1. Introduction

Olink® NEURO EXPLORATORY s a reagent kit that measures 92 human proteins simultaneously using just 1 µL 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[1-2], where 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their designated target protein, if it is present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA hybridization and polymerization event. This is then measured, 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. They have been designed to monitor the technical performance of the assay, as well as the quality of individual samples. This provides information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include two Incubation controls, one Extension control and one Detection control. The Incubation controls (two non-human proteins) monitor all three steps starting with the immuno reaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity-dependent hybridization and extension that does not require antibody binding to the target protein) monitors the extension and detection steps and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) specifically monitors the detection step. If one or more of the internal control values deviate from a pre-determined range, the sample will be flagged and may be removed before statistical analysis. An external control called the inter-plate control (IPC), is included on each plate and used in a second normalization step. The IPC is made up of a pool of probes similar to the Extension control (Ext Ctrl), except that it is 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 data that has been normalized as described above.

1.3 DATA ANALYSIS

The data analysis described in this document was performed using a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control was substracted, normalizing for technical variation within one run. Normalization between runs was then performed for each assay by substracting 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 were set relative to a correction factor that is determined by Olink for each batch of conjugated PEA probes. The Normalized Protein eXpression (NPX) unit generated by these procedures is on a log2 scale where a higher number represents a higher level of the target 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.

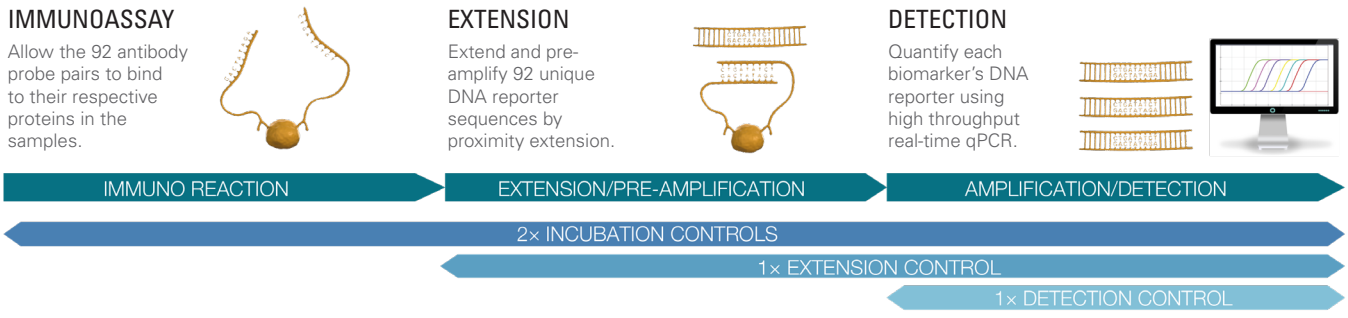

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Detection is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types with Olink NEURO EXPLORATORY was evaluated by collecting matched EDTA, acid citrate dextrose (ACD), and sodium heparin plasma and serum samples from 4 healthy individuals. Table 1 summarizes response values for 20 normal EDTA plasma samples expressed in NPX, as well as the relative differences for the other sample types compared to EDTA plasma. Variations observed between responses in the different sample types tested were generally small, and all assays will therefore function without limitation in these sample types. Table 2 shows detectability in CSF from healthy individuals tested in-house on 113 samples.

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, for which recombinant antigen was available, simultaneously in a multiplex format. For these assays, the Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL (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 reported, which can lead to misinterpretation of results. Therefore, the hook effect was determined for each analyte where applicable, 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 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 selected assays with their analytical data are shown in Figure 2 and the distribution of measuring ranges of 90 assays compared to endogenous plasma levels are shown in Figure 3. Where applicable, individual calibrator curves are available on the specific biomarker page on the Olink website.

