1. Introduction

Olink® Mouse Exploratory is a reagent kit measuring 92 mouse 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[1-2], where 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-mouse 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 IPC 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 $2^{\wedge NPX}$. Coefficient of variation (CV) calculations were performed on linearized values.

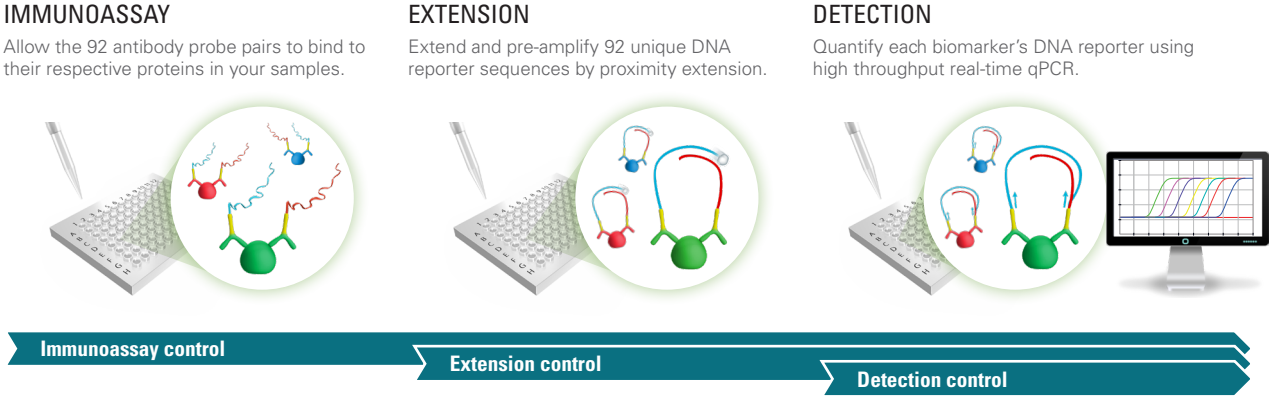

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and are used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Mouse Exploratory by collecting matched serum and EDTA plasma, bronchoalveolar lavage (BAL), cerebral spinal fluid (CSF), heart- and liver lysate samples as well as urine from healthy and diseased mice. Table 1 summarizes analytical measuring range for normal EDTA plasma samples expressed in NPX.

Detectability in each sample matrix is presented as the mean % of all samples included in the validation studies that could be measured above the limit of detection (LOD), see Figure 2.

Detectability diagrams for the various mouse sample types tested, are available for each individual assay at www.olink.com/mouse.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Calibrator curves were determined for 42 out of 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 3.

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 42 out of 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 log10, 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 3. Separate calibrator curves established for each assay may be viewed at www.olink.com/mouse

