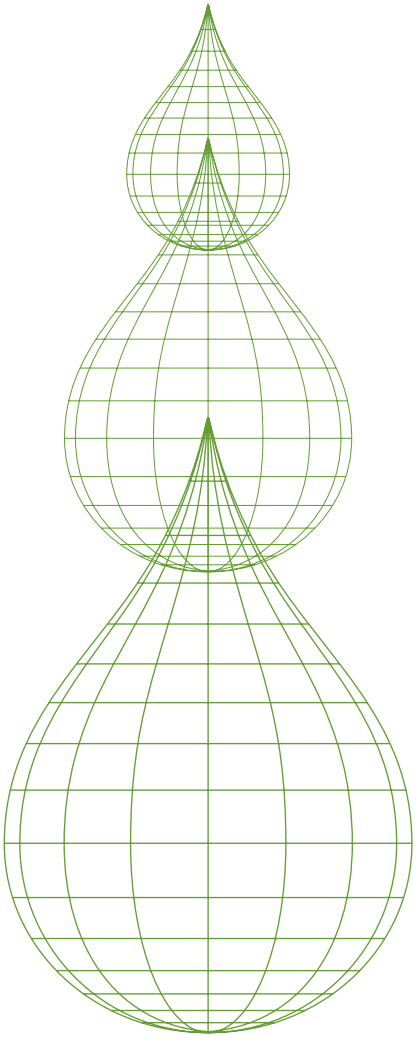
Proseek® Multiplex Oncology I v2 96×96

DATA PACKAGE



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TECHNICAL SUPPORT

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1. Introduction

Proseek® Multiplex Oncology I v2$^{96\times96}$ is a reagent kit measuring 92 oncology-related human protein biomarkers simultaneously in serum or 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[1-2], 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 correction factor determined by Olink. The generated Normalized Protein eXpression (NPX) unit is on a log2 scale where a larger number represents a higher protein level in the sample, typically with the background level at around zero. Linearization of data is performed by the mathematical operation 2^NPX. Statistical analyses, e.g. coefficient of variation (CV) calculations are performed on linearized values.

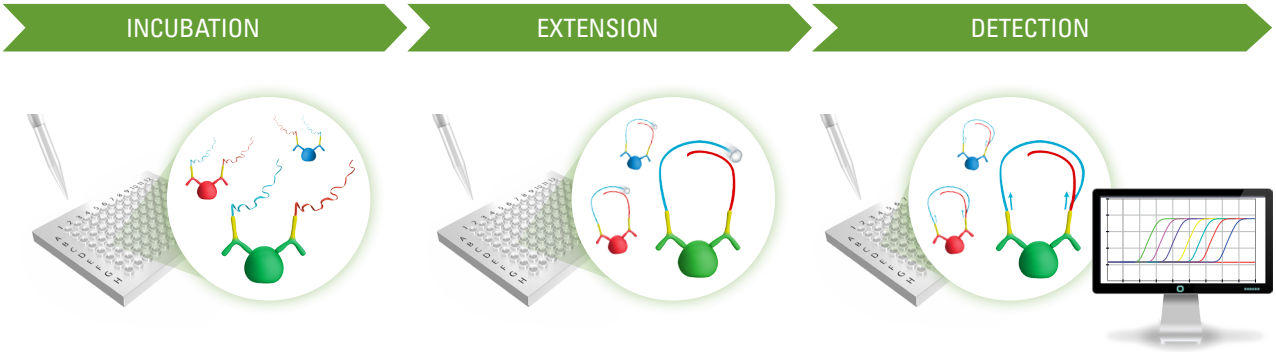

Fig 1. Proseek Multiplex assay procedure employs three core steps: Incubation, Extension and Detection. High throughput real-time qPCR is performed by using the Fluidigm® Biomark™ or Fluidigm® Biomark™ HD systems.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Proseek Multiplex Oncology I v2$^{96\times96}$ by collecting matched serum, ethylenediaminetetraacetic acid (EDTA), acid citrate dextrose (ACD), and sodium 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. Variations observed between responses in heparin and citrate plasma, as compared to EDTA plasma, were generally small, and most of the assays will therefore function without limitation in these sample types. Serum results in a higher signal compared to EDTA plasma for several assays. The results indicate that all plasma types and serum are suitable for the Proseek Multiplex Oncology I v2$^{96\times96}$ panel, although citrate and heparin plasma have not been fully validated.

