (NFATC1)	Q95644	0.2	0.7	1.2	67	52	71	15	244	244	250000	1000000	3.0	6	21
Oligodendrocyte-myelin glycoprotein (OMG)	P23515	1.4	2.3	2.9	97	98	112	15	7.6	15	15625	125000	3.0	5	19
Opticin (OPTC)	Q9UBM4	3.4	3.9	4.5	96	109	121	15	7.6	7.6	15625	62500	3.3	6	18
Peroxisome oxidase 6 (PROX6)	P30041	0.9	1.5	2.2	129	79	58	0	1953	3906	1000000	1000000	2.4	7	28
Podocalyxin-like protein 2 (PODXL2)	Q9NZ53	5.2	6	6.5	96	115	114	15	61	61	62500	125000	3.0	7	25
Polypeptide N-acetylgalactosaminyltransferase 2 (GALNT2)	Q10471	3.8	4.2	4.5	94	114	121	15	122	244	62500	250000	2.4	4	16
Probable carboxypeptidase X1 (CPXM1)	Q96SM3	1.8	2.9	4.3	29	104	568	15	244	244	125000	125000	2.7	7	20
Prokineticin-1 (PROK1)	P58294	1.7	3.2	4.5	95	80	93	15	244	488	62500	125000	2.1	6	16
Prolactin regulatory element-binding protein (PREB)	Q9HCU5	NA	0.3	0.5	NA	NA	NA	15	122	244	250000	250000	3.0	4	19
Protein FAM19A5 (FAM19A5)	Q725A7	1.9	2.9	3.6	77	71	74	15	122	122	31250	62500	2.4	6	13
Protein Wnt-9a (WNT9A)	Q14904	1	1.6	2	98	87	81	15	15	30	62500	125000	3.3	7	17
Protocadherin-17 (PCDH17)	Q14917	3.6	4.3	4.8	79	105	122	15	244	488	125000	500000	2.4	7	23
Ras GTPase-activating-like protein IQGAP2 (IQGAP2)	Q13576	NA	0.6	1.2	83	87	82	1.9	244	244	125000	1000000	2.7	6	20
Regulator of G-protein signaling 8 (RGS8)	P57771	NA	0.2	1	102	70	98	15	488	976	250000	1000000	2.4	9	21
Rho GTPase-activating protein 1 (ARHGAP1)	Q07960	1.1	1.5	2.1	95	134	136	0.5	255	244	250000	1000000	3.0	6	19
Rho guanine nucleotide exchange factor 12 (ARHGEF12)	Q9NZN5	NA	0.5	1.2	49	36	48	0.9	1953	1953	1000000	1000000	2.7	6	21
Seizure 6-like protein 2 (SEZ6L2)	Q6UXD5	0.8	1.1	1.3	104	71	104	15	122	244	125000	500000	2.7	5	24
Semaphorin-4C (SEMA4C)	Q9C0C4	1	1.4	1.9	96	130	88	15	30	61	62500	250000	3.0	8	17
Serine/threonine-protein kinase PAK 4 (PAK4)	Q96013	NA	NA	0.6	NA	NA	NA	15	488	1953	125000	250000	1.8	7	20
Sialic acid-binding Ig-like lectin 10 (SIGLEC10)	Q96LC7	2.6	3.3	3.7	101	103	106	15	61	61	1000000	1000000	4.2	6	23
Sialic acid-binding Ig-like lectin 6 (SIGLEC6)	Q43699	3.9	4.5	4.9	95	101	112	15	61	122	62500	125000	2.7	7	20