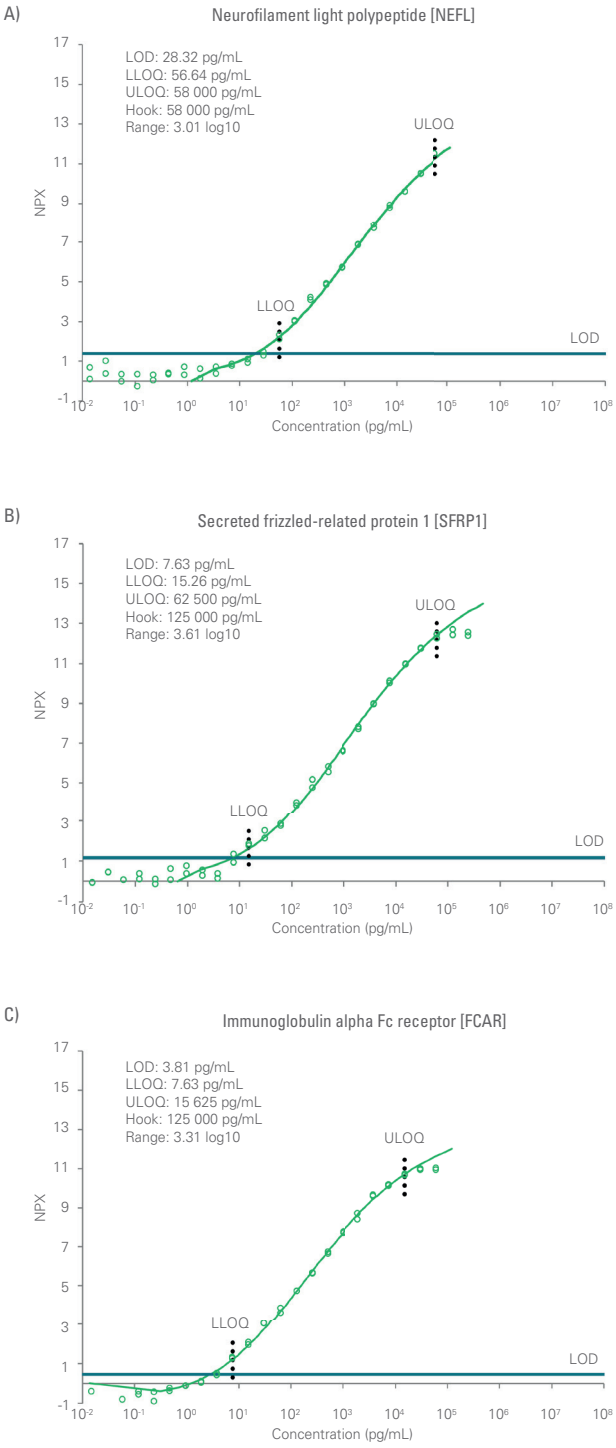

Fig 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

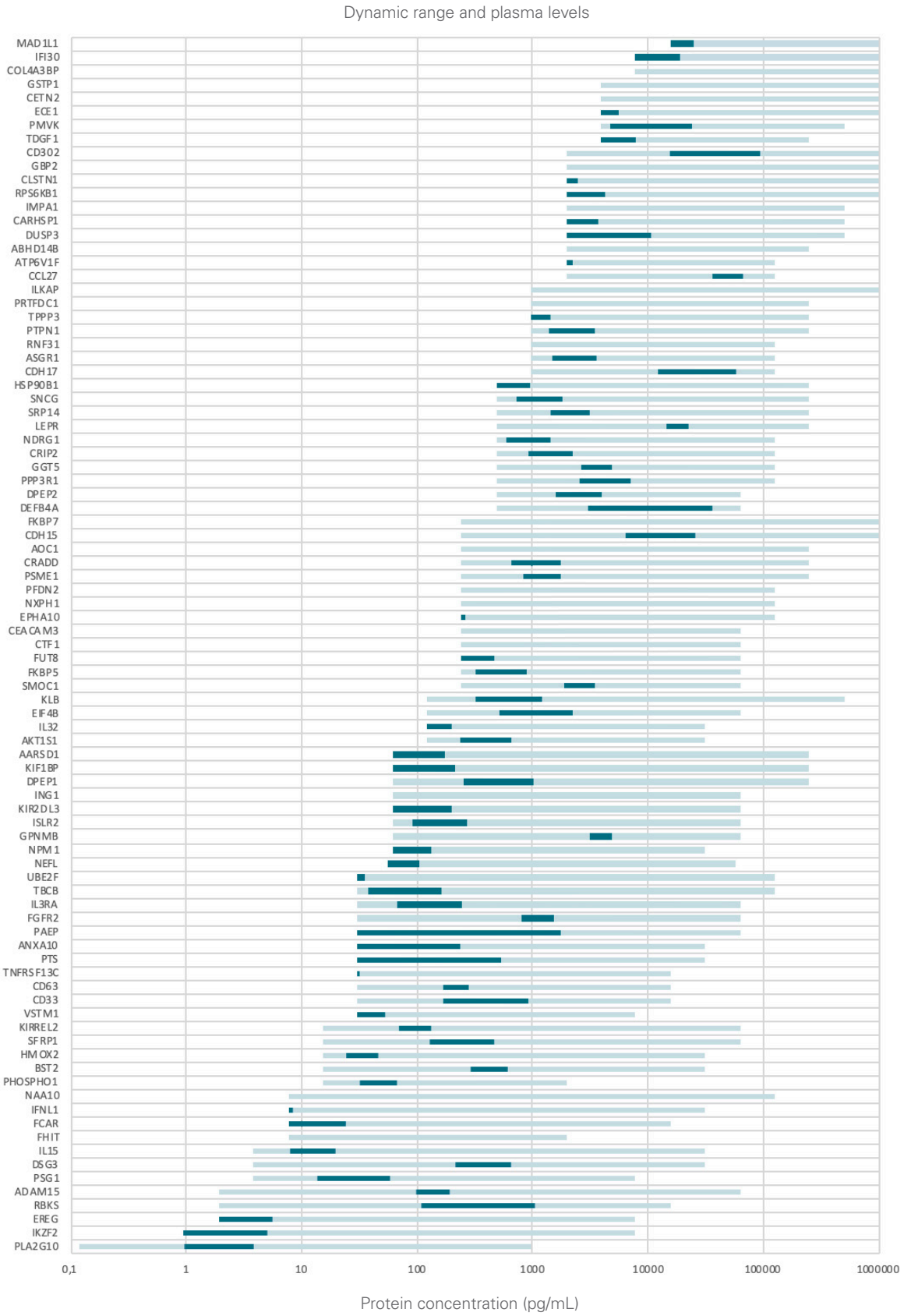

Fig 3. Distribution of analytical measuring range (light blue bars), defined by the lower and upper limits of quantification (LLOQ-ULOQ), and normal plasma levels (dark blue bars) for all assays with currently available data.