Tissues from 10 individuals and 8 different lysates were also evaluated on the Proseek Multiplex Oncology I v2$^{96\times96}$ panel. In tissue lysates 69 out of 92 assays were measured above Limit of Detection (LOD, defined as 3 standard deviations above background) and below hook. Similarly, in cell lysates 67 out of 92 assays were measured between LOD and hook. For more information about tissue and cell lysate contact support@olink.com.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Calibrator curves were determined for 90 out of 92 biomarkers simultaneously in a multiplex format. Two protein biomarkers lacked accessible recombinant antigens (CDKN1A and VIM). LOD was reported in pg/mL fo all assays except CA-125 which is in U/mL, see Table 1.

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 can be reported which leads to misinterpretation of results. Therefore, the hook effect was determined for each analyte, here reported in pg/mL fo all assays except CA-125 which is in U/mL, see 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/

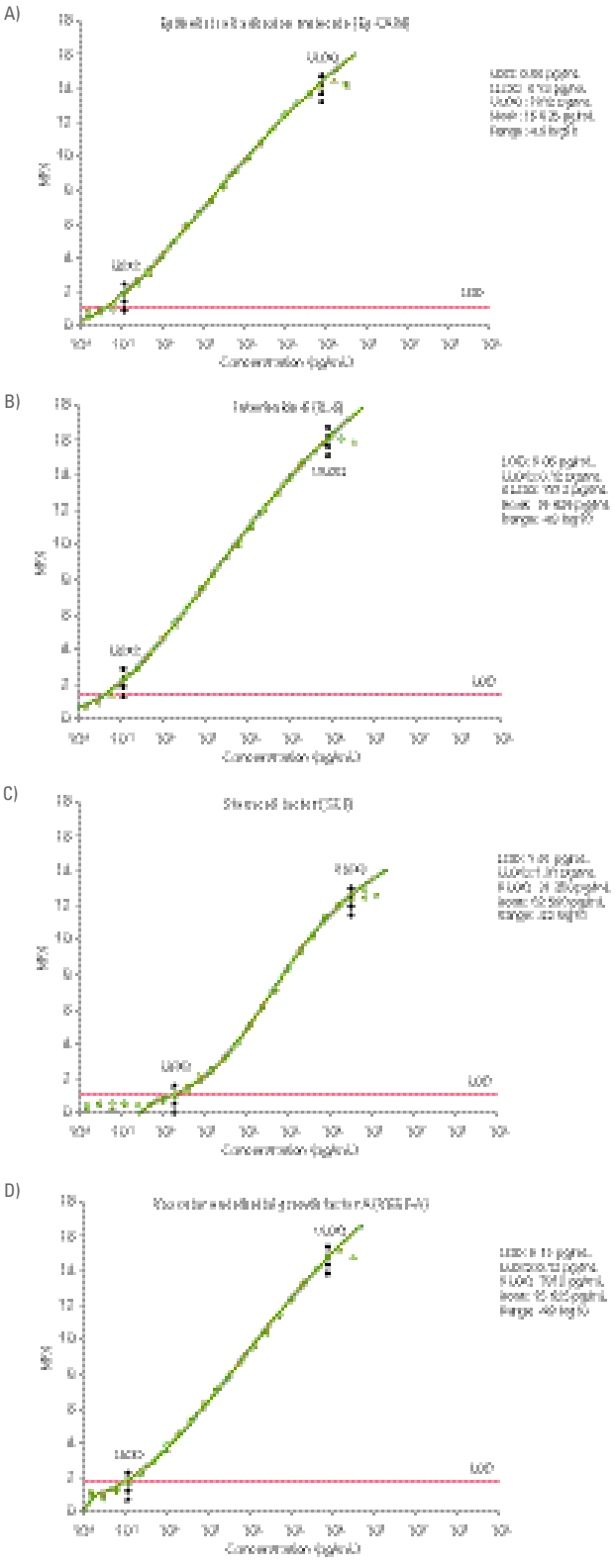

Fig 2. Calibrator curves for 4 representative assays and analytical data using a 4-parameter curve fitting model.

