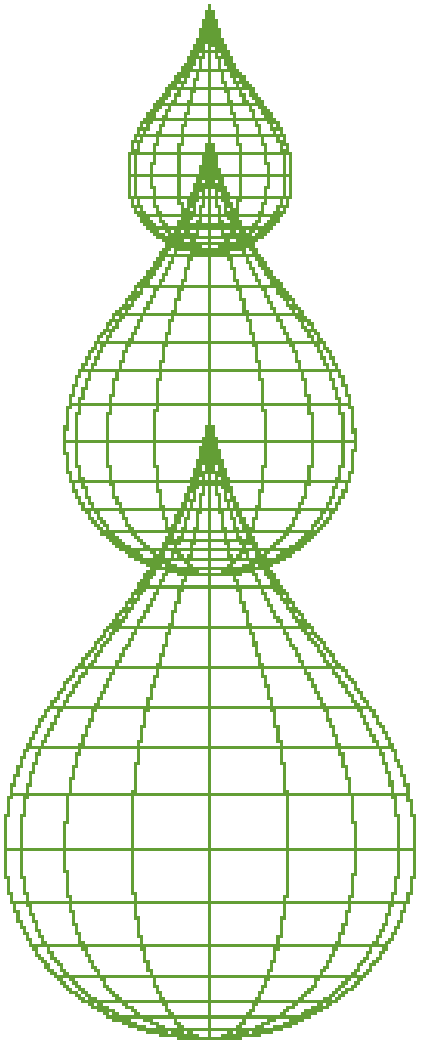
Proseek® Multiplex CVD I 96×96

DATA PACKAGE



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1. Introduction

Proseek® Multiplex CVD I 96×96 is a reagent kit measuring 92 cardiovascular disease related human protein biomarkers simultaneously in plasma samples. The analytical performance of the product has been carefully validated and the results are presented below.

1.1 TECHNOLOGY

The Proseek reagents are based on PEA, a Proximity Extension Assay technology[1], where 92 oligonucleotide labeled antibody probe pairs are allowed to bind to their respective target present in the sample. A PCR reporter sequence is formed by a proximity dependent DNA polymerization event and is 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 DATA ANALYSIS

Data analysis was performed by employing a pre-processing normalization procedure. For each data point, delta Cq (dCq) values were obtained by subtracting the value for the Extension control, thus normalizing each sample for technical variation within one run. Normalization between runs is then performed by subtraction of the Interplate Control (IPC) for each assay. In the final step of the pre-processing procedure the values are set relative to a fixed background level 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, although it might differ between runs. Linearization of data is performed by the mathematical operation 2 ^NPX. Statistical analyses, e.g. coefficient of variation (CV) calculations were performed on linearized values.

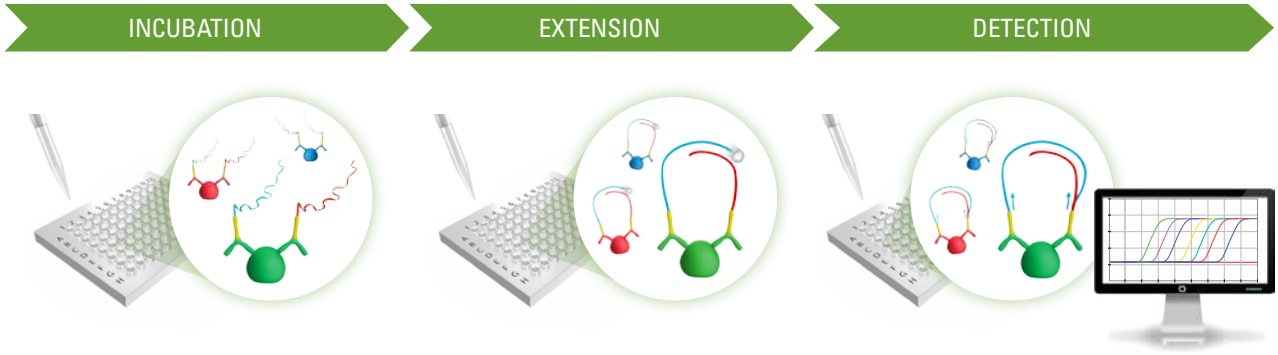


Fig 1. Proseek Multiplex assay procedure employs three core steps: Incubation, Extension and Detection. High throughput real-time qPCR is performed by using the Fluidigm® Biomark™ or Fluidigm® Biomark™ HD systems.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with the Proseek Multiplex CVD I 96×96 by collecting matched ethylenediaminetetraacetic acid (EDTA), acid citrate dextrose (ACD), and heparin plasma samples from 5 individuals. Table 1 shows signal to background values for each sample type and assay, as well as relative percentage differences compared to EDTA plasma. The results indicated that EDTA plasma is a suitable sample type for all assays. Variations observed between responses in heparin and citrate plasma, as compared to EDTA plasma, was generally small, and most of the assays will therefore function without limitation in these sample types.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Limit of detection (LOD) was defined as 3 standard deviations above background, and reported in pg/mL for 88 proteins out of 92, for which recombinant antigen was available, see Figure 2 and Table 1.

