

Validation data

Olink[®] Target 48 Neurodegeneration

Introduction

Olink[®] Target 48 Neurodegeneration is a reagent kit measuring 42 well-established protein biomarkers relevant in neurodegenerative diseases like Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis and traumatic brain injury. Olink Target 48 Neurodegeneration has been developed without any human plasma components. The analytical performance of the product has been carefully validated and the results are presented below.

Technology

The Olink reagents are based on the Proximity Extension Assay (PEA[™]) technology¹⁻², where oligonucleotide labeled antibody probe pairs are each allowed to bind to their respective target protein present in the sample. Following hybridization of the matched oligo sequences, a PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. These reporter sequences are then amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a 48-plex format without any need for washing or dilution steps (see Figure 1), and results are reported in both standard concentration units (pg/mL) and in relative concentration units (NPX).

Quality controls

Plasma-free internal and external controls have been developed by Olink to enable data normalization and quality control. These have been designed to enable monitoring of the technical performance of each run, as well as the individual performance of each sample, providing information about each step of the

Olink protocol (see Figure 1). The internal controls are added to each sample and include one Incubation Control, one Extension Control and one Detection Control. The Incubation Control (a non-human antigen) monitors all three steps starting with the immuno reaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity that is independent of antigen binding) monitors the extension and detection steps and is used for data normalization across samples. Finally, the Detection Control (a synthetic doublestranded reporter sequence template) monitors the detection step. Samples that deviate from a pre-determined range for one or more of the internal control values will result in a QC warning in the Olink[®] NPX Signature software.

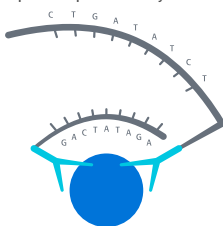
Eight external control samples are applied to each run. Triplicates of the Sample Control, duplicates of the Negative Control and triplicates of the Calibrator. The Calibrator is used in a second normalization step and is designed to improve inter-run precision, enabling optimal comparison of data derived from multiple runs and batches. The Sample Control is used to monitor and control the quality of reported output data by evaluating both accuracy and intra-run precision for all assays. Both the Sample Control and the Calibrator are composed of a pool of recombinant proteins, equivalent to the biomarkers targeted by the panel.

Data analysis and protein concentration calculation

Data analysis was performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Ct-value for the Extension control was subtracted, thus normalizing for technical variation within one run. Normalization between runs were then performed for each assay by subtracting the corresponding dCt-value for the median of the

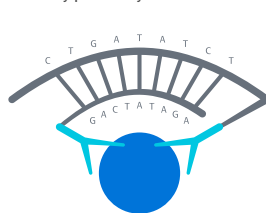
Immuno reaction

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



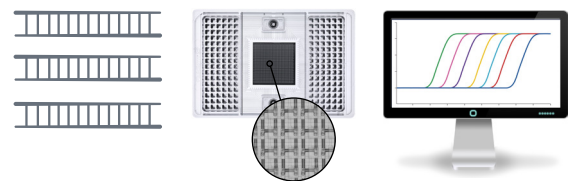
Extension and pre-amplification

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



Amplification and detection

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



Immuno/incubation control

Extension control

Detection control

Figure 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. Readout is performed by using Olink[®] Signature Q100.

three Calibrator replicates from the dCt-values generated. The next step in the pre-processing procedure was to set the values relative to a bridging factor that bridges the data between different kit batches. The Normalized Protein eXpression (NPX) unit generated is on a log₂ scale, where a larger number represents a higher protein level in the sample. The protein concentration in standard concentration units (pg/mL) is obtained by fitting the NPX-value to a standard curve, using four parameters in a non-linear logistic regression model. The standard curves are defined during the product verification and found via the product page (olink.com/target48human). Three examples are shown in Figure 2.

Performance characteristics

Sample information

The Olink Target 48 Neurodegeneration verification was done using 15 plasma samples and 4 cerebrospinal fluid (CSF) samples from adult control donors and 65 plasma samples and 27 CSF samples from adult patients diagnosed with various neurological impairments such as:

Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis and traumatic brain injury.

Sample types

The ability to use different sample types was evaluated by collecting matched serum and EDTA from 15 healthy individuals, Table 1 summarizes the response values for 15 normal EDTA plasma samples expressed in pg/mL, as well as relative differences between serum compared to EDTA plasma. Variations observed between responses in serum, as compared to EDTA plasma, were generally small and all assays should therefore function without limitation in serum.

