



# 1. Introduction

Olink® NEUROLOGY is a reagent kit measuring 92 neurology-related human protein biomarkers simultaneously, using just 1uL of serum, plasma or other human sample type. The analytical performance of the product has been carefully validated and the results are presented in this document. Please note that when a new panel is developed, both the individual assays and 92-plex panel as a whole are subject to our thorough validation procedure. If individual assays are subsequently improved or one or more assays are replaced in later versions of the panel, focus is placed on thoroughly validating the individual assays in question.

## 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 proteins, if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then 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.

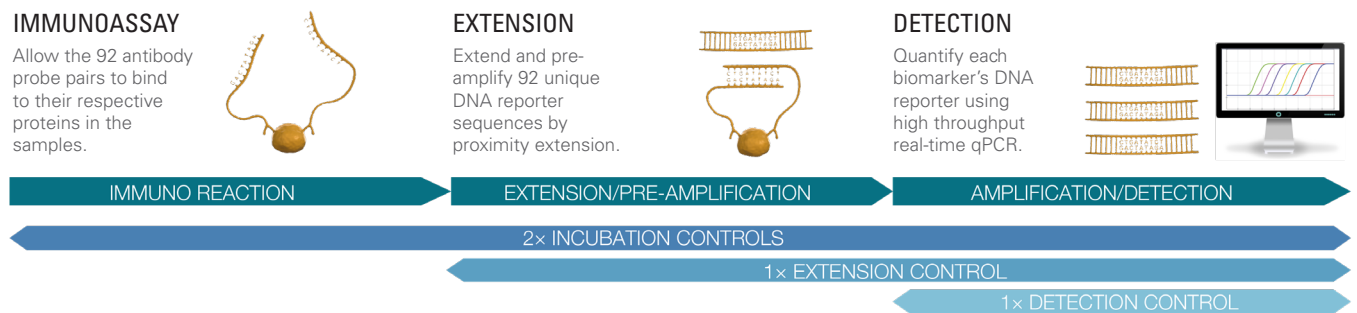
## 1.2 QUALITY CONTROLS

Internal and external controls have been developed by Olink for data normalization and quality control purposes. These controls are designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, and provide 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) monitors the extension and readout steps independent of antigen binding, 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 inter-plate control (IPC), is included on each plate and is 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. This 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 is performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control is subtracted, thereby 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 Normalized Protein eXpression (NPX) unit is generated 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 are performed on linearized values.



**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 NEUROLOGY by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Table 1 summarizes response values for 20 normal EDTA plasma samples expressed in NPX, as well as relative differences compared to EDTA plasma. Variations observed between responses in heparin, citrate plasma and serum, as compared to EDTA plasma, were generally small, and all assays will therefore function without limitation in these sample types.

### 2.2 ANALYTICAL MEASUREMENT

**NOTE:** The technical performance data based on *in vitro* assays using recombinant antigen must **NOT** be used to derive actual concentrations of native proteins in biological samples from the relative quantification NPX data that is obtained from an Olink assay.

#### DETECTION LIMIT

Calibrator curves were determined for all biomarkers simultaneously in a multiplex format. In cases where no suitable antigen was available, no calibrator data is presented. Limit of detection (LOD) was defined as 3 standard deviations above background, and reported in pg/mL, see Table 1.