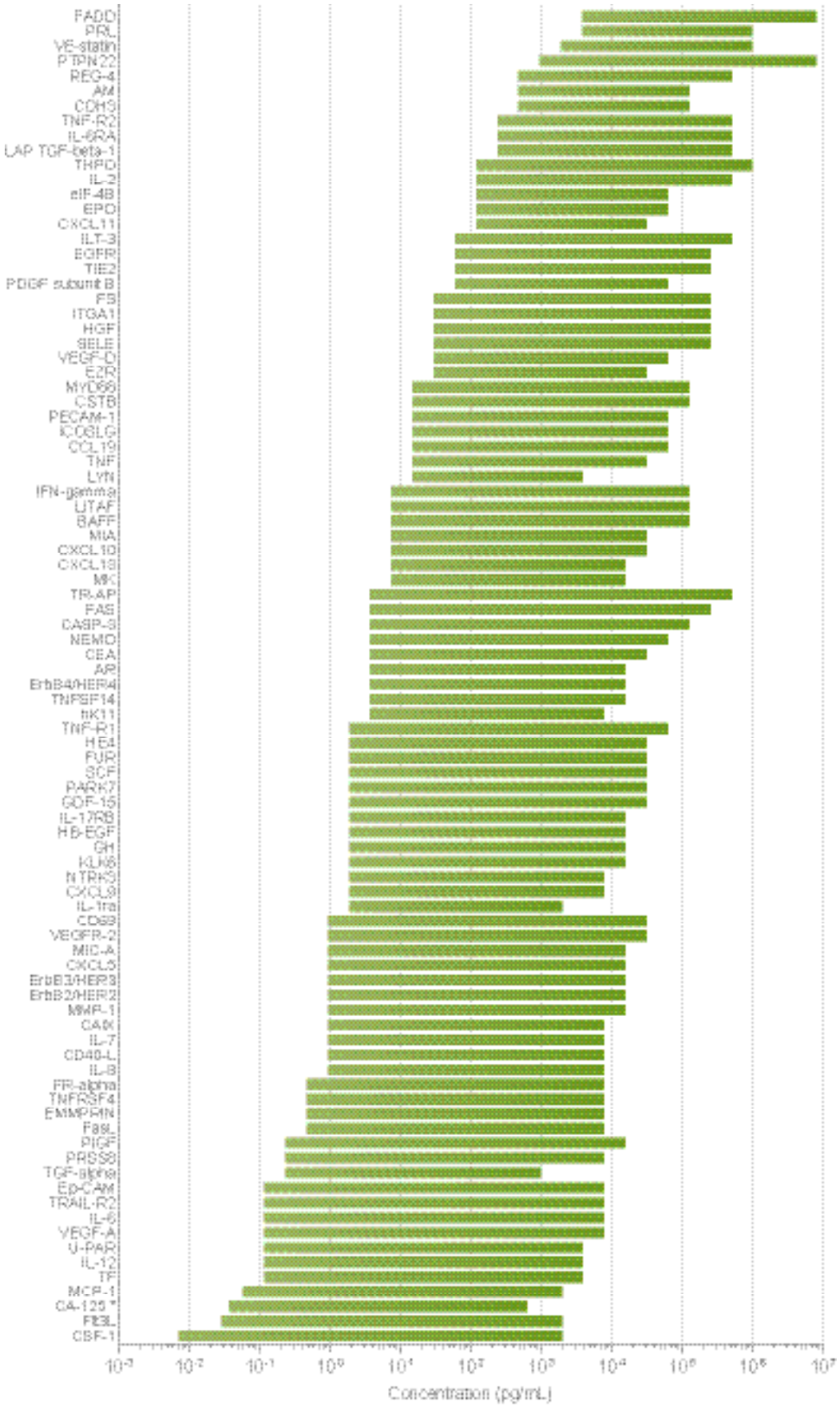

Fig 3. Distribution of analytical measuring range, defined by the limits of quantification LLOQ-ULOQ, for 90 out of 92 analytes.*=U/mL.

Table 1. Sample Types; acid citrate dextrose (ACD), ethylenediaminetetraacetic acid (EDTA), sodium heparin (heparin) and serum, Analytical Measurement; Limit of Detection (LOD), Lower/Upper Limit of Quantification (LLOQ/ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for 92 analytes. *=U/mL, **No antigen.