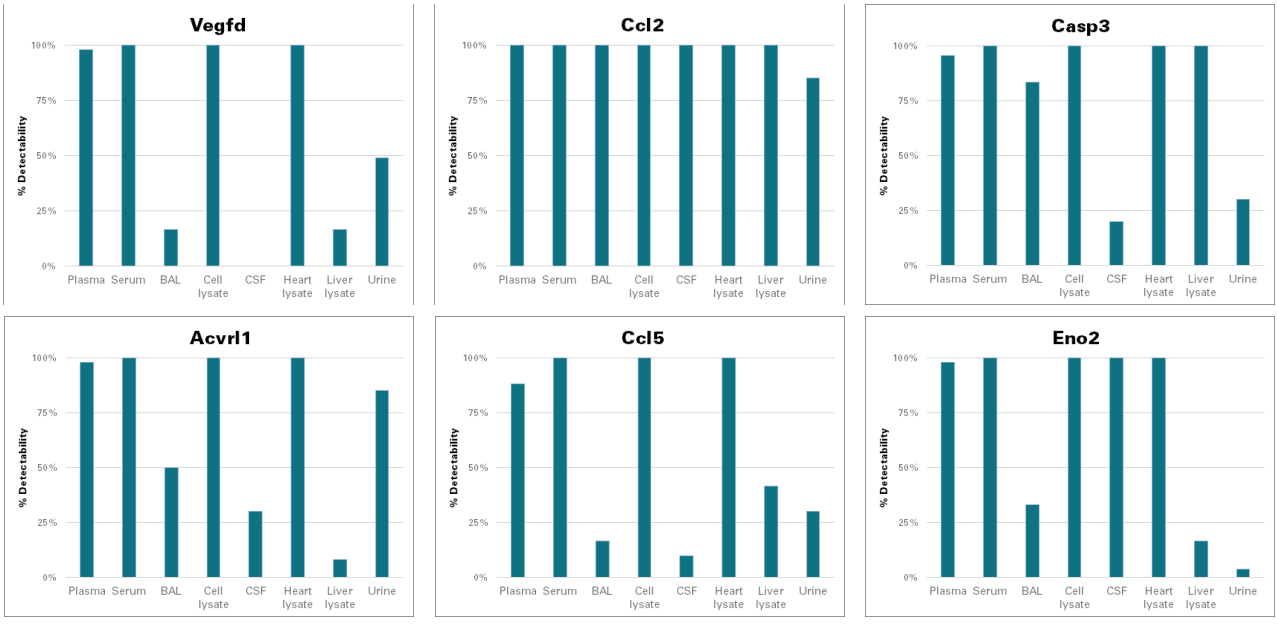

Fig 2. Detectability in each sample matrix for 6 assays.

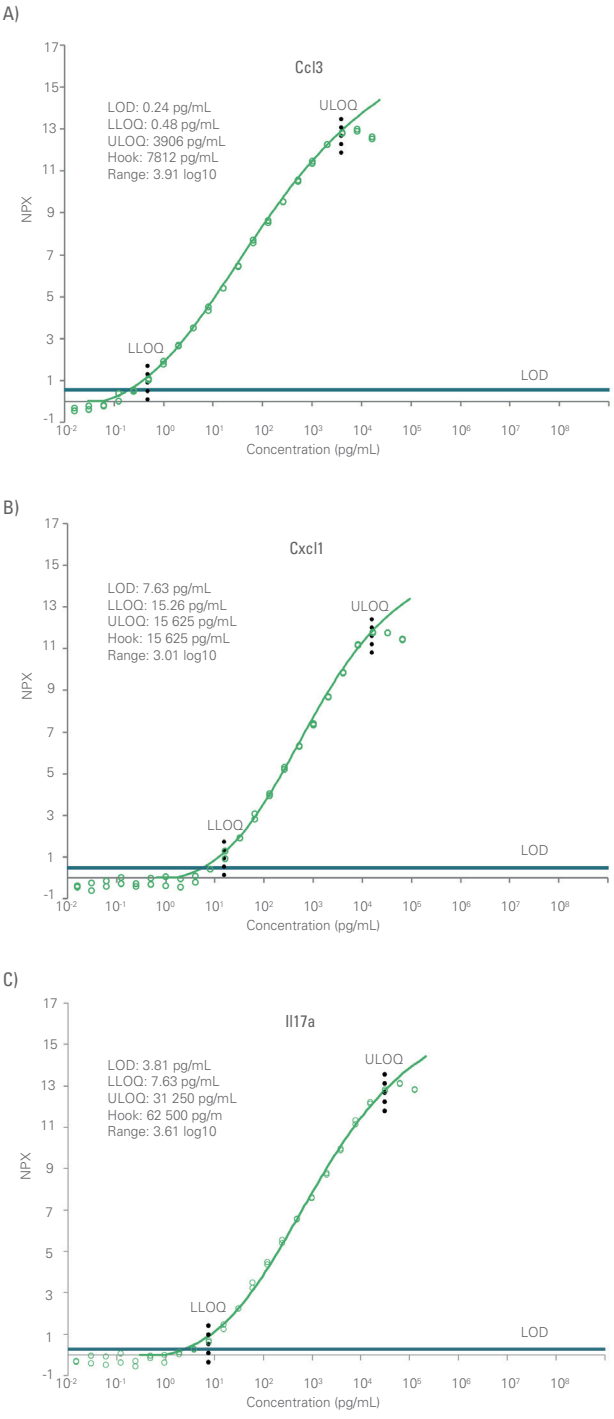

Fig 3. Calibrator curves from 3 assays and their corresponding analytical measurement data.