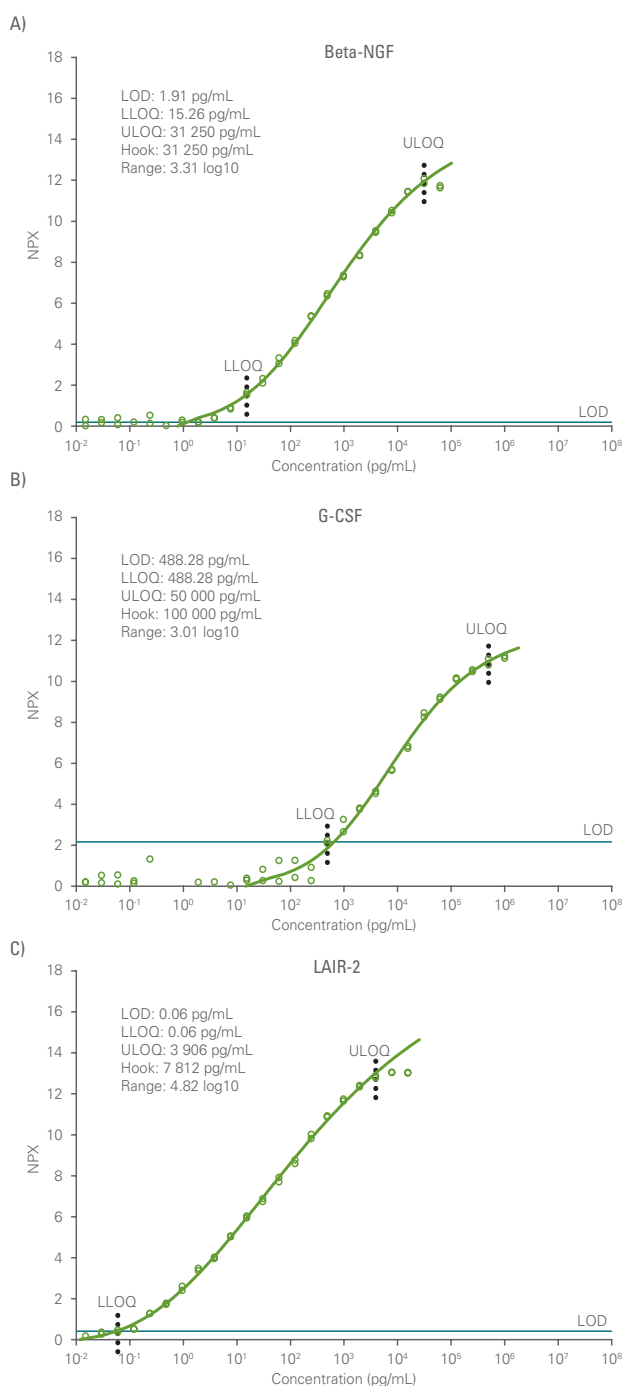
#### HIGH DOSE HOOK EFFECT

The high dose hook effect is seen when there is an antigen excess relative to the reagent antibodies, resulting in falsely lower values. In such cases, a significantly lower value can be reported that can lead to misinterpretation of results. Therefore, the hook threshold was determined for each analyte and reported in pg/mL, see Table 1.