		Sample types							Analytical measurement					Precision	
		Signal-to-background (2^{NPX})				Relative 2^{NPX} to EDTA			pg/mL				log10		
Target	UniProt No	ACD	EDTA	Heparin	Serum	ACD	Heparin	Serum	LOD	LLOQ	ULOQ	Hook	Range	Intra-assay	Inter-assay
Adrenomedullin (AM)	P35318	78	97	70	53	80%	72%	55%	488	488	125000	1000000	2.4	5%	20%
Amphiregulin (AR)	P15514	4	6	5	8	63%	79%	126%	3.8	3.8	15625	31250	3.6	5%	18%
Angiopoietin-1 receptor (TIE2)	Q02763	27	39	35	41	69%	91%	106%	61	61	250000	1000000	3.6	4%	15%
B-cell activating factor (BAFF)	Q9Y275	24	47	37	55	51%	80%	116%	7.6	7.6	125000	125000	4.2	6%	15%
Cadherin-3 (CDH3)	P22223	4	6	6	7	77%	96%	125%	488	488	125000	500000	2.4	5%	19%
Carbonic anhydrase IX (CAIX)	Q16790	2	4	3	4	57%	85%	112%	0.95	0.95	7812	62500	3.9	4%	19%
Carcinoembryonic antigen (CEA)	P06731	1	2	2	2	52%	75%	101%	3.8	3.8	31250	250000	3.9	5%	22%
Caspase-3 (CASP-3)	P42574	149	259	153	121	58%	59%	47%	3.8	3.8	125000	125000	4.5	5%	15%
C-C motif chemokine 19 (CCL19)	Q99731	723	824	654	937	88%	79%	114%	15	15	62500	62500	3.6	5%	27%
CD40 ligand (CD40-L)	P29965	11	38	87	346	29%	232%	920%	0.95	0.95	7812	15625	3.9	5%	19%
C-X-C motif chemokine 5 (CXCL5)	P42830	129	831	1359	2453	16%	163%	295%	0.95	0.95	15625	15625	4.2	5%	19%
C-X-C motif chemokine 9 (CXCL9)	Q07325	49	58	50	60	85%	87%	103%	0.95	1.9	7812	15625	3.6	5%	18%
C-X-C motif chemokine 10 (CXCL10)	P02778	130	143	108	198	91%	75%	138%	7.6	7.6	31250	62500	3.6	6%	17%
C-X-C motif chemokine 11 (CXCL11)	O14625	6	20	37	49	29%	185%	247%	122	122	31250	31250	2.4	5%	18%
C-X-C motif chemokine 13 (CXCL13)	O43927	312	366	259	512	85%	71%	140%	7.6	7.6	15625	15625	3.3	3%	19%
Cyclin-dependent kinase inhibitor 1 (CDKN1A) *	P38936	1	2	1	1	61%	52%	70%						4%	19%
Cystatin-B (CSTB)	P04080	29	55	40	57	52%	73%	103%	15	15	125000	250000	3.9	4%	16%
Early activation antigen CD69 (CD69)	Q07108	136	83	174	224	164%	210%	270%	0.95	0.95	31250	250000	4.5	5%	18%
Epidermal growth factor receptor (EGFR)	P00533	45	55	46	58	81%	84%	105%	61	61	250000	250000	3.6	5%	13%
Epididymal secretory protein E4 (HE4)	Q14508	81	101	96	117	80%	95%	116%	1.9	1.9	31250	250000	4.2	4%	20%
Epithelial cell adhesion molecule (Ep-CAM)	P16422	748	851	783	1060	88%	92%	125%	0.06	0.12	7812	15625	4.8	5%	21%
Erythropoietin (EPO)	P01588	1	2	1	2	68%	84%	110%	122	122	62500	125000	2.7	4%	17%
E-selectin (SELE)	P16581	106	158	127	159	67%	80%	101%	15	31	250000	250000	3.9	6%	14%
Eukaryotic translation initiation factor 4B (eIF-4B)	P23588	6	49	6	10	13%	12%	21%	122	122	62500	250000	2.7	5%	21%
Extracellular matrix metalloproteinase inducer (EMMPRIN)	P35613	82	90	98	100	91%	109%	111%	0.48	0.48	7812	15625	4.2	4%	19%
Ezrin (EZR)	P15311	19	32	28	32	59%	88%	100%	15	31	31250	125000	3.0	3%	21%
Fas antigen ligand (FasL)	P48023	4	4	3	4	98%	82%	100%	0.48	0.48	7812	15625	4.2	5%	14%
FAS-associated death domain protein (FADD)	Q13158	2	3	3	4	61%	101%	142%	1953	3906	8000000	8000000	3.3	5%	27%
Fms-related tyrosine kinase 3 ligand (Flt3L)	P49771	131	193	161	213	68%	84%	111%	0.03	0.03	1953	3906	4.8	5%	17%
Folate receptor alpha (FR-alpha)	P15328	18	26	22	29	69%	83%	110%	0.48	0.48	7812	31250	4.2	4%	21%
Follistatin (FS)	P19883	49	69	16	76	71%	24%	111%	31	31	250000	250000	3.9	5%	18%
Furin (FUR)	P09958	111	130	121	197	85%	93%	151%	1.9	1.9	31250	250000	4.2	5%	18%
Growth hormone (GH)	P01241	48	73	66	83	66%	90%	113%	1.9	1.9	15625	15625	3.9	4%	20%
Growth/differentiation factor 15 (GDF-15)	Q99988	137	185	146	166	74%	79%	90%	1.9	1.9	31250	62500	4.2	6%	17%
Heparin-binding EGF-like growth factor (HB-EGF)	Q99075	57	131	58	345	44%	44%	263%	1.9	1.9	15625	31250	3.9	5%	19%
Hepatocyte growth factor (HGF)	P14210	56	91	54	147	61%	59%	160%	7.6	31	250000	250000	3.9	5%	18%
ICOS ligand (ICOSLG)	O75144	9	12	14	19	76%	112%	156%	15	15	62500	125000	3.6	4%	22%
Immunoglobulin-like transcript 3 (ILT-3)	Q8NHJ6	3	5	4	4	66%	81%	74%	61	61	500000	1000000	3.9	4%	21%
Integrin alpha-1 (ITGA1)	P56199	83	113	84	119	73%	74%	105%	3.8	31	250000	1000000	3.9	4%	20%
Interferon gamma (IFN-gamma)	P01579	1	2	1	2	64%	79%	101%	3.8	7.6	125000	125000	4.2	5%	22%
Interleukin-1 receptor antagonist protein (IL-1ra)	P18510	12	15	16	27	79%	102%	176%	1.9	1.9	1953	7812	3.0	5%	22%
Interleukin-2 (IL-2)	P60568	1	1	1	1	62%	74%	106%	122	122	500000	2000000	3.6	9%	27%
Interleukin-6 (IL-6)	P05231	26	33	27	37	81%	83%	114%	0.06	0.12	7812	15625	4.8	5%	18%
Interleukin-7 (IL-7)	P13232	1	4	3	8	40%	72%	227%	0.95	0.95	7812	15625	3.9	5%	21%
Interleukin-8 (IL-8)	P10145	13	25	26	51	51%	104%	201%	0.95	0.95	7812	7812	3.9	5%	17%
Interleukin-12 (IL-12)	P29460; P29459	86	98	82	112	88%	84%	114%	0.12	0.12	3906	7812	4.5	5%	18%