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 4 individual samples run in triplicates within each of 6 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 all 92 analytes for which response levels could be measured in normal plasma, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 6.1% and 12.5%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

REPRODUCIBILITY

Inter-site variations (between-site) were investigated during the validation of previous panels in beta-site studies to estimate the expected variations 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 results, and were therefore not performed for Olink Mouse Exploratory. For information on performed beta-site studies, download our Data Validation documents at www.olink.com/data-validation

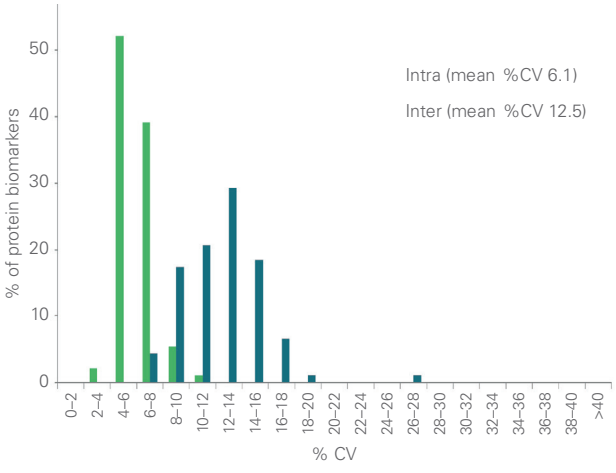

Fig 4. Distribution of intra-assay and inter-assay variations of Olink Mouse Exploratory

Table 1. EDTA plasma; Normalized Protein eXpression (NPX), 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.