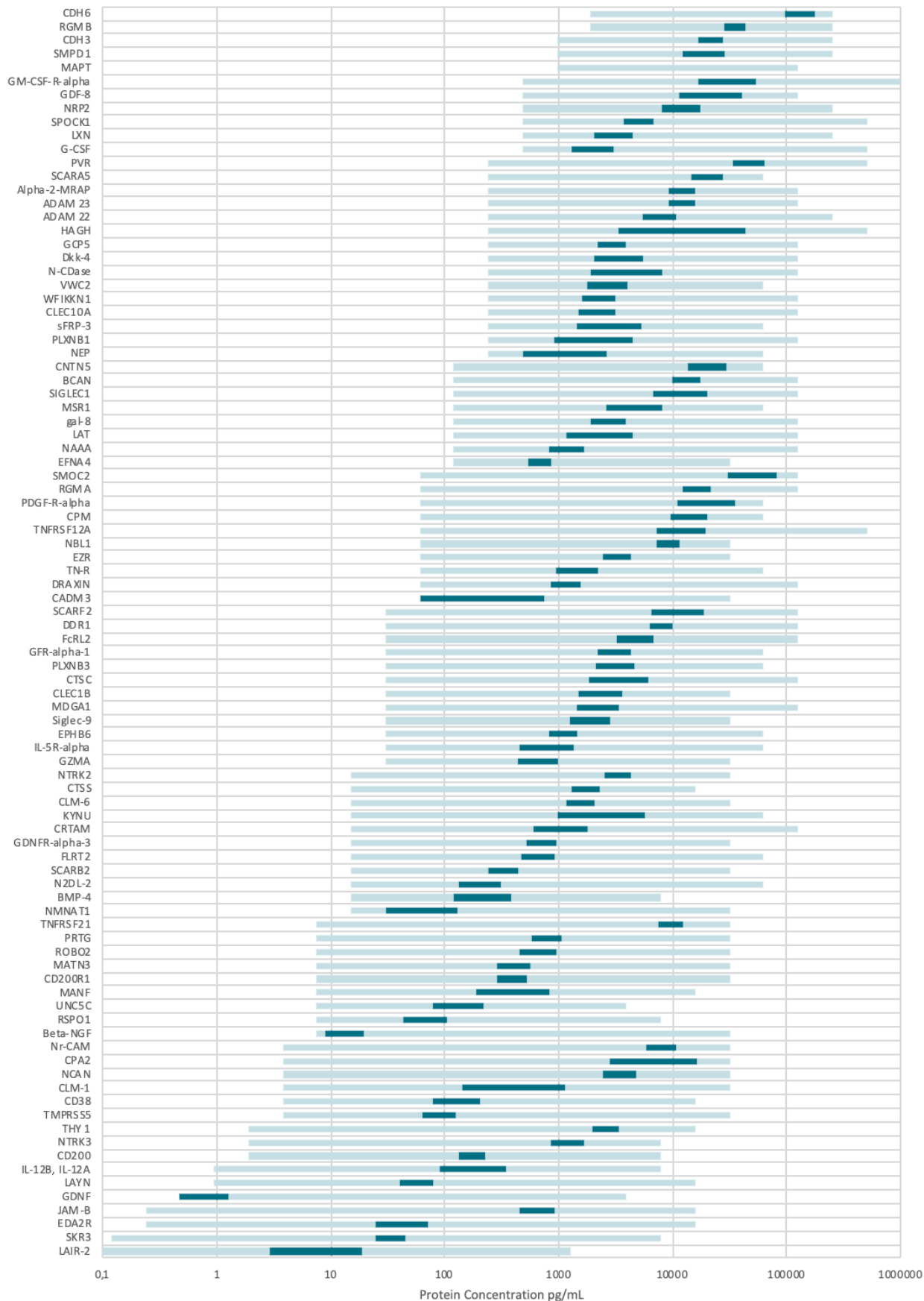
#### MEASURING RANGE

The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in pg/mL. Quantification limits of LLOQ and ULOQ were calculated with the following trueness and precision criteria; relative error  $\leq 30\%$  and CV  $\leq 30\%$ , of back-calculated values, respectively. Measuring ranges are presented in Table 1, ordered by LLOQ and displayed on a log<sub>10</sub> scale.

Example calibrator curves showing the measuring ranges for selected representative assays are shown in Figure 2. The overall distribution of measuring ranges for the assays with available recombinant antigens is 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 for representative assays using a 4-parameter curve fitting model.



**Fig 3.** Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ), and normal plasma levels where data is available (dark blue bars) for 92 analytes.