Analytical measurement

Detection limit

Standard curves were determined for the 42 biomarkers simultaneously in a multiplex format using recombinant proteins. 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 low values. In such cases, a significantly lower value can be reported, which leads to erroneous interpretation of results. Therefore, the hook effect was determined for each biomarker, 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 order of log₁₀, see Table 1. To ensure accurate quantification from lot to lot Olink establish release specifications for the limits of quantification (LOQ) for every manufactured lot. The analytical measuring data shown in Table 1 is based on the verification results obtained during product development. The upper and lower limits of quantification (ULOQ and LLOQ,

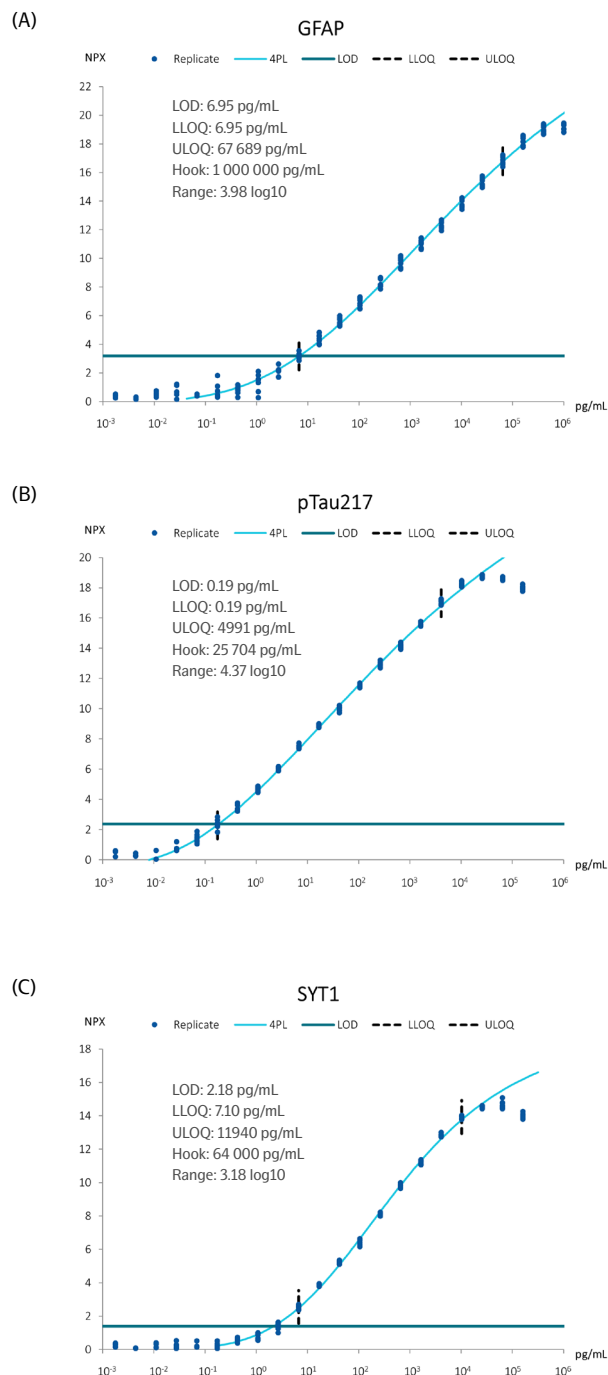


Figure 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

respectively) were calculated and reported in pg/mL with the following trueness and precision criteria relative error <30% and CV <30%, of back-calculated values (see Table 1). Separate calibrator curves were defined for each assay and can be accessed via the product page (olink.com/target48human) together with the analytical data for the assay. Three examples of assays and their analytical data are shown in Figure 2. The control and pathological plasma levels are listed in Table 1, and the control and pathological CSF levels are listed in Table 2. The distribution of measuring ranges of the 42 assays and endogenous plasma levels for control donors are shown in Figure 3 and CSF levels for both control and pathological donors are shown in Figure 4.

Table 1. Sample types; Control and pathological plasma levels, Serum detectability relative to plasma, Endogenous interference, Analytical measuring 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 42 protein biomarkers. Not available, NA.