MEASURING RANGES

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 ≤ 30% and CV ≤ 30%, of back-calculated values, respectively. Measuring ranges were reported in order of log10. See Figure 2 and Table 1.

Calibrator curves were determined for 88 protein biomarkers simultaneously in multiplex format. Two protein biomarkers lacked recombinant antigens and two were non-purified preparations. Representative assays with their analytical data are exemplified in Figure 2 and the distribution of their corresponding measuring range per assay is shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com/products/proseek-multiplex/proseek-multiplex-cvd-i

HIGH DOSE HOOK EFFECT

A high dose hook effect is a state of antigen excess relative to the reagent antibodies resulting in falsely lower values. If undetected, a significantly lower value will be reported which can lead to misinterpretation of results. Therefore, the high dose hook effect was determined for each analyte, here reported in pg/mL, see Figure 2 and Table 1.

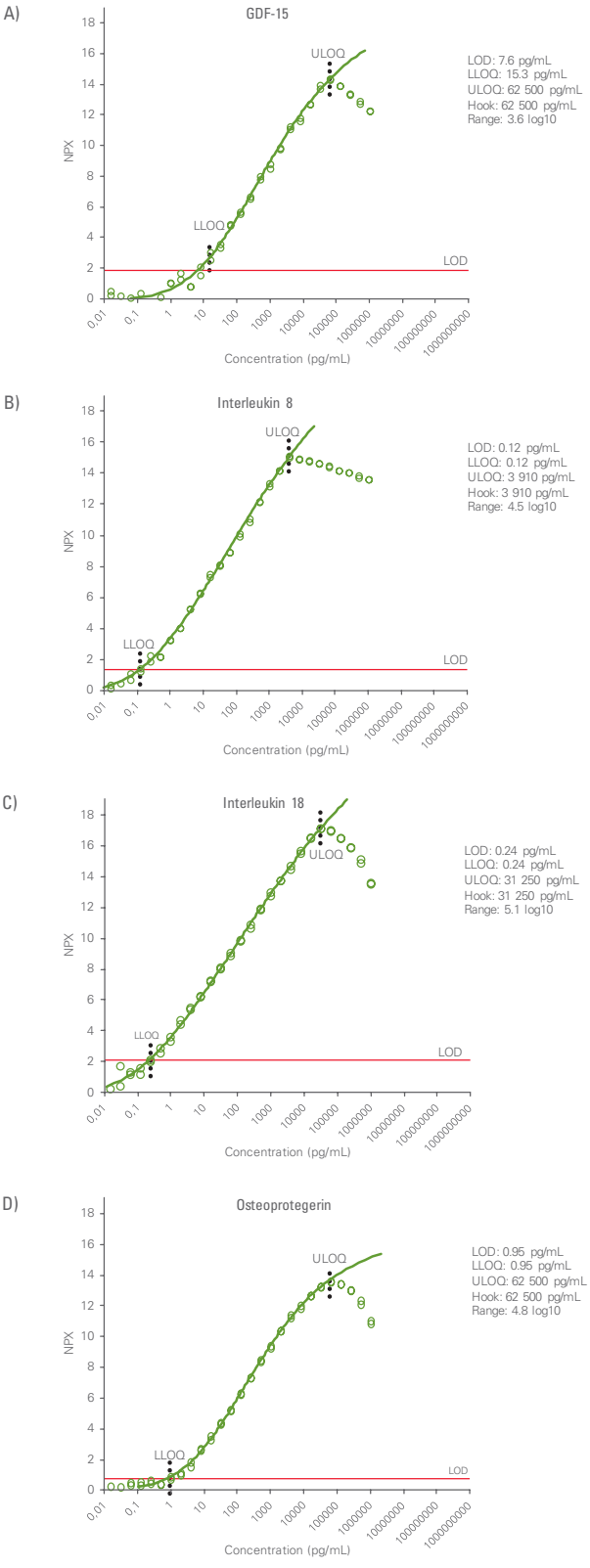


Fig 2. Calibrator curves from 4 representative assays and their corresponding analytical measurement data.