Table 1. Assay performance parameters. Sample types; Normalized Protein eXpression (NPX), Endogenous interference, Analytical range; 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 the analytes where applicable. Not available, NA

		Sample types						Endogenous interference	Analytical range					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
6-pyruvoyl tetrahydrobiopterin synthase (PTS)	Q03393	1.1	1.9	5.2	138	95	200	0	30.52	30.52	31250	62500	3	5	20
Adhesion G protein-coupled receptor B3 (ADGRB3)	O60242	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Alanyl-tRNA editing protein Aarsd1 (AARSD1)	Q9BTE6	0.8	1.3	2.1	54	NA	107	0	30.52	61.04	250000	250000	3.6	8	18
Alpha-(1,6)-fucosyltransferase (FUT8)	Q9BYC5	0.3	0.8	1.4	69	108	305	15	244.14	244.14	62500	125000	2.4	5	18
Amiloride-sensitive amine oxidase [copper-containing] (AOC1)	P19801	NA	0.5	1.4	79	NA	95	15	244.14	244.14	250000	500000	3	8	21
Annexin A10 (ANXA10)	Q9UJ72	0.4	1.3	2.9	103	NA	105	15	15.26	30.52	31250	125000	3	6	17
Asialoglycoprotein receptor 1 (ASGR1)	P07306	1.1	1.5	2	112	90	116	15	976.56	976.56	125000	250000	2.1	4	20
Beta-defensin 4A (DEFB4A)	O15263	2.4	4.7	5.5	105	100	116	15	488.28	488.28	62500	250000	2.1	6	24
Beta-klotho (KLB)	Q86Z14	1.3	1.6	2.8	63	47	72	15	122.07	122.07	500000	500000	3.6	4	30
Bis(5'-adenosyl)-triphosphatase (FHIT)	P49789	-0.1	0.1	0.3	89	NA	91	0.2	3.81	7.63	1953	3906	2.4	5	15
Bone marrow stromal antigen 2 (BST2)	Q10589	3.5	4.1	4.7	91	90	105	15	15.26	15.26	31250	62500	3.3	4	19
C-C motif chemokine 27 (CCL27)	Q9Y4X3	4.1	4.7	4.9	115	80	83	15	1953.12	1953.12	125000	250000	1.8	5	22
Cadherin-15 (CDH15)	P55291	4	5	5.9	89	108	116	7.5	244.14	244.14	1000000	2000000	3.6	6	20
Cadherin-17 (CDH17)	Q12864	4.2	5.5	6.5	97	111	127	15	488.28	976.56	125000	500000	2.1	5	17
Calcineurin subunit B type 1 (PPP3R1)	P63098	2.6	3.3	4.3	133	116	121	1.9	488.28	488.28	125000	250000	2.4	3	18
Calcium-regulated heat-stable protein 1 (CARHSP1)	Q9Y2V2	0.7	1.2	1.5	54	NA	95	0.2	1953.12	1953.12	500000	500000	2.4	6	24
Calsyntenin-1 (CLSTN1)	O94985	0.6	0.8	1.1	100	93	122	15	976.56	1953.12	1000000	1000000	2.7	5	19
Carcinoembryonic antigen-related cell adhesion molecule 3 (CEACAM3)	P40198	NA	NA	0.5	NA	NA	NA	15	244.14	244.14	62500	125000	2.4	5	20
Cardiotrophin-1 (CTF1)	Q16619	NA	NA	0.3	NA	NA	NA	15	122.07	244.14	62500	250000	2.4	5	21
CD302 antigen (CD302)	Q8IX05	2.4	2.8	3.6	106	92	122	15	976.56	1953.12	4000000	8000000	3.3	5	26
CD63 antigen (CD63)	P08962	3.2	3.8	4.2	78	120	303	15	15.26	30.52	15625	62500	2.7	5	19
Centrin-2 (CETN2)	P41208	NA	NA	0	NA	NA	NA	15	3906.25	3906.25	1000000	1000000	2.4	7	20
Collagen type IV alpha-3-binding protein (COL4A3BP)	Q9Y5P4	NA	0.4	0.8	88	NA	90	0.9	3906.25	7812.5	1000000	1000000	2.1	8	14