Target	Sample types						Endogenous interference		Analytical measuring range				Precision			
	UniProt	Control plasma levels (pg/mL)			Pathological plasma levels (pg/mL)			Relative plasma (%)	(mg/mL)	(pg/mL)			log10	% CV		
Protein name (gene name)		10 th %tile	Median	90 th %tile	10 th %tile	Median	90 th %tile	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Advanced glycosylation end product-specific receptor (AGER)	Q15109	827.60	1269.96	1590.72	773.52	1470.71	2657.16	103	15.0	2.98	2.98	28224	160000	3.98	5	5
Amyloid-beta precursor protein 40 (Abeta40)	P05067	48.88	69.61	93.61	118.56	175.85	251.12	67	15.0	3.30	7.23	11834	64000	3.18	4	5
Amyloid-beta precursor protein 42 (Abeta42)	P05067	25.68	38.42	50.77	58.08	81.08	106.84	87	15.0	7.29	19.03	11148	64000	2.79	5	6
Aromatic-L-amino-acid decarboxylase (DDC)	P20711	928.58	1468.90	4204.73	1033.90	2072.61	4637.19	131	15.0	2.08	6.58	81799	160000	3.98	6	6
Beta-nerve growth factor (NGF)	P01138	1.31	1.69	4.49	1.26	2.31	5.68	81	15.0	1.01	1.01	4101	64000	3.58	9	8
Beta-secretase 1 (BACE1)	P56817	835.04	961.12	1145.51	786.71	1049.65	1334.66	97	15.0	15.88	43.25	175062	400000	3.58	7	6
Bone morphogenetic protein 7 (BMP7)	P18075	5450.52	6782.33	8213.25	5975.34	7378.47	9832.06	63	15.0	866.58	866.58	417114	1000000	2.79	6	7
Calsyntenin-3 (CLSTN3)	Q9BQT9	526.62	683.64	777.77	474.54	602.06	817.49	122	15.0	119.05	119.05	12527	158489	1.99	7	6
Cellular tumor antigen p53 (TP53)	P04637	0.58	0.62	0.75	0.65	1.04	2.13	88	15.0	0.15	0.52	1730	10240	3.58	5	6
Forkhead box protein O3 (FOXO3)	O43524	18.02	18.88	30.19	23.79	45.85	125.29	82	7.5	5.50	16.93	26127	64000	3.18	5	6
Gamma-enolase (ENO2)	P09104	2380.19	3013.15	3831.42	1254.35	2597.59	5124.99	4	15.0	101.46	101.46	185451	1000000	3.18	7	4
Glial cell line-derived neurotrophic factor (GDNF)	P39905	0.12	0.14	0.16	0.14	0.22	0.44	NA	15.0	0.11	0.11	4244	9292	4.42	5	6
Glial fibrillary acidic protein (GFAP)	P14136	15.69	18.78	28.57	11.03	25.59	59.63	103	15.0	6.95	6.95	67689	1000000	3.98	6	7
Glutaredoxin-1 (GLRX)	P35754	88.92	138.91	281.51	159.48	287.20	592.43	84	0.0	6.26	18.82	10239	64000	2.79	7	5
HLA class II histocompatibility antigen, DR alpha chain (HLA-DRA)	P01903	80.23	85.98	143.53	105.35	165.58	374.33	162	15.0	71.74	71.74	2753721	6930504	4.59	7	5
Integrin alpha-M (ITGAM)	P11215	6182.91	7405.92	12874.56	3488.59	5161.22	7472.69	42	15.0	14.63	14.63	66289	400000	3.58	5	5
Integrin beta-2 (ITGB2)	P05107	16791.75	22909.27	51937.88	14509.92	21705.99	30679.26	63	15.0	142.36	142.36	429747	1000000	3.58	5	6
Interferon-induced, double-stranded RNA-activated protein kinase (EIF2AK2)	P19525	4157.33	6714.64	11087.25	8845.41	39605.12	104296.82	266	15.0	295.56	877.54	493333	1000000	2.79	8	8
Kallikrein-8 (KLK8)	O60259	1314.82	1614.20	2498.08	967.53	1625.79	2420.76	101	15.0	16.76	46.41	24903	160000	2.79	5	6
Leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2)	Q55007	NA	NA	NA	872.48	1526.63	3656.33	NA	15.0	854.25	854.25	1055572	2511886	3.18	5	6
Microtubule-associated protein tau phosphorylated at threonine 217 (pTau217)	P10636	0.24	0.34	0.48	0.38	0.56	1.59	82	15.0	0.19	0.19	4991	25704	4.37	6	6
Myelin-oligodendrocyte glycoprotein (MOG)	Q16653	23.43	25.78	31.54	25.44	37.95	60.83	103	15.0	0.56	0.56	4413	25704	3.98	7	8
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (DDAH1)	O94760	134.47	204.74	381.35	203.44	292.77	528.28	107	0.9	16.98	45.06	71582	1000000	3.18	6	6
Neurofilament light polypeptide (NEFL)	P07196	359.67	457.63	1013.36	476.69	1154.73	3255.42	85	15.0	75.55	259.74	412994	1000000	3.20	6	6
Neurologin-1 (NLGN1)	Q8N2Q7	442.27	442.27	442.27	295.07	346.66	706.01	107	15.0	282.91	282.91	418082	1000000	3.18	5	6
Neuronal pentraxin receptor (NPTXR)	O95502	925.30	1255.78	1605.00	1106.95	1645.45	2462.62	112	15.0	0.90	2.60	27669	160000	3.98	6	7
Neuronal pentraxin-1 (NPTX1)	Q15818	5135.38	7055.95	9187.48	6863.50	9236.78	13634.14	106	15.0	110.71	110.71	176905	1000000	3.18	4	5
Neuronal pentraxin-2 (NPTX2)	P47972	5224.66	8067.44	11475.27	6575.40	9367.45	13811.90	108	15.0	39.75	104.39	178611	1000000	3.18	6	5
Oligodendrocyte-myelin glycoprotein (OMG)	P23515	61.57	72.04	92.18	33.09	88.56	198.70	107	15.0	6.46	6.46	11516	64000	3.18	6	5
Protein S100-B (S100B)	P04271	NA	NA	NA	NA	NA	NA	NA	15.0	17.28	17.28	28102.8	160000	3.18	7	5
Reticulon-4 receptor (RTN4R)	Q9BZR6	367.40	527.93	720.21	444.96	609.97	807.48	111	15.0	44.94	44.94	11084	64000	2.39	5	7
Secretogranin-2 (SCG2)	P13521	2253.76	2761.86	3500.06	2391.66	3212.54	4549.87	72	15.0	12.85	44.61	28782	160000	2.79	5	5
SPARC-related modular calcium-binding protein 1 (SMOC1)	Q9H4F8	1585.99	2076.92	2430.79	1627.95	2477.78	3882.93	136	15.0	280.20	280.20	76176	400000	2.39	6	5
Stromelysin-2 (MMP10)	P09238	189.79	414.32	547.42	228.15	353.32	686.04	105	15.0	0.28	0.80	3723	21707	3.67	6	7
Synaptotagmin-1 (SYT1)	P21579	12.64	15.28	18.86	12.09	19.88	31.83	101	15.0	2.18	7.10	11940	64000	3.18	5	4
Syndecan-4 (SDC4)	P31431	2646.46	6187.71	7647.59	2155.48	4391.32	8344.25	250	15.0	317.99	317.99	414401	1000000	3.18	5	7
Synphilin-1 (SNCAIP)	Q9Y6H5	8.11	9.17	16.96	6.96	7.46	12.77	122	15.0	6.56	6.56	11255	64000	3.18	5	5
Syntaxin-1B (STX1B)	P61266	40.08	57.57	231.83	52.88	77.55	123.89	128	15.0	19.16	19.16	185270	1000000	3.98	7	5
Triggering receptor expressed on myeloid cells 1 (TREM1)	Q9NP99	169.26	245.75	359.15	140.85	225.34	410.22	103	15.0	0.15	0.47	13177	28986	4.43	6	6
Triggering receptor expressed on myeloid cells 2 (TREM2)	Q9NZC2	6082.76	12147.15	28836.97	8962.62	16154.08	37447.80	113	15.0	6.41	6.41	472281	887185	4.81	5	7
Visinin-like protein 1 (VSNL1)	P62760	25.88	40.50	59.35	24.89	40.81	63.33	110	15.0	1.17	3.64	29871	183953	3.83	7	5
WW domain-containing oxidoreductase (WWOX)	Q9NZC7	5.95	5.95	5.95	3.39	8.08	20.03	NA	3.8	2.34	2.34	12533	25600	3.58	5	5