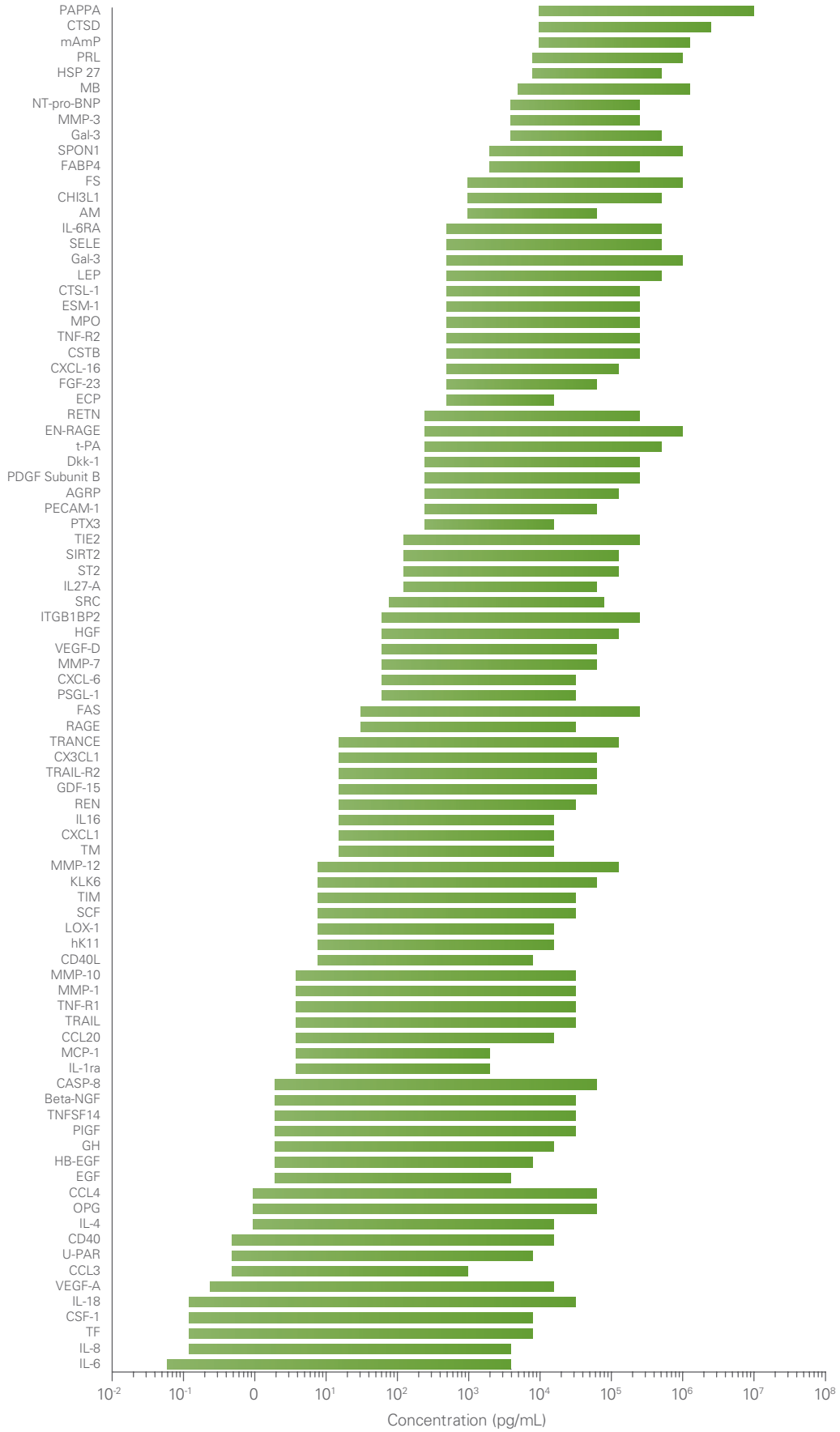


Fig 3. Distribution of analytical measuring range, defined by the limits of quantification LLOQ-ULOQ, for 88 out of 92 analytes.

Table 1. Sample Types, Analytical Measurement; Limit of Detection, LOD, Lower Limit of Quantification, LLOQ, Upper Limit of Quantification, ULOQ, High Dose Effect, Hook, and Precision indicative of assay performance are shown for 92 analytes. Values below limit of detection were not reported (NR).