**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		
		10th %tile	Median	90th %tile	ACD	Heparin	Serum	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1 (CD38)	P28907	3.8	4.5	5.3	84	77	54	15	3.81	3.81	15 625	31 250	3.6	7	8
Alpha-2-macroglobulin receptor-associated protein (Alpha-2-MRAP)	P30533	7	7.6	8.2	75	62	105	15	244.14	244.14	125 000	125 000	2.7	9	9
BDNF/NT-3 growth factors receptor (NTRK2)	Q16620	5.4	5.8	6	86	101	102	15	15.26	15.26	31 250	62 500	3.3	6	9
Beta-nerve growth factor (Beta-NGF)	P01138	1.1	1.4	1.7	96	89	112	15	1.91	7.63	31 250	31 250	3.6	8	7
Bone morphogenetic protein 4 (BMP-4)	P12644	4	5	5.8	80	23	59	15	15.26	15.26	7 812	31 250	2.7	6	6
Brevican core protein (BCAN)	Q96GW7	5.1	5.7	6.2	80	98	106	15	122.07	122.07	125 000	500 000	3	8	8
Brorin (VWC2)	Q2TAL6	4.1	4.9	6	79	98	79	15	244.14	244.14	62 500	125 000	2.4	7	7
Cadherin-3 (CDH3)	P22223	6.2	6.7	7.1	92	114	110	15	976.56	976.56	250 000	500 000	2.4	7	5
Cadherin-6 (CDH6)	P55285	4.9	5.5	5.7	89	107	118	15	1953.12	1953.12	250 000	500 000	2.1	5	5
Carboxypeptidase A2 (CPA2)	P48052	8.2	9.7	10.7	80	99	97	15	3.81	3.81	31 250	125 000	3.9	7	9
Carboxypeptidase M (CPM)	P14384	6.3	6.9	7.3	97	110	110	4	30.52	61.04	62 500	125 000	3	6	8
Cathepsin S (CTSS)	P25774	5.3	5.6	5.9	101	125	117	15	15.26	15.26	15 625	31 250	3	5	5
Cell adhesion molecule 3 (CADM3)	Q8N126	1.7	2.4	2.7	72	90	87	15	61.04	61.04	31 250	31 250	2.7	9	7
Cell surface glycoprotein CD200 receptor 1 (CD200R1)	Q8TD46	4.9	5.5	5.9	89	101	96	15	3.81	7.63	31 250	62 500	3.6	6	9
CMRF35-like molecule 1 (CLM-1)	Q8TDQ1	2.9	3.5	5.7	78	93	101	15	3.81	3.81	31 250	125 000	3.9	8	9
CMRF35-like molecule 6 (CLM-6)	Q08708	5	5.5	6	89	104	101	15	15.26	15.26	31 250	31 250	3.3	7	7
Contactin-5 (CNTN5)	Q94779	5.9	6.4	7	85	104	101	15	61.04	122.07	62 500	125 000	2.7	7	9
C-type lectin domain family 1 member B (CLEC1B)	Q9P126	7.5	8.4	9.3	69	198	323	15	15.26	30.52	31 250	125 000	3	9	11
C-type lectin domain family 10 member A (CLEC10A)	Q8IUN9	4.3	5.1	5.8	78	78	82	15	244.14	244.14	125 000	125 000	2.7	7	9
Cytotoxic and regulatory T-cell molecule (CRTAM)	Q95727	4.4	4.9	5.9	80	104	105	15	15.26	15.26	125 000	250 000	3.9	6	8
Dickkopf-related protein 4 (Dkk-4)	Q9UBT3	1.9	2.5	3.5	80	69	96	15	244.14	244.14	125 000	500 000	2.7	9	9
Dipeptidyl peptidase 1 (CTSC)	P53634	3	3.8	4.8	45	105	136	15	30.52	30.52	125 000	125 000	3.6	5	10
Disintegrin and metalloproteinase domain-containing protein 22 (ADAM 22)	Q9P0K1	3.9	4.7	5.1	80	99	103	15	122.07	244.14	250 000	500 000	3	7	7
Disintegrin and metalloproteinase domain-containing protein 23 (ADAM 23)	Q75077	4.3	5	5.5	81	96	61	15	244.14	244.14	125 000	500 000	2.7	7	12