Dynamic range and plasma levels

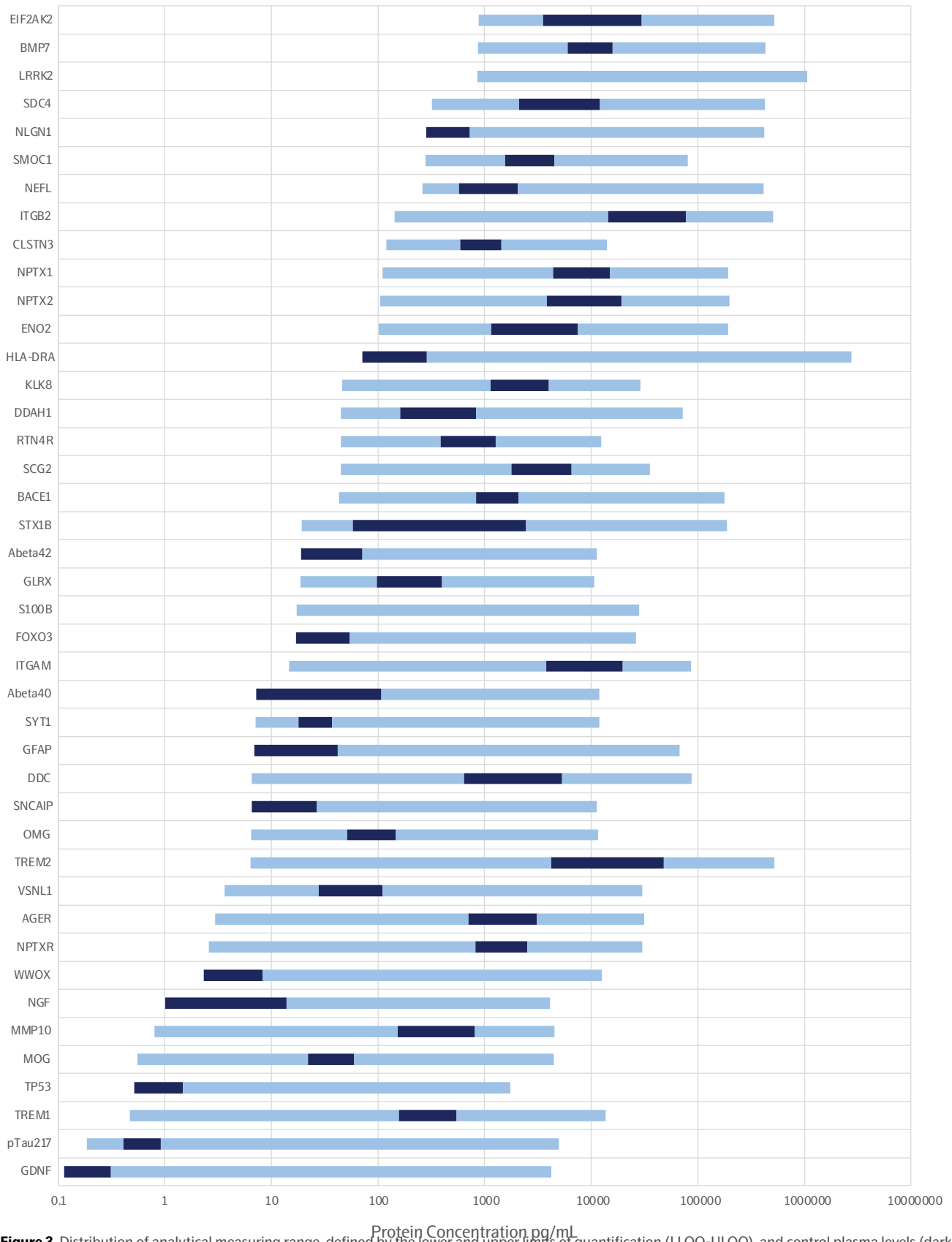


Figure 3. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ), and control plasma levels (darker bars) for the 42 protein biomarkers.

Table 2. Sample types; Control and pathological CSF levels for CSF indicative of assay performance are shown for the 42protein biomarkers. Not available, NA.