		Sample types					Analytical measurement					Precision		
		Signal-to-background (2^{ddCq})			Relative 2^{ddCq} to EDTA plasma		pg/mL				log10			
Target	UniProt No	ACD	EDTA	Heparin	ACD	Heparin	LOD	LLOQ	ULOQ	Hook	Range	Intra-assay	Inter-assay	Inter-site
Adrenomedullin	P35318	92	112	78	82%	69%	977	977	62500	250000	1.8	10%	18%	16%
Agouti-related protein	O00253	25	22	28	114%	127%	244	244	125000	250000	2.7	7%	12%	17%
Angiopoietin-1 receptor	Q02763	100	114	105	88%	92%	61	122	250000	1000000	3.3	8%	12%	10%
Beta-nerve growth factor	P01138	2	2	2	102%	81%	2	2	31250	62500	4.2	8%	25%	13%
Caspase-8	Q14790	2	2	2	84%	112%	2	2	62500	125000	4.5	7%	21%	25%
Cathepsin D	P07339	211	230	203	92%	88%	9766	9766	2500000	2500000	2.1	8%	16%	14%
Cathepsin L1	P07711	65	77	62	83%	80%	244	488	250000	500000	2.7	7%	14%	8%
C-C motif chemokine 3	P10147	3	5	4	74%	79%	0.24	0.48	977	7810	3.3	10%	18%	18%
C-C motif chemokine 4	P13236	257	452	385	57%	85%	0.95	0.95	62500	62500	4.8	8%	12%	8%
C-C motif chemokine 20	P78556	146	154	110	95%	72%	4	4	15630	31250	3.6	8%	9%	5%
CD40 ligand	P29965	54	172	391	31%	227%	8	8	7810	15630	3.0	8%	16%	10%
Chitinase-3-like protein 1	P36222	46	53	48	88%	91%	488	977	500000	500000	2.7	11%	15%	11%
C-X-C motife chemokine 1	P09341	67	208	350	32%	169%	15	15	15630	31250	3.0	6%	13%	18%
C-X-C motif chemokine 6	P80162	83	265	630	31%	238%	61	61	31250	31250	2.7	8%	12%	16%
C-X-C motif chemokine 16	Q9H2A7	41	46	39	90%	87%	488	488	125000	125000	2.4	10%	14%	14%
Cystatin-B	P04080	39	55	41	71%	74%	488	488	250000	500000	2.7	8%	13%	10%
Dickkopf-related protein 1	O94907	52	147	96	36%	65%	122	244	250000	500000	3.0	8%	15%	14%
Endothelial cell-specific molecule 1	Q9NQ30	5	8	5	65%	57%	244	488	250000	250000	2.7	9%	16%	11%
Eosinophil cationic protein	P12724	23	36	45	63%	124%	488	488	15630	15630	1.5	5%	22%	24%
Epidermal growth factor	P01133	23	81	130	29%	160%	0.95	2	3910	3910	3.3	5%	9%	5%
E-selectin	P16581	86	109	86	79%	78%	244	488	500000	500000	3.0	9%	13%	13%
Fatty acid-binding protein, adipocyte	P15090	11	13	11	82%	82%	977	1950	250000	250000	2.1	12%	14%	8%
Fibroblast growth factor 23	Q9GZV9	58	72	61	81%	85%	122	488	62500	125000	2.1	9%	21%	14%
Follistatin	P19883	22	31	9	70%	28%	977	977	1000000	2000000	3.0	10%	13%	14%
Fractalkine	P78423	36	44	39	82%	87%	15	15	62500	1000000	3.6	9%	14%	22%
Galanin peptides	P22466	75	94	80	80%	85%	488	488	1000000	1000000	3.3	9%	17%	9%
Galectin-3	P17931	49	57	50	87%	87%	3906	3906	500000	2000000	2.1	9%	12%	13%
Growth hormone	P01241	366	385	346	95%	90%	0.95	2	15630	31250	3.9	5%	14%	15%
Growth/differentiation factor 15	Q99988	325	382	298	85%	78%	8	15	62500	62500	3.6	9%	11%	14%
Heat shock 27 kDa protein	P04792	8	20	7	40%	35%	3910	7810	500000	500000	1.8	9%	13%	13%
Heparin-binding EGF-like growth factor	Q99075	57	93	44	62%	48%	2	2	7810	15630	3.6	5%	14%	11%
Hepatocyte growth factor	P14210	129	184	107	70%	58%	31	61	125000	250000	3.3	7%	12%	12%
Interleukin-1 receptor antagonist protein	P18510	28	37	37	76%	100%	4	4	1950	7810	2.7	6%	14%	16%
Interleukin-4	P05112	NR	NR	NR	NR	NR	0.95	0.95	15630	62500	4.2	5%	11%	13%
Interleukin-6	P05231	118	125	123	94%	98%	0.06	0.06	3910	15630	4.8	8%	10%	7%
Interleukin-6 receptor subunit alpha	P08887	97	107	93	91%	87%	488	488	500000	1000000	3.0	8%	14%	12%
Interleukin-8	P10145	37	58	60	65%	103%	0.12	0.12	3910	3910	4.5	8%	12%	8%
Interleukin-16	Q14005	30	34	34	90%	100%	15	15	15630	62500	3.0	5%	11%	10%
Interleukin-18	Q14116	843	942	842	89%	89%	0.24	0.24	31250	31250	5.1	8%	12%	14%
Interleukin-27 subunit alpha	Q8NEV9	10	9	6	109%	67%	122	122	62500	125000	2.7	8%	19%	22%
Kallikrein-6	Q92876	97	115	95	84%	82%	8	8	62500	62500	3.9	11%	22%	11%
Kallikrein-11	Q9UBX7	33	36	35	91%	97%	8	8	15630	62500	3.3	9%	19%	9%
Lectin-like oxidized LDL receptor 1	P78380	42	41	88	104%	216%	8	8	15630	15630	3.3	5%	12%	9%
Leptin	P41159	16	16	18	101%	114%	488	488	62500	1000000	2.1	8%	12%	21%
Macrophage colony-stimulating factor 1	P09603	505	546	523	92%	96%	0.12	0.12	7810	15630	4.8	7%	12%	14%
Matrix metalloproteinase-1	P03956	7	37	24	18%	64%	4	4	31250	62500	3.9	5%	31%	51%