		EDTA plasma			Analytical measurement					Precision	
		Normal plasma levels (NPX)			pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Amyloid beta A4 precursor protein-binding family B member 1-interacting protein (Apbb1ip)	Q8R5A3	0,8	1,1	1,9	NA	NA	NA	NA	NA	5	12
Appetite-regulating hormone (Ghrl)	Q9EQX0	1,9	3,6	4	NA	NA	NA	NA	NA	6	11
Aryl hydrocarbon receptor (Ahr)	P30561	1,3	1,8	2,2	NA	NA	NA	NA	NA	10	15
Axin-1 (Axin1)	O35625	0	1,3	2	NA	NA	NA	NA	NA	6	12
Cadherin-6 (Cdh6)	P97326	1,3	1,9	2,2	1953	1953	250000	1000000	2.1	5	15
Calsyntenin-2 (Clstn2)	Q9ER65	0,2	0,6	1	NA	NA	NA	NA	NA	7	14
Carbonic anhydrase 13 (Ca13)	Q9D6N1	0,4	1	1,8	NA	NA	NA	NA	NA	7	12
Carboxypeptidase E (Cpe)	Q00493	3,5	4,5	4,9	NA	NA	NA	NA	NA	8	18
Caspase-3 (Casp3)	P70677	2,4	3,8	4,7	NA	NA	NA	NA	NA	6	10
C-C motif chemokine 2 (Ccl2)	P10148	8,1	9	10,4	0.24	0.48	3906	7812	3.9	6	9
C-C motif chemokine 20 (Ccl20)	O89093	7,4	7,9	8,3	15	31	7812	7812	2.4	5	8
C-C motif chemokine 3 (Ccl3)	P10855	1,9	2,6	3,9	0.24	0.48	3906	7812	3.9	6	7
C-C motif chemokine 5 (Ccl5)	P30882	-0,1	0,2	0,7	244	244	31250	125000	2.1	5	9
Contactin-1 (Cntn1)	P12960	4,3	4,6	4,8	NA	NA	NA	NA	NA	5	12
Contactin-4 (Cntn4)	Q69Z26	1,7	2,2	2,5	61	61	62500	250000	3	6	10
CXADR-like membrane protein (Clmp)	Q8R373	4,7	5,1	5,5	1.9	3.8	7812	15625	3.3	6	12
C-X-C motif chemokine 9 (Cxcl9)	P18340	2,8	3,3	4,7	31	31	250000	250000	3.9	6	8
Cysteine-rich motor neuron 1 protein (Crim1)	Q9JLL0	-0,2	0,4	0,8	3.8	3.8	15625	62500	3.6	6	16
Cytosolic phospholipase A2 (Pla2g4a)	P47713	3	4,2	6,1	NA	NA	NA	NA	NA	5	13
Delta-like protein 1 (Dll1)	Q61483	2,8	3	3,2	31	31	7812	15625	2.4	5	13
Dihydropteridine reductase (Qdpr)	Q8BVI4	2	3,1	3,6	NA	NA	NA	NA	NA	7	13
Disintegrin and metalloproteinase domain-containing protein 23 (Adam23)	Q9R1V7	0,4	1	1,3	NA	NA	NA	NA	NA	6	16
Dual specificity mitogen-activated protein kinase kinase 6 (Map2k6)	P70236	2,7	3,8	4,8	NA	NA	NA	NA	NA	3	14
Dynactin subunit 2 (Dctn2)	Q99KJ8	0,2	0,5	1,2	NA	NA	NA	NA	NA	6	11
Epithelial cell adhesion molecule (Epcam)	Q99JW5	1	1,4	1,8	7.6	15	7812	7812	2.7	8	12
Erythropoietin (Epo)	P07321	1,8	2,7	4,1	1.9	1.9	3906	7812	3.3	6	11
Follistatin (Fst)	P47931	6,9	7,1	7,5	31	31	62500	125000	3.3	5	11
Follistatin-related protein 3 (Fstl3)	Q9EQC7	4,2	4,4	4,7	244	488	62500	125000	2.1	4	15
Forkhead box protein O1 (Foxo1)	Q9R1E0	-0,2	0,2	0,6	NA	NA	NA	NA	NA	6	11
Friend leukemia integration 1 transcription factor (Fli1)	P26323	-0,1	0,8	2,4	NA	NA	NA	NA	NA	5	10
Gamma-enolase (Eno2)	P17183	0,9	1,1	1,5	NA	NA	NA	NA	NA	6	13
GDNF family receptor alpha-1 (Gfra1)	P97785	1,5	1,9	2,7	NA	NA	NA	NA	NA	8	15
Glial cell line-derived neurotrophic factor (Gdnf)	P48540	-0,2	0,1	0,5	NA	NA	NA	NA	NA	7	8
Glucagon (Gcg)	P55095	0,4	1,2	2,5	NA	NA	NA	NA	NA	8	NA
Granulocyte-macrophage colony-stimulating factor (Csf2)	P01587	0,1	0,3	0,7	1.9	1.9	7812	15625	3.6	5	9
Growth-regulated alpha protein (Cxcl1)	P12850	5,9	6,5	7,7	7.6	15	15625	15625	3	6	12
Hepatocyte growth factor (Hgf)	Q08048	2,3	2,8	3,3	31	31	15625	62500	2.7	6	10
Immunoglobulin superfamily member 3 (Igsf3)	Q6ZQA6	2,1	2,6	2,9	NA	NA	NA	NA	NA	8	15
Integrin beta-1-binding protein 2 (Itgb1bp2)	Q9R000	-0,3	0,1	0,6	NA	NA	NA	NA	NA	10	12
Integrin beta-6 (Itgb6)	Q9Z0T9	-0,1	0,4	0,8	NA	NA	NA	NA	NA	6	13
Interleukin-1 (Il1a)	P01582	1,6	2,6	3,7	0.06	0.06	3906	7812	4.8	5	10
Interleukin-1 beta (Il1b)	P10749	-0,1	0,4	1,7	15	31	500000	1000000	4.2	5	8
Interleukin-10 (Il10)	P18893	-0,2	0,3	0,9	15	31	31250	62500	3	6	12
Interleukin-17A (Il17a)	Q62386	0,2	0,7	1,3	3.8	7.6	31250	62500	3.6	6	10
Interleukin-17F (Il17f)	Q7TNI7	0	0,4	1,5	3.8	3.8	7812	31250	3.3	6	9
Interleukin-23 receptor (Il23r)	Q8K4B4	3,4	3,8	4,4	3.8	3.8	15625	62500	3.6	5	13