Target	Sample types						
	UniProt	Control CSF levels (pg/mL)			Pathological CSF levels (pg/mL)		
Protein name (gene name)		10 th %tile	Median n=4	90 th %tile	10 th %tile	Median n=27	90 th %tile
Advanced glycosylation end product-specific receptor (AGER)	Q15109	5.82	9.67	12.87	5.63	9.44	15.61
Amyloid-beta precursor protein 40 (Abeta40)	P05067	3441.21	4347.48	5315.21	952.61	2663.30	4621.95
Amyloid-beta precursor protein 42 (Abeta42)	P05067	652.29	857.95	1059.33	183.64	618.98	1111.81
Aromatic-L-amino-acid decarboxylase (DDC)	P20711	9.02	12.58	15.11	12.01	19.56	32.29
Beta-nerve growth factor (NGF)	P01138	NA	NA	NA	NA	NA	NA
Beta-secretase 1 (BACE1)	P56817	1810.39	1923.18	2285.18	1142.44	2035.06	2607.37
Bone morphogenetic protein 7 (BMP7)	P18075	17091.63	21002.41	24987.30	8992.57	17872.67	24395.65
Calsyntenin-3 (CLSTN3)	Q9BQ19	552.24	646.48	784.02	426.92	638.59	767.20
Cellular tumor antigen p53 (TP53)	P04637	NA	NA	NA	NA	NA	NA
Forkhead box protein O3 (FOXO3)	O43524	NA	NA	NA	NA	NA	NA
Gamma-enolase (ENO2)	P09104	659.16	933.38	1345.77	824.91	1225.95	1894.59
Glial cell line-derived neurotrophic factor (GDNF)	P39905	NA	NA	NA	NA	NA	NA
Glial fibrillary acidic protein (GFAP)	P14136	449.32	696.91	968.53	169.16	547.79	1048.10
Glutaredoxin-1 (GLRX)	P35754	45.93	58.16	64.25	22.23	46.44	70.17
HLA class II histocompatibility antigen, DR alpha chain (HLA-DRA)	P01903	398.66	642.05	772.36	320.23	622.55	1493.94
Integrin alpha-M (ITGAM)	P11215	203.11	238.81	261.45	160.20	266.28	348.38
Integrin beta-2 (ITGB2)	P05107	443.20	505.62	639.54	396.64	687.63	992.59
Interferon-induced, double-stranded RNA-activated protein kinase (EIF2AK2)	P19525	NA	NA	NA	NA	NA	NA
Kallikrein-8 (KLK8)	O60259	133.01	145.46	155.64	113.22	171.06	256.10
Leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2)	Q55007	NA	NA	NA	NA	NA	NA
Microtubule-associated protein tau phosphorylated at threonine 217 (pTau217)	P10636	14.03	15.23	19.53	9.46	17.60	42.11
Myelin-oligodendrocyte glycoprotein (MOG)	Q16653	2812.10	3213.43	3485.89	1704.39	2532.23	4085.55
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (DDAH1)	O94760	2172.56	2235.08	2255.36	1299.67	2183.14	3232.85
Neurofilament light polypeptide (NEFL)	P07196	9837.04	10774.17	12635.31	5746.57	13169.72	63822.81
Neuroigin-1 (NLGN1)	Q8N2Q7	371.83	371.83	371.83	338.51	359.08	601.71
Neuronal pentraxin receptor (NPTXR)	O95502	23565.93	24525.48	25485.04	19196.98	24402.54	26605.70
Neuronal pentraxin-1 (NPTX1)	Q15818	46149.38	59296.33	65469.36	28369.08	52981.15	79085.75
Neuronal pentraxin-2 (NPTX2)	P47972	10102.96	13996.93	21679.44	3564.84	10549.73	20796.18
Oligodendrocyte-myelin glycoprotein (OMG)	P23515	NA	NA	NA	NA	NA	NA
Protein S100-B (S100B)	P04271	35.27	51.37	66.32	25.66	37.64	56.02
Reticulon-4 receptor (RTN4R)	Q9BZR6	1811.38	1962.16	2469.06	1467.48	2393.82	2650.46
Secretogranin-2 (SCG2)	P13521	133.01	145.68	7640.40	13991.87	21346.22	23814.03
SPARC-related modular calcium-binding protein 1 (SMOC1)	Q9H4F8	2815.32	3135.16	3613.10	1821.57	2874.26	3878.70
Stromelysin-2 (MMP10)	P09238	10.15	10.68	13.05	5.93	9.41	18.64
Synaptotagmin-1 (SYT1)	P21579	663.88	802.72	922.41	415.52	700.51	1078.80
Syndecan-4 (SDC4)	P31431	353.72	429.58	489.41	344.25	437.77	584.59
Synphilin-1 (SNCAIP)	Q9YGH5	NA	NA	NA	NA	NA	NA
Syntaxin-1B (STX1B)	P61266	2493.39	3005.87	3511.67	1532.43	2960.61	3879.15
Triggering receptor expressed on myeloid cells 1 (TREM1)	Q9NP99	9.59	9.86	13.03	8.68	14.24	24.75
Triggering receptor expressed on myeloid cells 2 (TREM2)	Q9NZC2	4295.43	4926.85	28348.64	2128.73	4776.22	7491.63
Visinin-like protein 1 (VSNL1)	P62760	37.32	41.95	53.69	18.00	37.12	67.47
WW domain-containing oxidoreductase (WWOX)	Q9NZC7	NA	NA	NA	NA	NA	NA