Draxin (DRAXIN)	Q8NB13	2.9	3.6	4.1	82	40	69	15	61.04	61.04	125 000	125 000	3.3	9	10
Ephrin type-B receptor 6 (EPHB6)	Q15197	3.9	4.4	4.9	83	98	96	15	30.52	30.52	62 500	125 000	3.3	8	7
Ephrin-A4 (EFNA4)	P52798	3.5	3.9	4.3	85	98	105	15	61.04	122.07	31 250	31 250	2.4	9	10
Epithelial discoidin domain-containing receptor 1 (DDR1)	Q08345	6.4	6.8	7	86	97	102	15	30.52	30.52	125 000	500 000	3.6	6	7
Ezrin (EZR)	P15311	5.1	5.6	6.1	72	100	94	1	30.52	61.04	31 250	250 000	2.7	7	10
Fc receptor-like protein 2 (FcRL2)	Q96LA5	4.5	5.5	6	84	100	93	15	15.26	30.52	125 000	500 000	3.6	7	9
Galectin-8 (gal-8)	Q00214	5.6	6.2	6.9	88	88	84	4	30.52	122.07	125 000	125 000	3	7	7
GDNF family receptor alpha-1 (GFR-alpha-1)	P56159	6.3	6.6	7.3	78	88	99	15	15.26	30.52	62 500	125 000	3.3	7	12
GDNF family receptor alpha-3 (GDNFR-alpha-3)	Q60609	4	4.5	4.9	88	73	105	15	15.26	15.26	31 250	31 250	3.3	8	7
Glial cell line-derived neurotrophic factor (GDNF)	P39905	1.5	2.1	2.6	92	54	79	15	0.24	0.48	3 906	7 812	3.9	9	9
Glypican-5 (GCP5)	P78333	3.5	3.8	4.5	69	107	138	15	244.14	244.14	125 000	250 000	2.7	8	10
Granulocyte Colony-Stimulating Factor (G-CSF)	P09919	3	3.5	4.3	91	97	106	15	488.28	488.28	500 000	1 000 000	3	6	10
Granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GM-CSF-R-alpha)	P15509	5.2	6	6.9	88	101	107	15	488.28	488.28	1 000 000	1 000 000	3.3	6	5
Granzyme A (GZMA)	P12544	4	4.5	5.2	84	92	97	15	7.63	30.52	31 250	31 250	3	8	7
Growth/differentiation factor 8 (GDF-8)	Q14793	3.6	5.2	6.1	91	100	114	15	488.28	488.28	125 000	500 000	2.4	6	7
Hydroxyacylglutathione hydrolase, mitochondrial (HAGH)	Q16775	2.3	3.1	6.3	3	3	7	0	122.07	244.14	500 000	500 000	3.3	8	12
Interleukin-12 subunit beta, Interleukin-12 subunit alpha (IL12)	P29460, P29459	6.9	8	8.9	80	88	95	15	0.48	0.95	7 812	15 625	3.9	7	5
Interleukin-5 receptor subunit alpha (IL-5R-alpha)	Q01344	3.6	4.6	5.5	75	96	98	15	30.52	30.52	62 500	125 000	3.3	8	7
Junctional adhesion molecule B (JAM-B)	P57087	7.6	8	8.4	86	94	90	15	0.24	0.24	15 625	31 250	4.8	8	8
Kynureninase (KYNU)	Q16719	4.5	5.9	7.3	77	93	95	15	15.26	15.26	62 500	500 000	3.6	7	8
Latexin (LXN)	Q9BS40	2.2	2.5	3.2	69	28	73	0	244.14	488.28	250 000	1 000 000	2.7	9	7
Layilin (LAYN)	Q6UX15	4.7	5.1	5.7	87	106	100	15	0.95	0.95	15 625	125 000	4.2	9	6
Leucine-rich repeat transmembrane protein FLRT2 (FLRT2)	Q43155	2.4	2.8	3.3	82	88	103	15	15.26	15.26	62 500	250 000	3.6	6	7
Leukocyte-associated immunoglobulin-like receptor 2 (LAIR-2)	Q6ISS4	3.6	4.7	6.2	73	95	91	15	0.04	0.04	1 250	1 250	4.5	7	7