Cysteine-rich protein 2 (CRIP2)	P52943	1.7	2.5	3	180	105	114	7.5	488.28	488.28	125000	125000	2.4	8	13
Death domain-containing protein (CRADD)	P78560	1.7	2.3	3	68	51	98	0	244.14	244.14	250000	1000000	3	6	18
Desmoglein-3 (DSG3)	P32926	5.4	6.2	7.2	99	106	122	15	1.91	3.81	31250	62500	3.9	5	19
Dipeptidase 1 (DPEP1)	P16444	2.1	2.9	3.5	140	118	147	15	30.52	61.04	250000	1000000	3.6	5	21
Dipeptidase 2 (DPEP2)	Q9H4A9	2.4	3.4	3.9	28	23	33	15	244.14	488.28	62500	125000	2.1	6	18
Disintegrin and metalloproteinase domain-containing protein 15 (ADAM15)	Q13444	7.6	8.1	8.6	101	103	108	15	0.48	1.91	62500	125000	4.5	4	24
Dual specificity protein phosphatase 3 (DUSP3)	P51452	NA	NA	2.2	NA	NA	NA	15	1953.12	1953.12	500000	1000000	2.4	11	30
E3 ubiquitin-protein ligase RNF31 (RNF31)	Q96EP0	NA	0.1	0.7	107	NA	106	15	976.56	976.56	125000	500000	2.1	6	21
Endoplasmin (HSP90B1)	P14625	NA	NA	1.2	NA	NA	NA	15	488.28	488.28	250000	1000000	2.7	5	22
Endothelin-converting enzyme 1 (ECE1)	P42892	NA	NA	0.8	NA	NA	NA	15	3906.25	3906.25	1000000	4000000	2.4	7	22
Ephrin type-A receptor 10 (EPHA10)	Q5JZY3	NA	NA	1.2	NA	NA	NA	15	244.14	244.14	125000	1000000	2.7	9	19
Eukaryotic translation initiation factor 4B (EIF4B)	P23588	2.3	3.9	5.2	27	12	21	0.2	122.07	122.07	62500	125000	2.7	7	18
Fibroblast growth factor receptor 2 (FGFR2)	P21802	3.4	3.8	4.3	95	101	110	15	30.52	30.52	62500	125000	3.3	5	22
Gamma-interferon-inducible lysosomal thiol reductase (IFI30)	P13284	0.5	0.8	1.3	94	95	118	15	7812.5	7812.5	4000000	8000000	2.7	7	20
Gamma-synuclein (SNCG)	O76070	0.7	1.3	1.8	104	76	107	15	488.28	488.28	250000	1000000	2.7	5	20
Glutathione hydrolase 5 proenzyme (GGT5)	P36269	1.6	2.1	2.3	97	80	102	15	488.28	488.28	125000	1000000	2.4	7	21
Glutathione S-transferase P (GSTP1)	P09211	NA	NA	0.1	NA	NA	NA	15	1953.12	3906.25	8000000	8000000	3.3	7	20
Glycodelin (PAEP)	P09466	NA	0.5	5.5	79	67	53	15	30.52	30.52	62500	125000	3.3	4	25
Group 10 secretory phospholipase A2 (PLA2G10)	O15496	3.2	4	5	91	89	115	7.5	0.12	0.12	977	1953	3.9	4	17
Guanylate-binding protein 2 (GBP2)	P32456	NA	NA	NA	NA	NA	NA	15	1953.12	1953.12	1000000	2000000	3	8	21
Heme oxygenase 2 (HMOX2)	P30519	2.3	2.5	3.1	102	121	116	15	3.81	15.26	31250	125000	3.3	4	20
Immunoglobulin alpha Fc receptor (FCAR)	P24071	1.1	1.7	2.4	97	97	114	15	3.81	7.63	15625	125000	3.3	5	25
Immunoglobulin superfamily containing leucine-rich repeat protein 2 (ISLR2)	Q6UXK2	1.2	1.8	2.4	94	88	131	15	61.04	61.04	62500	250000	3	8	20
Inhibitor of growth protein 1 (ING1)	Q9UK53	NA	NA	NA	NA	NA	NA	15	61.04	61.04	62500	125000	3	7	17
Inositol monophosphatase 1 (IMPA1)	P29218	0	0.1	0.4	NA	NA	NA	1.9	1953.12	1953.12	500000	500000	2.4	4	21