Dynamic range and CSF levels

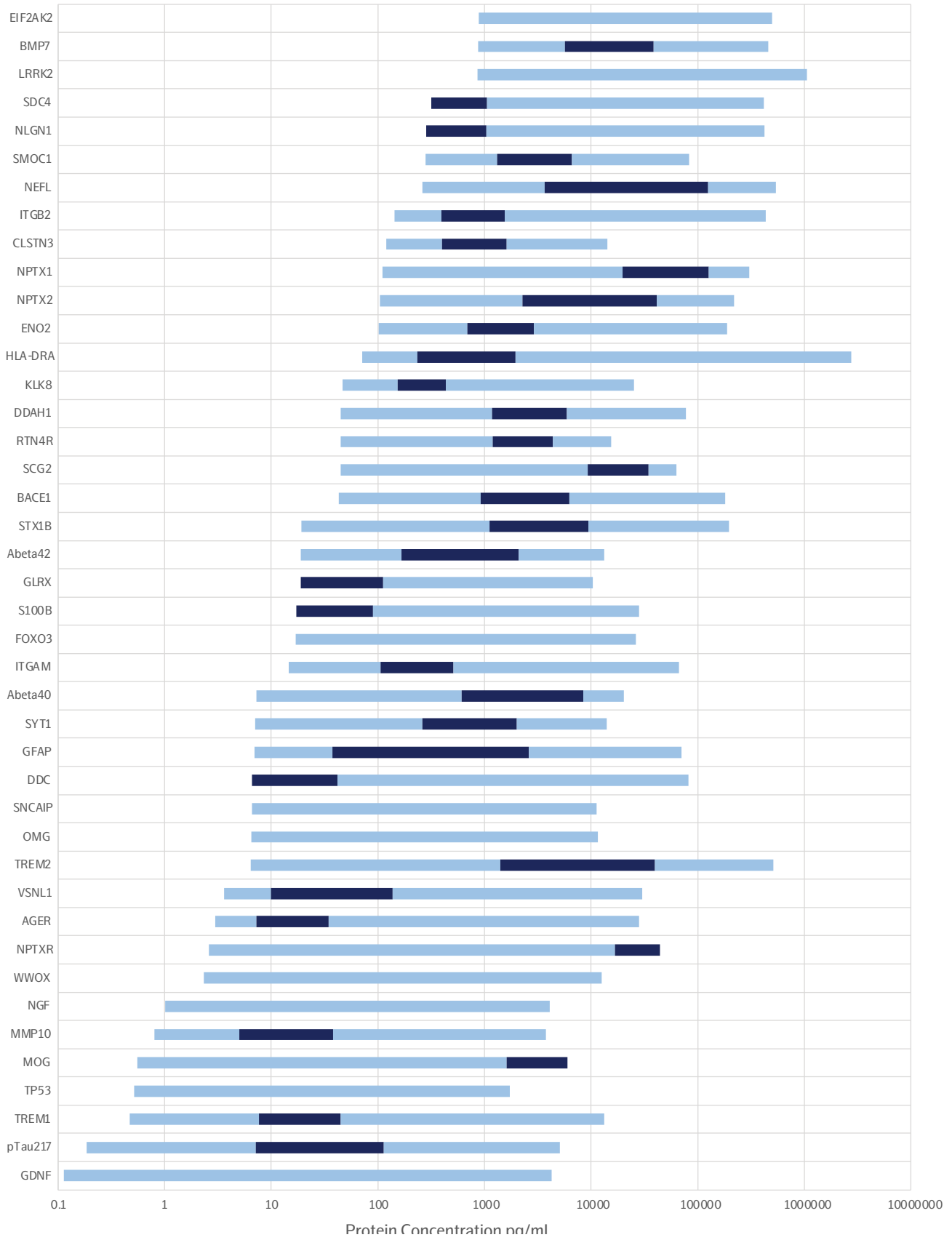


Figure 4. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ), and CSF levels for both control and pathological samples (darker bars) for the 42 protein biomarkers.

Precision

Repeatability

Inter-run (between run) and intra-run (within run) CV were assessed by evaluating triplicate measurements of the Sample Control on each plate, based on 24 plate runs performed by three different operators. Each operator performed a minimum of three runs.

Inter-run CV values were calculated between runs done by the same operator. The inter-run CV reported here is the average of the three operators' CV. CV calculations were performed on data in pg/mL for the 42 analytes for which response levels within LOQ were detected, see Table 1.

Across the 42 assays, both the mean intra-run and inter-run variations observed were 6%. The distribution of both intra-run and inter-run variations are shown in Figure 5.

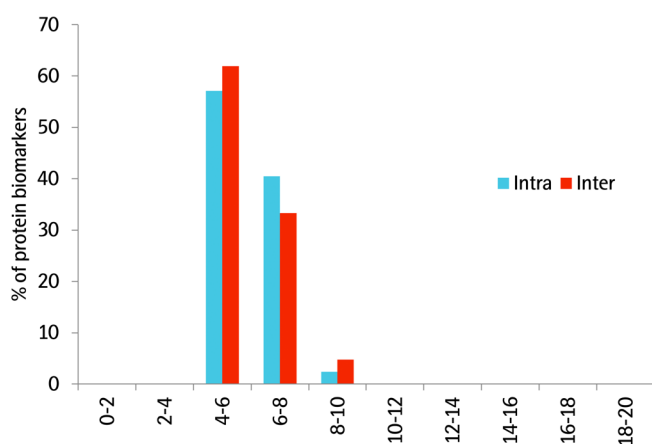


Figure 5. Distribution of intra-run and inter-run variations of Olink® Target 48 Neurodegeneration based on Sample Control data.

Reproducibility

To determine CV inter-operator (between operators) and CV inter-site (between sites), identical sample plates were sent to 5 laboratories (sites) together with Olink Target 48 Neurodegeneration kits. Ten individual plasma samples (in triplicates) and a pooled plasma sample in five different dilutions (1:1, 1:2, 1:4, 1:8 and 1:16, in duplicate) were provided. At four sites, two operators per site executed one experiment each, using one sample plate each. At one site, two operators executed one experiment each, using the same sample plate. Inter-operator and inter-site CV were calculated based on the undiluted plasma samples (the 10 individual plasma samples and the pool with a 1:1 dilution) and Olink's Sample Control, provided with the kit. All samples and controls showed good CV between operators and sites (see Table 3). Note that one run did not pass QC, so the average inter-operator CV is based on data from four sites.