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	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Linker for activation of T-cells family member 1 (LAT)	O43561	2.9	3.6	4.7	30	90	41	15	122.07	122.07	125 000	500 000	3	7	17
Lysosome membrane protein 2 (SCARB2)	Q14108	3.4	3.7	4.3	80	89	96	15	15.26	15.26	31 250	125 000	3.3	6	9
Macrophage scavenger receptor types I and II (MSR1)	P21757	4.1	5.1	5.9	88	93	90	15	30.52	122.07	62 500	250 000	2.7	6	5
MAM domain-containing glycosylphosphatidylinositol anchor protein 1 (MDGA1)	Q8NFP4	4.3	4.9	6	84	103	102	15	30.52	30.52	125 000	250 000	3.6	9	14
Matrilin-3 (MATN3)	O15232	7.4	8.6	9.1	86	89	94	15	3.81	7.63	31 250	250 000	3.9	8	5
Mesencephalic astrocyte-derived neurotrophic factor (MANF)	P55145	4.1	4.9	6.8	54	66	89	2	7.63	7.63	15 625	31 250	3.3	9	12
Microtubule-associated protein tau (MAPT)	P10636	NA	NA	NA	NA	NA	NA	15	976.56	976.56	125 000	125 000	2.1	9	13
N-acyl ethanolamine-hydrolyzing acid amidase (NAAA)	Q02083	3	3.7	4.3	80	105	105	15	61.04	122.07	125 000	250 000	3	8	7
Neprilysin (NEP)	P08473	2	2.9	3.8	83	95	94	15	61.04	244.14	62 500	500 000	2.4	6	5
Netrin receptor UNC5C (UNC5C)	Q95185	3	3.6	4.3	72	93	95	15	3.81	7.63	3 906	15 625	2.7	6	9
Neuroblastoma suppressor of tumorigenicity 1 (NBL1)	P41271	5.3	5.5	5.7	101	113	110	15	61.04	61.04	31 250	125 000	2.7	4	4
Neurocan core protein (NCAN)	O14594	7.3	7.9	8.4	90	96	104	15	3.81	3.81	31 250	62 500	3.9	6	7
Neuronal cell adhesion molecule (Nr-CAM)	Q92823	8.8	9.2	9.4	95	104	103	15	3.81	3.81	31 250	62 500	3.9	5	8
Neuropilin-2 (NRP2)	O60462	3.9	4.3	4.9	82	159	171	15	488.28	488.28	250 000	500 000	2.7	6	5
Neutral ceramidase (N-CDase)	Q9NR71	2.5	3.7	4.5	87	95	100	15	244.14	244.14	125 000	500 000	2.7	5	12
Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 (NMNAT1)	Q9HAN9	2.1	2.9	4.1	90	248	371	1	7.63	15.26	31 250	31 250	3.3	9	5
NKG2D ligand 2 (N2DL-2)	Q9BZM5	2.5	3.1	3.6	86	101	98	15	7.63	15.26	62 500	125 000	3.6	8	9
NT-3 growth factor receptor (NTRK3)	Q16288	7.1	7.5	7.9	81	97	100	15	1.91	1.91	7 812	31 250	3.6	6	11
OX-2 membrane glycoprotein (CD200)	P41217	6	6.4	6.7	84	97	99	15	1.91	1.91	7 812	31 250	3.6	7	8
Platelet-derived growth factor receptor alpha (PDGF-R-alpha)	P16234	4.5	5.1	5.8	87	103	105	15	61.04	61.04	62 500	1 000 000	3	7	8
Plexin-B1 (PLXNB1)	O43157	1.4	1.9	3.3	96	171	112	15	244.14	244.14	125 000	500 000	2.7	7	8
Plexin-B3 (PLXNB3)	Q9ULL4	4.3	4.8	5.3	83	110	134	15	30.52	30.52	62 500	500 000	3.3	7	7
Poliovirus receptor (PVR)	P15151	7.4	7.8	8.1	87	104	100	15	61.04	244.14	500 000	1 000 000	3.3	6	8
Protogenin (PRTG)	Q2VWP7	5.6	6	6.7	86	87	99	15	7.63	7.63	31 250	125 000	3.6	7	8
Repulsive guidance molecule A (RGMA)	Q96B86	7.8	8.2	8.7	85	114	100	15	61.04	61.04	125 000	500 000	3.3	6	9
RGM domain family member B (RGM B)	Q6NWW40	5.2	5.6	6.1	81	96	83	15	30.52	1953.12	250 000	500 000	2.1	8	11
Roundabout homolog 2 (ROBO2)	Q9HCK4	4.8	5.3	5.9	71	86	97	15	7.63	7.63	31 250	31 250	3.6	8	11
R-spondin-1 (RSP01)	Q2MKA7	2.6	3.1	3.9	67	39	104	15	1.91	7.63	7 812	15 625	3	9	12
Scavenger receptor class A member 5 (SCARA5)	Q6ZMJ2	7.4	7.8	8.3	89	103	104	15	122.07	244.14	62 500	125 000	2.4	6	10
Scavenger receptor class F member 2 (SCARF2)	Q96GP6	5.3	6.1	6.7	88	104	104	15	30.52	30.52	125 000	250 000	3.6	7	5
Secreted frizzled-related protein 3 (sFRP-3)	Q92765	2.6	3.5	4.5	74	32	52	15	122.07	244.14	62 500	62 500	2.4	9	8
Serine/threonine-protein kinase receptor R3 (SKR3)	P37023	6.9	7.4	7.7	88	108	102	15	0.06	0.12	7 812	15 625	4.8	8	6
Sialic acid-binding Ig-like lectin 9 (Siglec-9)	Q9Y336	5.2	5.8	6.4	99	113	108	15	30.52	30.52	31 250	31 250	3	5	7
Sialoadhesin (SIGLEC1)	Q9BZZ2	4.9	5.6	6.5	91	95	103	15	61.04	122.07	125 000	250 000	3	6	9
SPARC-related modular calcium-binding protein 2 (SMOC2)	Q9H3U7	7.6	8.6	9.3	78	94	92	15	61.04	61.04	125 000	500 000	3.3	9	11
Sphingomyelin phosphodiesterase (SMPD1)	P17405	4.1	5	5.5	90	88	87	15	488.28	976.56	250 000	1 000 000	2.4	6	6
Tenascin-R (TN-R)	Q92752	3.8	4.4	5.1	86	104	101	15	61.04	61.04	62 500	125 000	3	7	8
Testican-1 (SPOCK1)	Q08629	3.2	3.7	4.1	80	91	81	15	30.52	488.28	500 000	500 000	3	9	10
Thy-1 membrane glycoprotein (THY 1)	P04216	9.3	9.7	10	96	107	106	15	1.91	1.91	15 625	125 000	3.9	6	9
Transmembrane protease serine 5 (TMPRSS5)	Q9H3S3	3.7	4.2	4.6	84	107	100	15	3.81	3.81	31 250	31 250	3.9	6	9
Tumor necrosis factor receptor superfamily member 12A (TNFRSF12A)	Q9NP84	5.4	6.1	6.8	88	107	99	15	61.04	61.04	500 000	1 000 000	3.9	6	10
Tumor necrosis factor receptor superfamily member 21 (TNFRSF21)	O75509	9.1	9.5	9.8	86	92	105	15	7.63	7.63	31 250	125 000	3.6	7	10
Tumor necrosis factor receptor superfamily member 27 (EDA2R)	Q9HAV5	3.3	3.9	4.8	82	98	98	15	0.24	0.24	15 625	15 625	4.8	8	6
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 1 (WFIKKN1)	Q96NZ8	3.4	3.9	4.4	60	55	99	15	61.04	244.14	125 000	250 000	2.7	6	9