		Sample types						Endogenous interference	Analytical range					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Integrin-linked kinase-associated serine/threonine phosphatase 2C (ILKAP)	Q9H0C8	NA	0.2	0.4	NA	NA	NA	15	976.56	976.56	1000000	1000000	3	8	19
Interferon lambda-1 (IFNL1)	Q8IU54	NA	0.6	1	104	NA	117	15	7.63	7.63	31250	31250	3.6	7	19
Interleukin-15 (IL15)	P40933	1.3	1.7	2.1	95	86	106	15	1.91	3.81	31250	125000	3.9	6	18
Interleukin-3 receptor subunit alpha (IL3RA)	P26951	0.9	1.5	2.1	102	83	104	15	30.52	30.52	62500	500000	3.3	4	20
Interleukin-32 (IL32)	P24001	0.5	0.9	1.4	103	85	108	15	122.07	122.07	31250	125000	2.4	5	17
KIF1-binding protein (KIF1BP)	Q96EK5	0.7	1.2	1.8	63	44	36	15	61.04	61.04	250000	1000000	3.6	6	21
Killer cell immunoglobulin-like receptor 2DL3 (KIR2DL3)	P43628	NA	1.5	2.2	98	NA	104	15	30.52	61.04	62500	250000	3	6	22
Kin of IRRE-like protein 2 (KIRREL2)	Q6UWL6	1.9	2.3	2.6	96	84	109	15	15.26	15.26	62500	125000	3.6	5	22
Latent-transforming growth factor beta-binding protein 3 (LTBP3)	Q9NS15	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Leptin receptor (LEPR)	P48357	4.9	5.2	5.4	96	95	117	15	488.28	488.28	250000	500000	2.7	4	25
Mitotic spindle assembly checkpoint protein MAD1 (MAD1L1)	Q9Y6D9	0.6	0.9	1.3	100	114	141	3.8	15625	15625	1000000	1000000	1.8	5	21
Myeloid cell surface antigen CD33 (CD33)	P20138	2.6	4	4.8	98	94	115	15	15.26	30.52	15625	15625	2.7	4	15
N-alpha-acetyltransferase 10 (NAA10)	P41227	NA	NA	0.7	80	NA	73	0.9	7.63	7.63	125000	1000000	4.2	6	22
NEDD4-like E3 ubiquitin-protein ligase (WWP2)	O00308	3.1	3.7	4.4	133	81	199	0.9	NA	NA	NA	NA	NA	6	15
NEDD8-conjugating enzyme UBE2F (UBE2F)	Q969M7	0	NA	0.7	95	NA	95	7.5	15.26	30.52	125000	500000	3.6	5	20
Neurexophilin-1 (NXPH1)	P58417	NA	NA	0.1	NA	NA	NA	15	244.14	244.14	125000	125000	2.7	5	25
Neurofilament light polypeptide (NEFL)	P07196	1.2	1.9	2.7	90	78	104	15	28.32	56.64	58000	58000	3	8	20
Nucleophosmin (NPM1)	P06748	NA	0.6	1.1	95	201	176	7.5	61.04	61.04	31250	250000	2.7	4	16
Peptidyl-prolyl cis-trans isomerase FKBP5 (FKBP5)	Q13451	1.4	1.9	2.9	85	118	167	0.9	122.07	244.14	62500	125000	2.4	4	21
Peptidyl-prolyl cis-trans isomerase FKBP7 (FKBP7)	Q9Y680	NA	0.5	0.9	101	NA	97	15	122.07	244.14	1000000	1000000	3.6	5	21
Phosphoethanolamine/phosphocholine phosphatase (PHOSPHO1)	Q8TCT1	2.5	2.8	3.3	109	92	116	15	7.63	15.26	1953	7812	2.1	6	18
Phosphomevalonate kinase (PMVK)	Q15126	1	1.7	2.8	111	50	58	3.8	1953.12	3906.25	500000	2000000	2.1	8	21
Phosphoribosyltransferase domain-containing protein 1 (PRTFDC1)	Q9NRG1	NA	NA	1	95	NA	NA	15	244.14	976.56	250000	500000	2.7	13	22
Prefoldin subunit 2 (PFDN2)	Q9UHV9	0.3	0.5	0.8	93	75	105	0.9	122.07	244.14	125000	1000000	2.7	7	21
Pregnancy-specific beta-1-glycoprotein 1 (PSG1)	P11464	2	2.7	3.8	104	77	119	15	1.91	3.81	7812	15625	3.3	5	17
Proepiregulin (EREG)	O14944	NA	0.4	1	113	207	2524	15	1.91	1.91	7812	31250	3.6	5	19
Proline-rich AKT1 substrate 1 (AKT1S1)	Q96B36	1.5	2.2	2.8	58	38	111	7.5	122.07	122.07	31250	125000	2.4	4	22
Proteasome activator complex subunit 1 (PSME1)	Q06323	2.3	2.7	3.3	65	61	98	0	122.07	244.14	250000	500000	3	4	18
Protein ABHD14B (ABHD14B)	Q96IU4	0.5	0.8	1.2	51	54	58	0	1953.12	1953.12	250000	500000	2.1	5	16
Protein NDRG1 (NDRG1)	Q92597	0.8	1.3	1.7	106	NA	84	15	488.28	488.28	125000	250000	2.4	6	19
Ribokinase (RBKS)	Q9H477	5.1	6.1	8.1	78	76	143	0	1.91	1.91	15625	31250	3.9	4	20
Ribosomal protein S6 kinase beta-1 (RPS6KB1)	P23443	NA	0.7	1	100	NA	76	0.9	1953.12	1953.12	1000000	2000000	2.7	7	24
Secreted frizzled-related protein 1 (SFRP1)	Q8N474	3.8	4.6	5.7	80	65	41	15	7.63	15.26	62500	125000	3.6	7	17
Signal recognition particle 14 kDa protein (SRP14)	P37108	1.5	2	2.4	87	45	302	15	488.28	488.28	250000	1000000	2.7	5	20
SPARC-related modular calcium-binding protein 1 (SMOC1)	Q9H4F8	2.9	3.8	4	130	71	59	7.5	244.14	244.14	62500	125000	2.4	6	18
Teratocarcinoma-derived growth factor 1 (TDGF1)	P13385	NA	NA	1.6	NA	NA	NA	15	3906.25	3906.25	250000	500000	1.8	8	22
Transmembrane glycoprotein NMB (GPNMB)	Q14956	5.6	5.9	6.1	98	88	116	15	30.52	61.04	62500	125000	3	4	20
Tubulin polymerization-promoting protein family member 3 (TPPP3)	Q9BW30	0.3	0.8	1.2	104	88	112	15	976.56	976.56	250000	500000	2.4	6	21
Tubulin-folding cofactor B (TBCB)	Q99426	1.5	2.5	3.2	59	42	40	0.2	15.26	30.52	125000	250000	3.6	7	23
Tumor necrosis factor receptor superfamily member 13C (TNFRSF13C)	Q96RJ3	NA	0.2	1	104	NA	106	15	15.26	30.52	15625	31250	2.7	5	15
Tyrosine-protein phosphatase non-receptor type 1 (PTPN1)	P18031	1	1.7	2	142	92	153	0.9	488.28	976.56	250000	500000	2.4	6	19
V-set and transmembrane domain-containing protein 1 (VSTM1)	Q6UX27	0.4	0.8	1.1	99	81	117	15	15.26	30.52	7812	31250	2.4	5	21
V-type proton ATPase subunit F (ATP6V1F)	Q16864	NA	0.3	0.8	88	NA	91	0.2	976.56	1953.12	125000	1000000	1.8	6	22
Zinc finger protein Helios (IKZF2)	Q9UKS7	NA	NA	1.7	NA	NA	NA	7.5	0.95	0.95	7812	31250	3.9	7	21