SLAM family member 8 (SLAMF8)	Q9P0V8	1.5	1.9	2.5	105	91	108	15	3.8	7.6	7812	31250	3.0	21	35
SLIT and NTRK-like protein 2 (SLITRK2)	Q9H156	3.1	3.8	4.2	81	87	117	15	61	61	250000	500000	3.6	7	18
SLIT and NTRK-like protein 6 (SLITRK6)	Q9H5Y7	NA	0.6	1	NA	NA	101	15	15	30	62500	125000	3.3	7	20
Src kinase-associated phosphoprotein 1 (SKAP1)	Q86WV1	3.9	4.6	5.6	97	171	54	15	15	30	250000	250000	3.9	6	16
Syntaxin-6 (STX6)	Q14662	NA	NA	0.7	NA	NA	NA	3.8	488	976	500000	1000000	2.7	16	37
Syntaxin-16 (STX16)	Q43752	NA	1.1	1.8	104	89	96	0.5	244	244	125000	250000	2.7	7	17
T-cell leukemia/lymphoma protein 1B (TCL1B)	Q95988	NA	NA	1.1	NA	NA	NA	15	122	244	1000000	1000000	3.6	8	19
Transcription factor AP-1 (JUN)	P05412	NA	NA	2.7	100	99	101	15	976	1953	1000000	1000000	2.7	11	18
Transforming acidic coiled-coil-containing protein 3 (TACC3)	Q9Y6A5	NA	0.4	1	100	97	58	1.9	7.6	16	15625	125000	3.0	7	24
Tudor and KH domain-containing protein (TDRKH)	Q9Y2W6	NA	0.4	0.8	95	NA	93	15	15	15	31250	62500	3.3	4	16
Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A)	Q00220	2.1	2.5	3.1	103	90	110	15	7.6	15	31250	31250	3.3	5	12
Tumor-associated calcium signal transducer 2 (TACSTD2)	P09758	3.7	4.2	4.5	90	99	115	15	0.95	1.9	15625	31250	3.9	5	19
Vascular endothelial growth factor D (VEGFD)	Q43915	8.1	8.6	8.9	104	115	130	15	30	30	31250	125000	3.0	4	20
Vesicle-associated membrane protein 5 (VAMP5)	Q95183	0.3	0.8	1.9	109	NA	89	15	122	488	125000	125000	2.4	5	12
Wiskott-Aldrich syndrome protein family member 1 (WASF1)	Q92558	NA	0.2	1.1	92	NA	56	15	1953	3906	1000000	1000000	2.4	11	23
Wiskott-Aldrich syndrome protein family member 3 (WASF3)	Q9UJY6	NA	NA	NA	NA	NA	NA	15	NA	NA	NA	NA	NA	NA	NA
VPS10 domain-containing receptor SorCS2 (SORCS2)	Q96PQ0	0.9	1.7	2.2	92	68	85	15	122	122	250000	1000000	3.3	6	18
Zinc finger and BTB domain-containing protein 16 (ZBTB16)	Q05516	NA	NA	NA	134	94	NA	15	61	61	62500	250000	3.0	9	35
Zinc finger and BTB domain-containing protein 17 (ZBTB17)	Q13105	1.1	1.8	2.5	86	90	110	0	1.9	3.8	15625	31250	3.6	5	13