		Sample types					Analytical measurement					Precision		
		Signal-to-background (2^{ddCq})			Relative 2^{ddCq} to EDTA plasma		pg/mL				log10			
Target	UniProt No	ACD	EDTA	Heparin	ACD	Heparin	LOD	LLOQ	ULOQ	Hook	Range	Intra-assay	Inter-assay	Inter-site
Matrix metalloproteinase-3	P08254	3	4	3	90%	76%	3910	3910	250000	250000	1.8	7%	20%	16%
Matrix metalloproteinase-7	P09237	163	37	154	439%	414%	31	61	62500	125000	3.0	11%	16%	15%
Matrix metalloproteinase-10	P09238	111	111	115	100%	104%	4	4	31250	31250	3.9	7%	15%	18%
Matrix metalloproteinase-12	P39900	233	164	199	142%	121%	8	8	125000	250000	4.2	9%	39%	26%
Melusin	Q9UKP3	NR	8	NR	NR	NR	122	122	125000	250000	3.0	13%	21%	31%
Membrane-bound aminopeptidase P	O43895	9	12	9	74%	75%	9766	9766	1250000	1250000	2.1	13%	22%	12%
Monocyte chemotactic protein 1	P13500	42	46	33	91%	72%	2	4	1950	1950	2.7	7%	18%	26%
Myeloperoxidase	P05164	14	15	15	92%	101%	488	488	250000	250000	2.7	5%	18%	10%
Myoglobin	P02144	41	48	41	86%	85%	4883	4883	1250000	1250000	2.4	9%	17%	10%
Natriuretic peptides B	P16860	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
NF-kappa-B essential modulator	Q9Y6K9	9	14	7	67%	52%	NR	NR	NR	NR	NR	8%	20%	15%
N-terminal pro-B-type natriuretic peptide		NR	NR	NR	NR	NR	3910	3910	250000	250000	1.8	NR	NR	NR
Osteoprotegerin	O00300	1314	1470	1094	89%	74%	0.95	0.95	62500	62500	4.8	7%	11%	7%
Ovanrian cancer-related tumor marker CA 125	Q8WXI7	43	53	45	81%	86%	NR	NR	NR	NR	NR	10%	20%	21%
Pappalysin-1	Q13219	4	6	NR	68%	NR	4883	9766	10000000	10000000	3.0	11%	21%	5%
Pentraxin-related protein PTX3	P26022	NR	2	NR	NR	NR	244	244	15630	62500	1.8	8%	20%	14%
Placenta growth factor	P49763	164	207	151	79%	73%	0.48	2	31250	31250	4.2	8%	13%	7%
Platelet endothelial cell adhesion molecule	P16284	78	94	74	84%	79%	244	244	62500	1000000	2.4	8%	16%	13%
Platelet-derived growth factor subunit B	P01127	30	237	132	13%	56%	244	244	250000	250000	3.0	9%	14%	17%
Prolactin	P01236	21	23	18	89%	79%	3906	7812	1000000	2000000	2.1	9%	14%	9%
Protein S100-A12	P80511	69	43	88	162%	206%	244	244	1000000	1000000	3.6	8%	22%	30%
Proteinase-activated receptor 1	P25116	153	193	201	80%	104%	NR	NR	NR	NR	NR	6%	14%	17%
Proto-oncogene tyrosine-protein kinase Src	P12931	96	152	68	64%	45%	76	76	78125	156250	3.0	4%	12%	15%
P-selectin glycoprotein ligand 1	Q14242	2	2	1	98%	57%	61	61	31250	62500	2.7	8%	20%	30%
Receptor for advanced glycosylation end products	Q15109	25	28	13	90%	46%	15	31	31250	1000000	3.0	8%	13%	10%
Renin	P00797	117	134	134	87%	100%	15	15	31250	250000	3.3	7%	13%	9%
Resistin	Q9HD89	188	235	178	80%	75%	122	244	250000	500000	3.0	9%	18%	21%
SIR2-like protein	Q8IXJ6	3	12	3	29%	27%	122	122	125000	250000	3.0	11%	20%	22%
Spondin-1	Q9HCB6	25	32	10	77%	31%	3906	3906	1000000	4000000	2.4	10%	13%	13%
ST2 protein	Q01638	14	17	13	84%	75%	122	122	125000	250000	3.0	9%	14%	13%
Stem cell factor	P21583	238	256	243	93%	95%	0.12	0.12	7810	7810	7810	6%	13%	16%
Thrombomodulin	P07204	1032	1125	1040	92%	92%	8	15	15625	250000	3.0	8%	14%	13%
TIM-1	Q96D42	36	40	36	91%	90%	8	8	31250	62500	3.6	10%	14%	13%
Tissue factor	P13726	60	70	64	86%	91%	0.06	0.12	7810	31250	4.8	7%	16%	17%
Tissue-type plasminogen activator	P00750	355	293	106	121%	36%	244	244	500000	500000	3.3	9%	14%	23%
TNF-related activation-induced cytokine	O14788	29	34	29	87%	85%	15	15	125000	125000	3.9	9%	16%	13%
TNF-related apoptosis-inducing ligand	P50591	710	762	678	93%	89%	4	4	31250	62500	3.9	7%	15%	12%
TNF-related apoptosis-inducing ligand receptor 2	O14763	4	4	3	90%	82%	8	15	62500	62500	3.6	7%	15%	12%
Tumor necrosis factor receptor 1	P19438	8469	9172	8775	92%	96%	2	4	31250	62500	3.9	7%	11%	7%
Tumor necrosis factor receptor 2	P20333	25	30	26	84%	85%	488	488	250000	250000	2.7	9%	14%	14%
Tumor necrosis factor receptor superfamily member 5	P25942	517	583	541	89%	93%	0.24	0.48	15630	31250	4.5	7%	12%	14%
Tumor necrosis factor receptor superfamily member 6	P25445	156	182	157	85%	86%	31	31	250000	250000	3.9	8%	12%	13%
Tumor necrosis factor ligand superfamily member 14	O43557	8	11	15	73%	130%	2	2	31250	31250	4.2	8%	25%	17%
Urokinase plasminogen activator surface receptor	Q03405	1435	1612	1516	89%	94%	0.24	0.48	7810	31250	4.2	5%	12%	9%
Vascular endothelial growth factor A	P15692	1517	2491	1916	61%	77%	0.12	0.24	15630	31250	4.8	8%	13%	12%
Vascular endothelial growth factor D	O43915	128	128	142	100%	111%	61	61	62500	125000	3.0	7%	13%	11%