*These recently updated assays were subject to rigorous validation and QC during their development, but final validation data in full-panel context is not yet available. This will be updated as soon as possible.

Table 2. Detectability in CSF from healthy individuals tested in-house on 113 samples. Percentage of samples with values over LOD. Not available, NA.

		Detectability in CSF		
Target	UniProt No	No detectability	Detected in 1-50% of the samples	Detected in 51-100% of the samples
6-pyruvoyl tetrahydrobiopterin synthase (PTS)	Q03393			X
Adhesion G protein-coupled receptor B3 (ADGRB3)	O60242	NA	NA	NA
Alanyl-tRNA editing protein Aarsd1 (AARSD1)	Q9BTE6	X		
Alpha-(1,6)-fucosyltransferase (FUT8)	Q9BYC5		X	
Amiloride-sensitive amine oxidase [copper-containing] (AOC1)	P19801	X		
Annexin A10 (ANXA10)	Q9UJ72	X		
Asialoglycoprotein receptor 1 (ASGR1)	P07306		X	
Beta-defensin 4A (DEFB4A)	O15263		X	
Beta-klotho (KLB)	Q86Z14	X		
Bis(5'-adenosyl)-triphosphatase (FHIT)	P49789		X	
Bone marrow stromal antigen 2 (BST2)	Q10589			X
Cadherin-15 (CDH15)	P55291			X
Cadherin-17 (CDH17)	Q12864	X		
Calcineurin subunit B type 1 (PPP3R1)	P63098			X
Calcium-regulated heat-stable protein 1 (CARHSP1)	Q9Y2V2	X		
Calsyntenin-1 (CLSTN1)	O94985			X
Carcinoembryonic antigen-related cell adhesion molecule 3 (CEACAM3)	P40198		X	
Cardiotrophin-1 (CTF1)	Q16619	X		
C-C motif chemokine 27 (CCL27)	Q9Y4X3		X	
CD302 antigen (CD302)	Q8IX05			X
CD63 antigen (CD63)	P08962			X
Centrin-2 (CETN2)	P41208	X		
Collagen type IV alpha-3-binding protein (COL4A3BP)	Q9Y5P4	X		
Cysteine-rich protein 2 (CRIP2)	P52943		X	
Death domain-containing protein CRADD (CRADD)	P78560		X	
Desmoglein-3 (DSG3)	P32926			X
Dipeptidase 1 (DPEP1)	P16444	X		
Dipeptidase 2 (DPEP2)	Q9H4A9		X	
Disintegrin and metalloproteinase domain-containing protein 15 (ADAM15)	Q13444			X
Dual specificity protein phosphatase 3 (DUSP3)	P51452	X		
E3 ubiquitin-protein ligase RNF31 (RNF31)	Q96EP0		X	
Endoplasmin (HSP90B1)	P14625		X	
Endothelin-converting enzyme 1 (ECE1)	P42892	X		
Ephrin type-A receptor 10 (EPHA10)	Q5JZY3			X
Eukaryotic translation initiation factor 4B (EIF4B)	P23588		X	
Fibroblast growth factor receptor 2 (FGFR2)	P21802			X
Gamma-interferon-inducible lysosomal thiol reductase (IFI30)	P13284			X
Gamma-synuclein (SNCG)	O76070			X
Glutathione hydrolase 5 proenzyme (GGT5)	P36269		X	
Glutathione S-transferase P (GSTP1)	P09211		X	
Glycodelin (PAEP)	P09466		X	
Group 10 secretory phospholipase A2 (PLA2G10)	O15496		X	
Guanylate-binding protein 2 (GBP2)	P32456		X	
Heme oxygenase 2 (HMOX2)	P30519			X
Immunoglobulin alpha Fc receptor (FCAR)	P24071		X	
Immunoglobulin superfamily containing leucine-rich repeat protein 2 (ISLR2)	Q6UXK2			X
Inhibitor of growth protein 1 (ING1)	Q9UK53	X		
Inositol monophosphatase 1 (IMPA1)	P29218			X