		EDTA plasma			Analytical measurement					Precision	
		Normal plasma levels (NPX)			pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Interleukin-5 (Il5)	P04401	0,3	1,3	8,4	31	31	31250	125000	3	8	7
Interleukin-6 (Il6)	P08505	-0,4	0,2	3,2	61	61	62500	500000	3	5	13
Kit ligand (Kitlg)	P20826	-0,4	-0,1	0,2	31	61	15625	62500	2.4	5	15
Latency-associated peptide transforming growth factor beta-1 (Tgfb1)	P04202	1,2	1,4	1,8	NA	NA	NA	NA	NA	6	12
Legumain (Lgmn)	O89017	3,6	4,1	4,7	488	976	250000	1000000	2.4	3	13
Leucine-rich repeat transmembrane protein FLRT2 (Flrt2)	Q8BLU0	2	2,5	2,8	NA	NA	NA	NA	NA	6	12
Lipoprotein lipase (Lpl)	P11152	1,3	2,1	2,7	NA	NA	NA	NA	NA	10	13
Matrilin-2 (Matn2)	O08746	1,5	2,3	2,8	976	976	125000	250000	2.1	7	15
Melanoma-derived growth regulatory protein (Mia)	Q61865	1,9	2,9	3,3	NA	NA	NA	NA	NA	5	14
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (Ddah1)	Q9CWS0	0,3	1,3	2,7	NA	NA	NA	NA	NA	6	8
NAD kinase (Nadk)	P58058	1,7	6,1	7,4	NA	NA	NA	NA	NA	4	15
Neurogenic locus notch homolog protein 3 (Notch3)	Q61982	2,9	3,2	3,4	122	122	62500	250000	2.7	5	15
Neurotrophin-3 (Ntf3)	P20181	0,3	0,7	1	NA	NA	NA	NA	NA	6	10
Perilipin-1 (Plin1)	Q8CGN5	0,2	0,8	1,6	NA	NA	NA	NA	NA	8	15
Peroxiredoxin-5, mitochondrial (Prdx5)	P99029	2,5	4,2	5,9	NA	NA	NA	NA	NA	6	27
Platelet-derived growth factor subunit B (Pdgfb)	P31240	2,4	3,1	5,4	NA	NA	NA	NA	NA	5	9
Plexin-A4 (Plxna4)	Q80UG2	0,3	0,9	1,4	NA	NA	NA	NA	NA	7	13
Poly [ADP-ribose] polymerase 1 (Parp1)	P11103	3	4,2	5,7	NA	NA	NA	NA	NA	5	10
Protein CYR61 (Cyr61)	P18406	2,4	3,6	4,9	NA	NA	NA	NA	NA	6	12
Protein delta homolog 1 (Dlk1)	Q09163	1,3	1,6	1,8	976	976	62500	250000	1.8	7	13
Protein phosphatase inhibitor 2 (Ppp1r2)	Q9DCL8	-0,1	0,9	1,7	NA	NA	NA	NA	NA	7	16
Protein S100-A4 (S100a4)	P07091	4,2	4,8	5,7	61	61	15625	125000	2.4	6	14
Protransforming growth factor alpha (Tgfa)	P48030	3,7	4,1	5,5	NA	NA	NA	NA	NA	6	9
Receptor tyrosine-protein kinase erbB-4 (Erbb4)	Q61527	2,7	2,9	3,2	31	61	31250	125000	2.7	5	10
Repulsive guidance molecule A (Rgma)	Q6PCX7	1,1	1,4	1,9	NA	NA	NA	NA	NA	8	12
Ribosomal oxygenase 2 (Riox2)	Q8CD15	0,1	1,8	3,5	NA	NA	NA	NA	NA	8	17
Seizure 6-like protein 2 (Sez6l2)	Q4V9Z5	2,1	2,9	3,2	NA	NA	NA	NA	NA	6	16
Serine/threonine-protein kinase PAK 4 (Pak4)	Q8BTW9	-0,4	0,1	0,5	NA	NA	NA	NA	NA	9	18
Serine/threonine-protein kinase receptor R3 (Acvrl1)	Q61288	2,2	2,6	2,8	31	31	15625	62500	2.7	7	12
Soluble calcium-activated nucleotidase 1 (Cant1)	Q8VCF1	0,2	0,4	0,6	NA	NA	NA	NA	NA	7	18
Synaptosomal-associated protein 29 (Snap29)	Q9ERB0	2	4	5,1	NA	NA	NA	NA	NA	7	16
Tenascin-R (Tnr)	Q8BYI9	2,7	3	3,3	NA	NA	NA	NA	NA	6	13
Transforming growth factor beta receptor type 3 (Tgfbr3)	O88393	2,3	2,6	2,9	1953	1953	250000	500000	2.1	5	13
Tripeptidyl-peptidase 1 (Tpp1)	O89023	4,7	5,2	5,7	NA	NA	NA	NA	NA	5	14
Troponin I, cardiac muscle (Tnni3)	P48787	0,5	6,3	9,5	NA	NA	NA	NA	NA	7	14
Tumor necrosis factor (Tnf)	P06804	-0,2	0	0,2	15	31	31250	125000	3	5	13
Tumor necrosis factor ligand superfamily member 12 (Tnfsf12)	O54907	1,4	2	2,4	61	122	62500	250000	2.7	7	12
Tumor necrosis factor receptor superfamily member 11B (Tnfrsf11b)	O08712	3,3	3,9	5,2	15	30	15625	15625	2.7	5	10
Tumor necrosis factor receptor superfamily member 12A (Tnfrsf12a)	Q9CR75	3,3	3,7	4,3	1953	1953	1000000	1000000	2.7	6	11
Tumor necrosis factor receptor superfamily member 27 (Eda2r)	Q8BX35	4,7	5,6	6,1	3.8	3.8	7812	7812	3.3	5	12
Tumor necrosis factor receptor superfamily member 6 (Fas)	P25446	2,6	2,9	3,3	15	15	31250	62500	3.3	5	9
Tyrosine-protein kinase Yes (Yes1)	Q04736	0,2	0,6	1	NA	NA	NA	NA	NA	5	12
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2 (Wfikkn2)	Q7TQN3	3,1	3,4	3,6	NA	NA	NA	NA	NA	6	11
Vascular endothelial growth factor D (Vegfd)	P97946	2,2	2,5	2,8	488	488	31250	250000	1.8	5	15
WNT1-inducible-signaling pathway protein 1 (Wisp1)	O54775	2,4	2,8	3,2	31	61	7812	15625	2.1	5	14
V-set and immunoglobulin domain-containing protein 2 (Vsig2)	Q9Z109	2	2,6	3,2	NA	NA	NA	NA	NA	7	14