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean coefficient of variation (% CV) for 7 individual samples, within each of the 9 separate runs during the validation studies. Inter-assay variation (between-run was calculated as the mean coefficient of variation (% CV), for the same 7 individual samples, between the 9 separate runs during the validation studies. Variation calculations were assessed on linearized values for 90 out of 92 analytes. Assays with values below limit of detection were not reported, see Table 1.

Across 90 assays, the mean CV intra-assay and inter-assay variations were observed to be 8% and 15%, respectively. The distribution of both inter-assay and inter-assay variations per assay is shown in Figure 4.

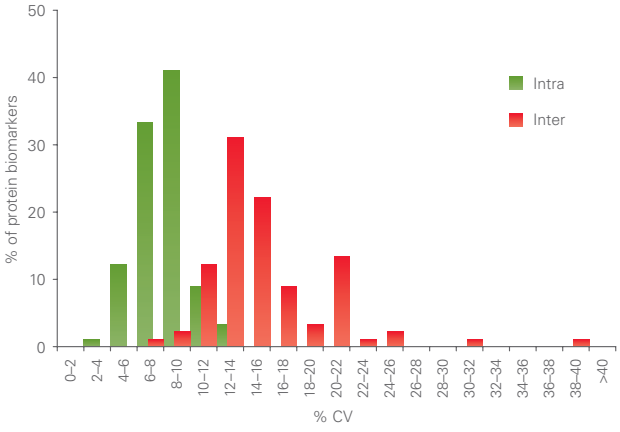


Fig 4. Distribution of intra-assay and inter-assay variations of Proseek Multiplex CVD I 96×96

REPRODUCIBILITY

Inter-site variation (between-site) was also investigated during the validation in a β-site study, to estimate the expected variations in values between different laboratories, with different operators and using different equipment. Seven individual samples were distributed to each site together with Proseek Multiplex CVD I 96×96 reagent kits. Each site was instructed to perform the analysis of the 7 individual samples according to the same run design. Each site was also asked to perform two independent runs.

The overall design of the β-site study enabled the estimation of both the intra-assay and inter-assay variations for 3 sites including Olink Bioscience, and the inter-site variation for each site, here shown in Figure 5.

The mean % CV value in the first analysis ranged from 6% to 9% intra-assay. The mean % CV ranged from 13% to 17% inter-assay, and 10% to 16% inter-site, here shown in direct comparison to Olink Bioscience in Figure 5.

Overall, the Proseek Multiplex CVD I 96×96 showed very good reproducibility and repeatability with average inter-site variation of 15%.

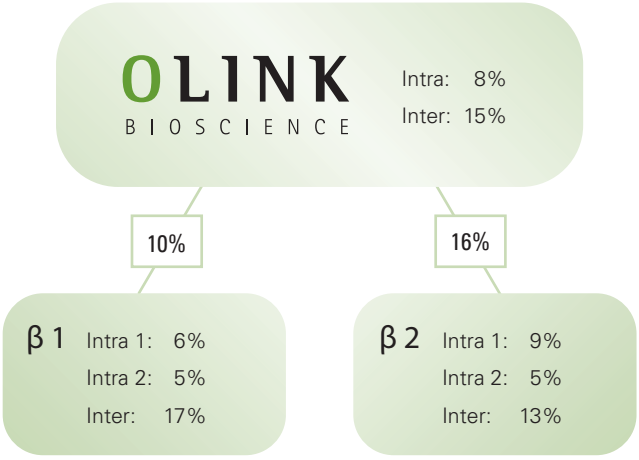


Fig 5. Validation of the Proseek Multiplex CVD I 96×96 at 2 (β1-β2) different laboratories. Larger boxes shows intra-assay and inter-assay variations for each site and small boxes represent the inter-site run variations in direct comparison to Olink Bioscience.