		Detectability in CSF		
Target	UniProt No	No detectability	Detected in 1-50% of the samples	Detected in 51-100% of the samples
Integrin-linked kinase-associated serine/threonine phosphatase 2C (ILKAP)	Q9H0C8	X		
Interferon lambda-1 (IFNL1)	Q8IU54	X		
Interleukin-15 (IL15)	P40933			X
Interleukin-3 receptor subunit alpha (IL3RA)	P26951		X	
Interleukin-32 (IL32)	P24001	X		
KIF1-binding protein (KIF1BP)	Q96EK5	X		
Killer cell immunoglobulin-like receptor 2DL3 (KIR2DL3)	P43628	X		
Kin of IRRE-like protein 2 (KIRREL2)	Q6UWL6			X
Latent-transforming growth factor beta-binding protein 3 (LTBP3)	Q9NS15	NA	NA	NA
Leptin receptor (LEPR)	P48357		X	
Mitotic spindle assembly checkpoint protein MAD1 (MAD1L1)	Q9Y6D9		X	
Myeloid cell surface antigen CD33 (CD33)	P20138			X
N-alpha-acetyltransferase 10 (NAA10)	P41227	X		
NEDD4-like E3 ubiquitin-protein ligase WWP2 (WWP2)	O00308			X
NEDD8-conjugating enzyme UBE2F (UBE2F)	Q969M7	X		
Neurexophilin-1 (NXPH1)	P58417			X
Neurofilament light polypeptide (NEFL)	P07196			X
Nucleophosmin (NPM1)	P06748		X	
Peptidyl-prolyl cis-trans isomerase FKBP5 (FKBP5)	Q13451		X	
Peptidyl-prolyl cis-trans isomerase FKBP7 (FKBP7)	Q9Y680			X
Phosphoethanolamine/phosphocholine phosphatase (PHOSPHO1)	Q8TCT1			X
Phosphomevalonate kinase (PMVK)	Q15126		X	
Phosphoribosyltransferase domain-containing protein 1 (PRTFDC1)	Q9NRG1	X		
Prefoldin subunit 2 (PFDN2)	Q9UHV9		X	
Pregnancy-specific beta-1-glycoprotein 1 (PSG1)	P11464		X	
Proepiregulin (EREG)	O14944	X		
Proline-rich AKT1 substrate 1 (AKT1S1)	Q96B36		X	
Proteasome activator complex subunit 1 (PSME1)	Q06323			X
Protein ABHD14B (ABHD14B)	Q96IU4		X	
Protein NDRG1 (NDRG1)	Q92597			X
Ribokinase (RBKS)	Q9H477			X
Ribosomal protein S6 kinase beta-1 (RPS6KB1)	P23443		X	
Secreted frizzled-related protein 1 (SFRP1)	Q8N474			X
Signal recognition particle 14 kDa protein (SRP14)	P37108		X	
SPARC-related modular calcium-binding protein 1 (SMOC1)	Q9H4F8			X
Teratocarcinoma-derived growth factor 1 (TDGF1)	P13385			X
Transmembrane glycoprotein NMB (GPNMB)	Q14956			X
Tubulin polymerization-promoting protein family member 3 (TPPP3)	Q9BW30		X	
Tubulin-folding cofactor B (TBCB)	Q99426		X	
Tumor necrosis factor receptor superfamily member 13C (TNFRSF13C)	Q96RJ3			X
Tyrosine-protein phosphatase non-receptor type 1 (PTPN1)	P18031		X	
V-set and transmembrane domain-containing protein 1 (VSTM1)	Q6UX27		X	
V-type proton ATPase subunit F (ATP6V1F)	Q16864	X		
Zinc finger protein Helios (IKZF2)	Q9UKS7		X	

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 different samples, run in triplicate, in 8 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 %CV for three different operators. These calculations were performed on linearized values for all analytes where 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 5.9% and 20.2%, respectively. The reason for the inter-assay variation, was due to inter-operative variation of 10%, 18% and 32%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

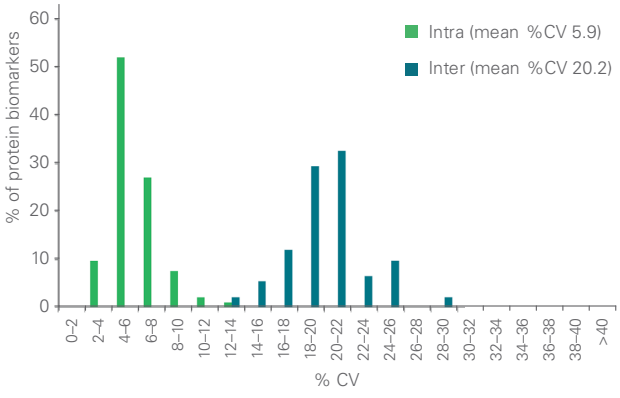

Fig 4. Distribution of intra-assay and inter-assay variations of Olink NEURO EXPLORATORY

REPRODUCIBILITY

Variations due to different operators in different laboratories using different equipment are another potential source of assay variation. 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 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.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

The antibodies selected for use in Olink NEURO EXPLORATORY have previously been evaluated against the 92 panel-specific proteins as well as against an additional 107 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 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, see Figure 5.