## 2.3 PRECISION

### REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 individual samples run, within each of 8 separate runs during the validation studies. Inter-assay variation (between-run) was calculated between experiments with the same operator. The reported inter-assay mean %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 observed were 7.1% and 8.4%, respectively. The distributions of intra-assay and inter-assay variations are shown in Figure 4.

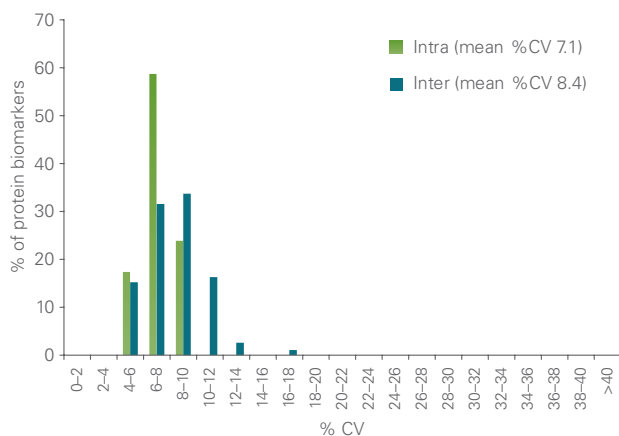


Fig 4. Distribution of intra-assay and inter-assay variations of Olink NEUROLOGY

### 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 shown reproducibility and repeatability in line with Olink results. For more information, download our Data Validation documents at [www.olink.com/data-validation](http://www.olink.com/data-validation)

Olink has Analysis Service labs in Sweden and the USA, and in addition there are many Olink-certified core laboratories around the world running the Olink platform (see [www.olink.com/service](http://www.olink.com/service) for details). Our experience over several years is that inter-site reproducibility is very good providing that operators are properly trained. For more information please contact [support@olink.com](mailto:support@olink.com).

## 2.4 ANALYTICAL SPECIFICITY

### ASSAY SPECIFICITY

To test the target-protein specificity of the PEA probes used in the panel, all of the antibodies used were tested for cross-reactivity against all of the recombinant proteins used during assay validation. The probes were also checked for cross-reactivity to more than 100 additional proteins (data not shown). This was carried out by creating a test sample consisting of a pool of antigens, which was then incubated with all 92 antibody probe pairs from the panel. To optimize this analysis, 10 sub-pools of antigen were evaluated to cover the 92 assays (see Figure 5).

The lack of significant signal from these tests indicates that each probe pair is specific for its target antigen, demonstrating the readout specificity of the PEA method.