2.4 ANALYTICAL SPECIFICITY

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. 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 here is by antibody probe pairs being brought into proximity, by cross-binding substances other than antigens, e.g. heterophilic antibodies and similarly acting rheumatoid factor. Two different "mismatched" probe pairs of varying antibody host species origin were designed and evaluated with a Heterophilic Assessment Panel from Scantibodies Laboratory Inc. (part no. 3KG027) and two sets of samples known to contain rheumatoid factor (<20-320 International Units/mL (IU/mL)) and rheumatoid arthritis (375-600 arbitrary units (AU)). No interference could be detected for any of the panel samples, indicating a sufficient blocking ability in all assays in the Proseek Multiplex CVD I 96×96.

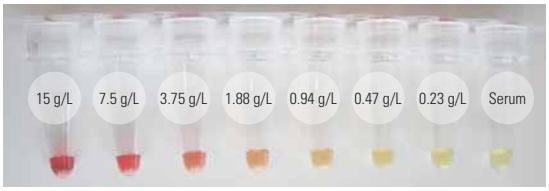
Table 2. Performance characteristics. Endogenous interference was performed by addition of hemolysate, lipids and bilirubin in serum matrix. Reported are the highest tested concentrations without impact on assay performance.

	Endogenous interference		
Targets 1-46	g/L Hemolysate	mg/mL Lipids	µg/mL Bilirubin
Adrenomedullin	4	20	630
Agouti-related protein	15	20	630
Angiopoietin-1 receptor	15	20	630
Beta-nerve growth factor	15	20	630
Caspase-8	0.23	20	630
Cathepsin D	15	20	630
Cathepsin L1	15	20	630
C-C motif chemokine 3	15	20	630
C-C motif chemokine 4	15	20	630
C-C motif chemokine 20	15	20	315
CD40 ligand	15	20	630
Chitinase-3-like protein 1	15	20	630
C-X-C motife chemokine 1	0.47	20	630
C-X-C motif chemokine 6	2	20	630
C-X-C motif chemokine 16	15	20	630
Cystatin-B	0	20	630
Dickkopf-related protein 1	15	20	630
Endothelial cell-specific molecule 1	15	20	630
Eosinophil cationic protein	0.47	20	315
Epidermal growth factor	15	20	630
E-selectin	15	20	630
Fatty acid-binding protein, adipocyte	15	20	630
Fibroblast growth factor 23	15	20	630
Follistatin	15	20	630
Fractalkine	4	20	630
Galanin peptides	15	20	630
Galectin-3	0.94	20	630
Growth hormone	15	20	630
Growth/differentiation factor 15	15	20	630
Heat shock 27 kDa protein	0	20	630
Heparin-binding EGF-like growth factor	15	20	630
Hepatocyte growth factor	15	20	630
Interleukin-1 receptor antagonist protein	15	20	630
Interleukin-4	15	20	630
Interleukin-6	15	20	630
Interleukin-6 receptor subunit alpha	15	20	630
Interleukin-8	8	20	630
Interleukin-16	4	20	630
Interleukin-18	4	20	630
Interleukin-27 subunit alpha	8	20	630
Kallikrein-11	15	20	630
Kallikrein-6	15	20	630
Lectin-like oxidized LDL receptor 1	0.23	20	630
Leptin	15	20	630
Macrophage colony-stimulating factor 1	15	20	630
Matrix metalloproteinase-1	15	20	630

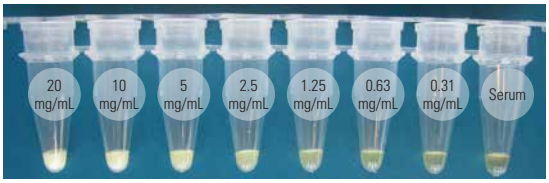
	Endogenous interference		
Targets 47-92	g/L Hemolysate	mg/mL Lipids	µg/mL Bilirubin
Matrix metalloproteinase-3	15	20	630
Matrix metalloproteinase-7	15	20	630
Matrix metalloproteinase-10	15	20	630
Matrix metalloproteinase-12	15	20	630
Melusin	15	20	630
Membrane-bound aminopeptidase P	15	20	630
Monocyte chemotactic protein 1	15	20	630
Myeloperoxidase	0.94	20	630
Myoglobin	15	20	630
Natriuretic peptides B	15	20	630
NF-kappa-B essential modulator	0	20	630
N-terminal pro-B-type natriuretic peptide	15	20	630
Osteoprotegerin	8	20	630
Ovanrian cancer-related tumor marker CA 125	8	20	630
Pappalysin-1	15	20	630
Pentraxin-related protein PTX3	15	20	630
Placenta growth factor	8	20	630
Platelet endothelial cell adhesion molecule	8	20	630
Platelet-derived growth factor subunit B	15	20	630
Prolactin	15	20	630
Protein S100-A12	4	20	630
Proteinase-activated receptor 1	15	20	630
Proto-oncogene tyrosine-protein kinase Src	15	20	315
P-selectin glycoprotein ligand 1	15	20	630
Receptor for advanced glycosylation end products	15	20	630
Renin	15	20	630
Resistin	15	20	630
SIR2-like protein	0	20	630
Spondin-1	15	20	630
ST2 protein	15	20	630
Stem cell factor	15	20	630
Thrombomodulin	15	20	630
TIM-1	15	20	630
Tissue factor	15	20	630
Tissue-type plasminogen activator	15	20	630
TNF-related activation-induced cytokine	15	20	630
TNF-related apoptosis-inducing ligand	15	20	630
TNF-related apoptosis-inducing ligand receptor 2	15	20	630
Tumor necrosis factor receptor 1	15	20	630
Tumor necrosis factor receptor 2	15	20	630
Tumor necrosis factor receptor superfamily member 5	15	20	630
Tumor necrosis factor receptor superfamily member 6	15	20	630
Tumor necrosis factor ligand superfamily member 14	4	20	630
Urokinase plasminogen activator surface receptor	15	20	630
Vascular endothelial growth factor A	8	20	315
Vascular endothelial growth factor D	15g	20	630