Table 3. The average CV intra-run was determined for each assay on each run (n=9), and values shown represent the average of all runs. CV inter-run is the average of all runs. Inter-operator CV was determined per site (four sites). CV inter-operator is the average of inter-operator CV from all sites. The CV inter-site was determined pairwise, between all sites (five sites). CV inter-site is the average of all pairwise calculations.

%CV	Plasma samples	Pooled plasma	Sample Control
Intra-run	9.16	7.27	4.60
Inter-operator	7.44	6.54	6.04
Inter-site	10.51	11.13	4.12

There are many Olink-certified core laboratories around the world running Olink panels (see www.olink.com/service for details). Our experience over several years is that inter-site reproducibility is very good provided that operators are properly trained. For more information please contact support@olink.com.

Analytical Specificity

Assay specificity

To test the specificity of the PEA probes of Olink Target 48 Neurodegeneration, all antibodies used were tested for cross reactivity against all proteins targeted. To confirm that the antibodies implemented into Olink Target 48 Neurodegeneration are specific for their targets, detection of the 42 proteins were determined applying recombinant proteins solitary to the multiplex. These tests revealed that one assay showed cross-binding to another protein, with 10% (OMG detecting ITGB2). No other assays had any unspecific signal.

Endogenous interference

Bilirubin, lipids and hemolysate, are plasma and serum components that are known to interfere with some analytical assays. An example of the hemolysate levels tested is shown in Figure 6. These additions represent different health conditions and/or sample collection irregularities. In 4 out of 42 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.

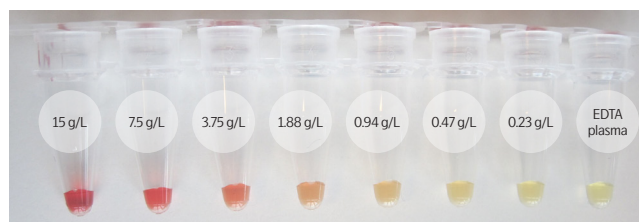


Figure 6. Endogenous interference. Levels of hemolysis tested, corresponding to 0.23–15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

Interference by bilirubin and lipids has previously been evaluated, and disturbance was only observed at extreme levels corresponding to 8 or 10 times normal values^{3,4}. This test was therefore not repeated for Olink Target 48 Neurodegeneration.

Linearity

Linearity was assessed in true matrix conditions by diluting one sample in another sample. A native plasma sample containing a relatively high endogenous level of the target protein is mixed with a native plasma sample containing a relatively low level of the protein, at different ratios, to give three equally spaced intermediate concentrations. Native samples were chosen to obtain as wide a range as possible, requiring several different sample combinations to be included in the test. The difference between the “theoretical” concentration and the “measured” concentration was analyzed. The expected (theoretical) concentrations were based on the measured concentration of the highest and lowest sample, and the theoretically calculated expected concentrations for the intermediate data points, (see Figure 7).

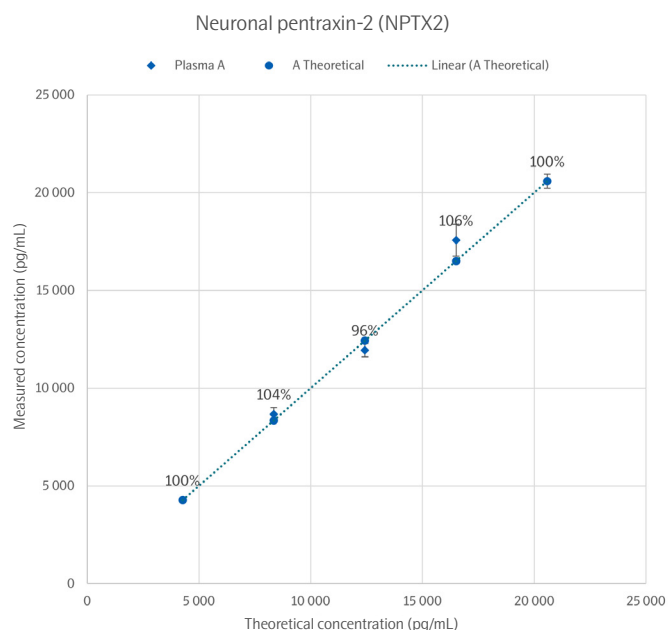


Figure 7. The difference between the “theoretical” concentration and the “measured” concentration.

References

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