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

To test that the antibodies selected for use in our Olink Mouse Exploratory assays are specific for their desired targets, we measured each assay response to 50 mouse proteins. 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, a reporter sequence will be created and serve as a template for subsequent real-time qPCR. None of the Olink Mouse Exploratory showed significant signal from the proteins tested.

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 (data not shown). Previously, we have shown that a step-wise increase of multiplex grade (8, 24, 48, 72 and 96) does not compromise assay performance. 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, see Figure 5. 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.

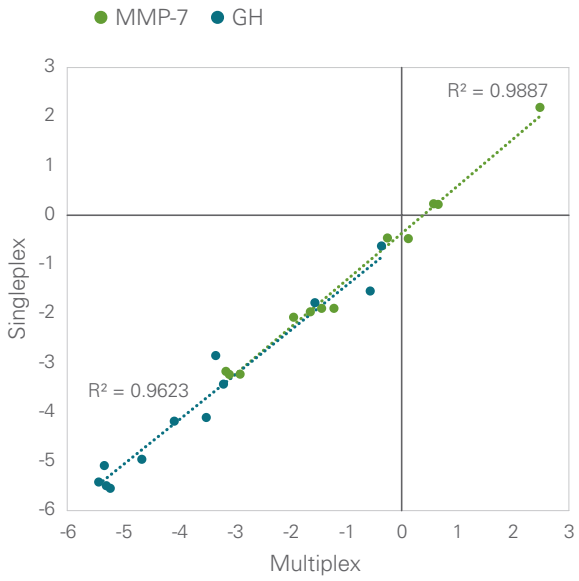

Fig 5. Scalability of the Olink technology platform. This experiment was performed using the Olink CVD II panel. Human plasma samples were analyzed for two representative assays in singleplex and with the complete Olink CVD II panel. The observed dCq (log2) values were plotted, and the correlation coefficient R^2 value was generated by linear regression.

3. References

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TECHNICAL SUPPORT

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