The potential impact of certain known interfering serum and plasma components was evaluated by using serial dilutions of bilirubin, hemolysate, and lipids, respectively in EDTA plasma, as shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. No interference was detected by addition of lipids while 2 assays were observed to be affected by bilirubin and 23 assays out 92 were altered by hemolysate. The latter is probably due to actual analyte leaking out from the disrupted blood cells rather than disturbance of the assay mechanism. Table 2 shows the highest concentrations without impact on assay performance for each component.

A) Hemolysate



B) Lipids



C) Bilirubin

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin, lipids 0.3-20 mg/mL and bilirubin 10-630 µg/mL. The highest hemolysate concentration translates to about 10% hemolysis.

2.5 SCALABILITY

Assay performance was further evaluated with regard to scalability, meaning the capability of the Proseek Multiplex technology to maintain the same quality of performance irrespective of multiplex grade. A step-wise increase of multiplex grade (24, 48, 72 and 96) was performed and the observed dCq values for the 24-plex were plotted against the 48-plex, 72-plex and 96-plex for each analyte. The correlation coefficient R^2 value generated by linear regression analysis reflects the correlation between the multiplex assays. The R^2 values were >0.99 for the different multiplex blocks, as shown in Figure 7, demonstrating the scalability of the system.

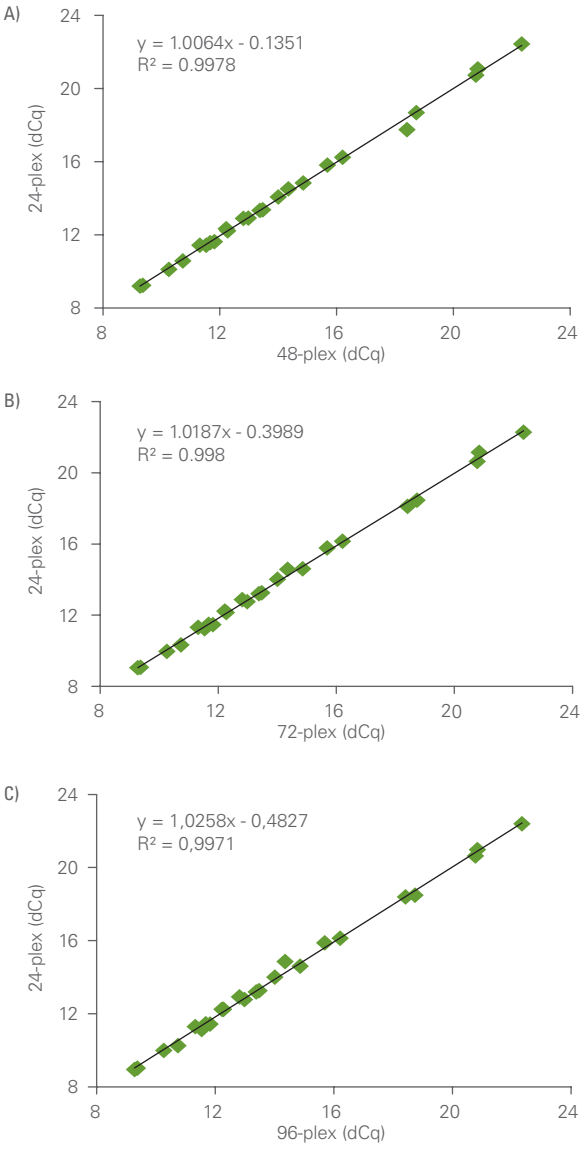


Fig 7. Scalability of the Proseek Mutliplex technology platform. This experiment was performed using the Proseek Multiplex Oncology I $^{96\times96}$ panel. Human serum samples were analyzed with a 24-plex, 48-plex and 72-plex assay and the complete Proseek Mutliplex Oncology I $^{96\times96}$ panel. The observed dCq (log2) values were plotted, and the correlation coefficient R^2 value was generated by linear regression.

3. References

1. Lundberg M, Eriksson A, Tran B, Assarsson E and Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of owabundant proteins in human blood. *Nucleic Acid Res* 6 June (2011). doi: 10.1093/nar/gkr424