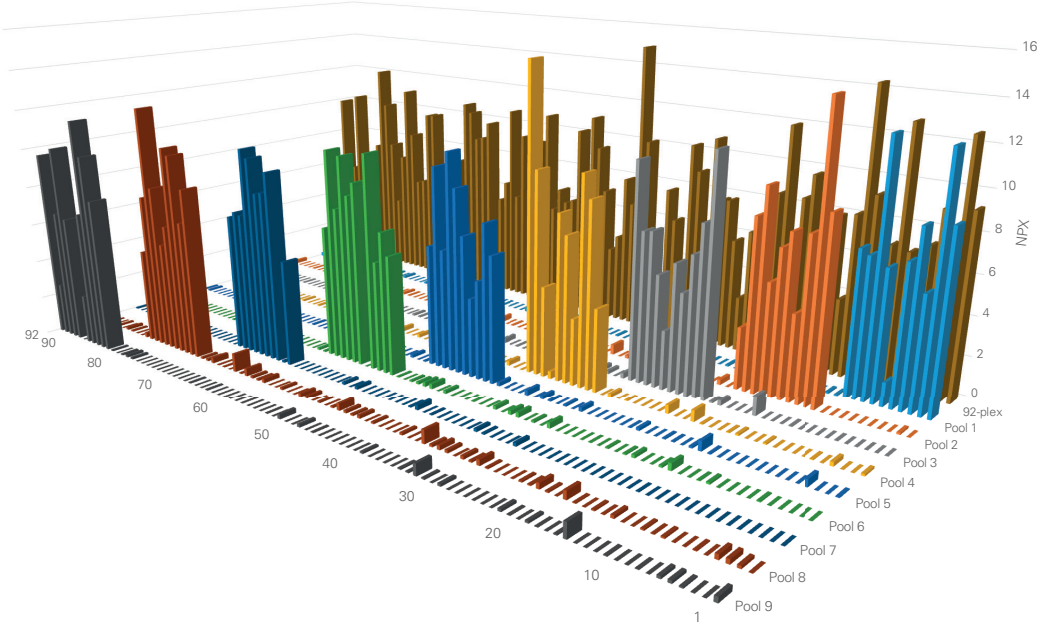

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool.

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).

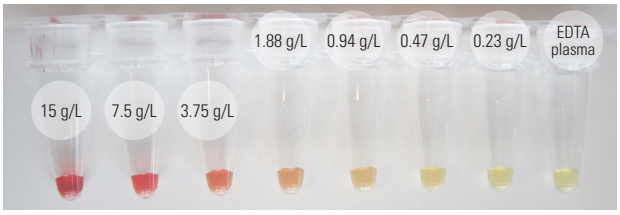

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

Bilirubin, lipids and hemolysate, are plasma and serum components that are known to interfere with some analytical assays. These were evaluted for potential impact on the Olink assays at different added concentrations. An example of the hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. In 7 out of 92 assays, altered signal was observed by the addition of hemolysate. The reason is most likely due to the specific analytes 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.

Interference by bilirubin and lipids has previously been evaluated, and disturbance was only observed at extreme levels corresponding to 8 or 10 times normal [3, 4] values. This test was not therefore repeated for Olink NEURO EXPLORATORY.

2.5 SCALABILITY

Assay performance has been previously 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-plex 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.

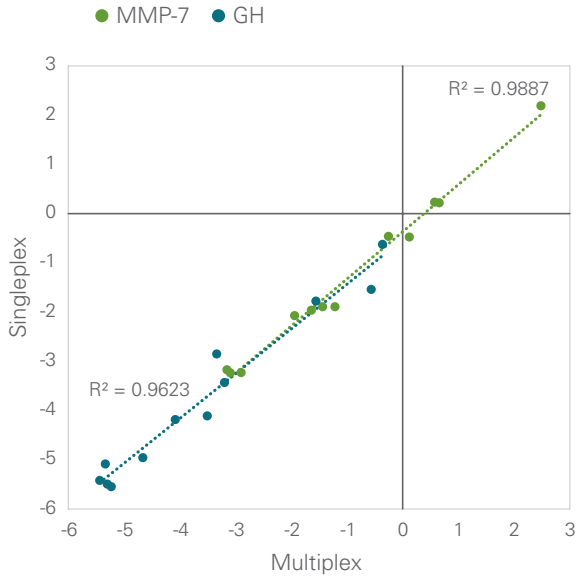

Fig 7. Scalability of the Olink technology platform. The experiment was performed using the Olink CARDIOVASCULAR II panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The 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.

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