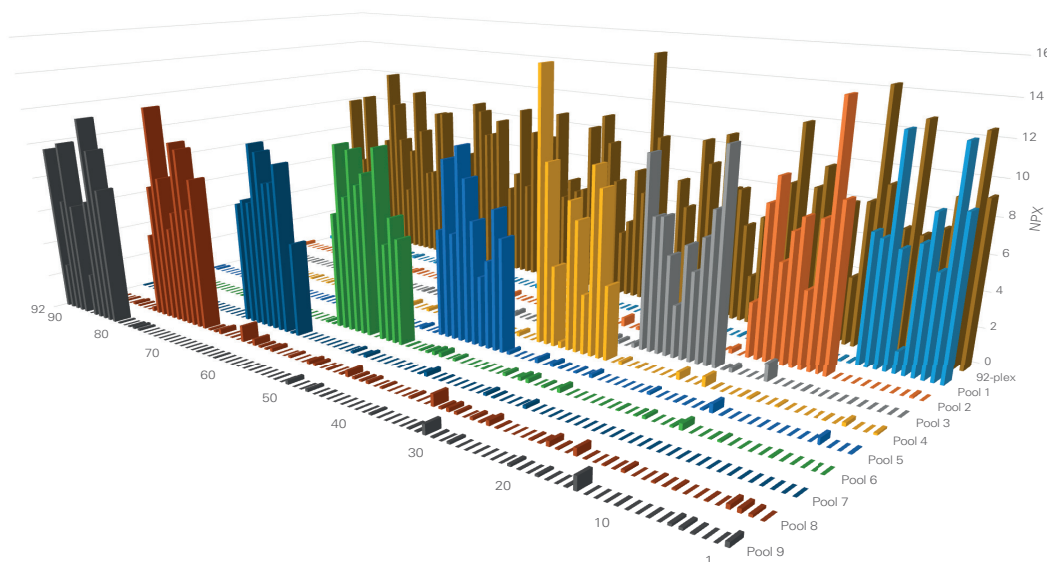


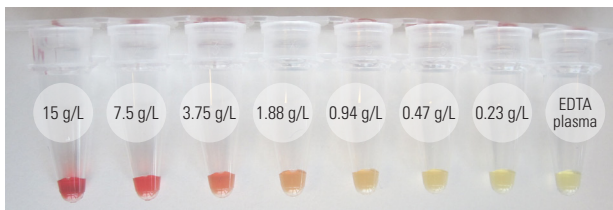
Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.



## ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are known to cause problems in immunoassays.

To evaluate the potential impact of this specific interference, a special “mismatch” system was designed. The only way to generate a signal in this system is to bring antibody probe pairs into proximity, by cross-binding substances other than antigens, e.g. heterophilic antibodies or rheumatoid factor. No interference due to HAMA or RF could be detected for any of the samples in any of the previously tested panels, indicating sufficient blocking of these agents (data not shown).



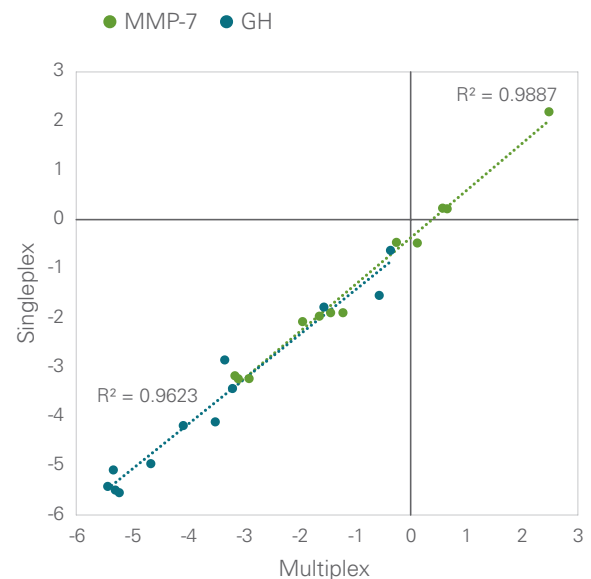
**Fig 6.** Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of some known interfering serum and plasma components was evaluated using serial dilutions of hemolysate, lipids and bilirubin, respectively in EDTA plasma and serum

An example of hemolysate levels tested is shown in Figure 6. These additions simulate different patient health conditions and/or sample collection irregularities. Interference 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 NEUROLOGY. In 7 out of 92 assays, altered values were recorded after the addition of hemolysate. The reason is most likely due to more of the measured 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 grade. 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 the full Olink CVD II panel. 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. This experiment was performed using the Olink CVD II 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.

## 3. References

---

1. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Bucht Thorsen S, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson AC, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. *PLoS One* April (2014). doi: 10.1371/journal.pone.0095192.
2. Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low abundant proteins in human blood. *Nucleic Acid Res* June (2011). doi: 10.1093/nar/gkr424.
3. <http://emedicine.medscape.com/article/2074115-overview>
4. <http://www.nlm.nih.gov/medlineplus/ency/article/003479.htm>

### TECHNICAL SUPPORT

For technical support, please contact us at [support@olink.com](mailto:support@olink.com)

For Research Use Only. Not for Use in Diagnostic Procedures.

This product includes a license for non-commercial use of Olink products. Commercial users may require additional licenses. Please contact Olink Proteomics AB for details.

There are no warranties, expressed or implied, which extend beyond this description. Olink Proteomics AB is not liable for property damage, personal injury, or economic loss caused by this product.

The following trademarks are owned by Olink AB: Olink® and Olink Bioscience™.

This product is covered by several patents and patent applications including US 6,511,809, US 7,306,904 and related US and foreign patents.

This product is sold under license from PHRI Properties, Inc. and may be used under PHRI Properties patent rights outside the field of human in vitro diagnostics.

Components in the Olink Probe Kit utilise Lightning-Link™ technology and are provided under license from Innova Biosciences.

© Copyright 2019 Olink Proteomics AB. All third party trademarks are the property of their respective owners.

1037, v2.1, 2019-11-25