\*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 7.1% and 19.7%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

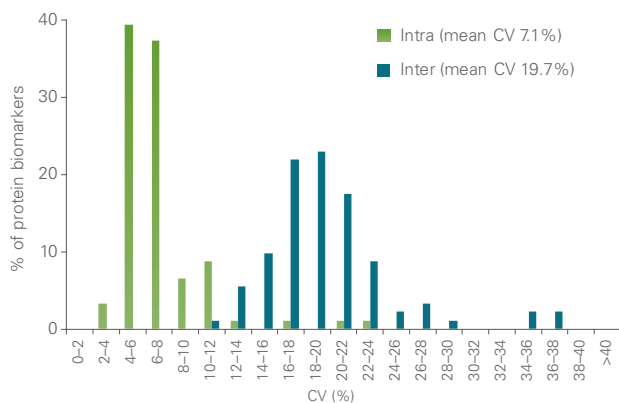


Fig 4. Distribution of intra-assay and inter-assay variations of Olink Cell Regulation.

### 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 Cell Regulation. 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 Cell Regulation 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.

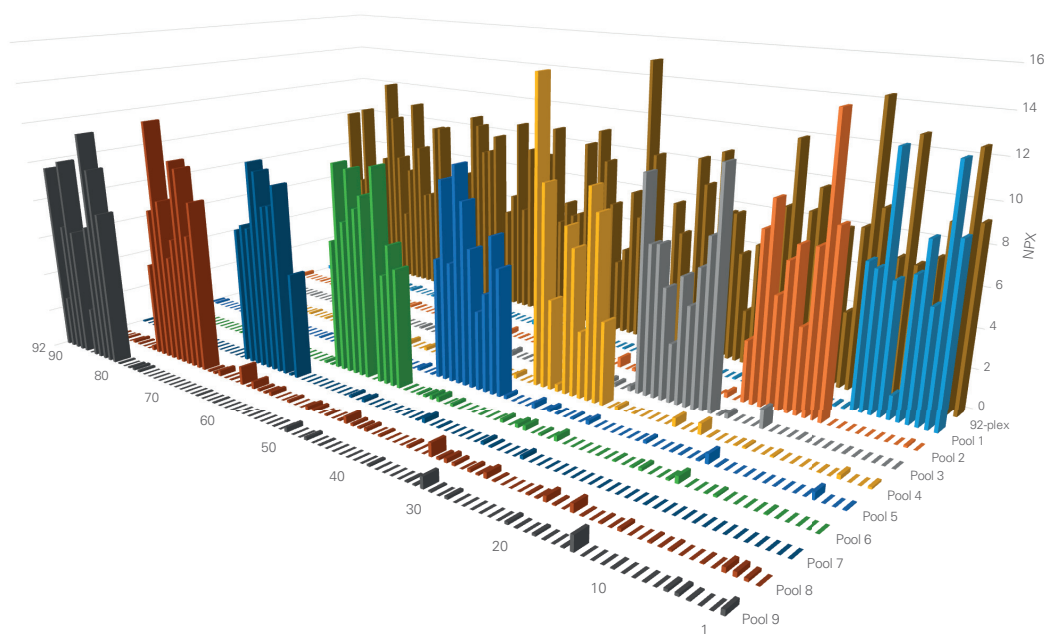
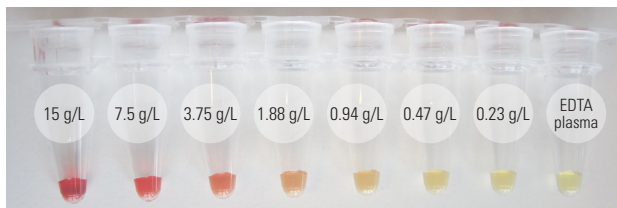


Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

## 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).



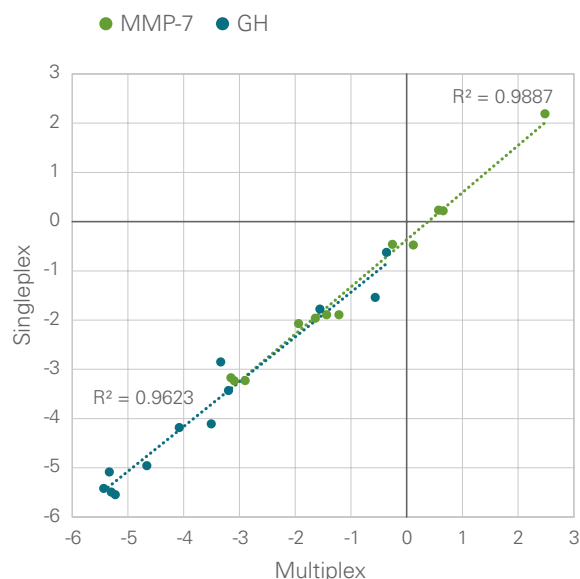
**Fig 6.** Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

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 Cell Regulation.

In 16 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

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