		Sample types							Analytical measurement					Precision	
		Signal-to-background (2^{NPX})				Relative 2^{NPX} to EDTA			pg/mL				log10		
Target	UniProt No	ACD	EDTA	Heparin	Serum	ACD	Heparin	Serum	LOD	LLOQ	ULOQ	Hook	Range	Intra-assay	Inter-assay
Interleukin-17 receptor B (IL-17RB)	Q9NRM6	1	2	2	3	59%	89%	112%	1.9	1.9	15625	62500	3.9	4%	20%
Interleukin-6 receptor subunit alpha (IL-6RA)	P08887	44	59	49	63	74%	83%	108%	244	244	500000	500000	3.3	5%	14%
Kallikrein-11 (hK11)	Q9UBX7	19	23	22	26	83%	98%	114%	3.8	3.8	7812	15625	3.3	5%	18%
Kallikrein-6 (KLK6)	Q92876	18	28	24	31	64%	88%	112%	1.9	1.9	15625	31250	3.9	5%	16%
Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1)	P01137	30	71	80	149	42%	112%	210%	122	244	500000	500000	3.3	4%	22%
Lipopolysaccharide-induced tumor necrosis factor-alpha factor (LITAF)	Q99732	2	2	1	3	71%	67%	125%	7.6	7.6	125000	125000	4.2	4%	18%
Macrophage colony-stimulating factor 1 (CSF-1)	P09603	248	279	273	325	89%	98%	117%	0.01	0.01	1953	7812	5.4	5%	16%
Matrix metalloproteinase-1 (MMP-1)	P03956	18	110	58	121	16%	53%	109%	0.95	0.95	15625	31250	4.2	5%	36%
Melanoma-derived growth regulatory protein (MIA)	Q16674	23	32	21	31	73%	67%	98%	7.6	7.6	31250	62500	3.6	5%	15%
MHC class I polypeptide-related sequence A (MIC-A)	Q29983	2	3	3	4	55%	78%	116%	0.95	0.95	15625	125000	4.2	4%	23%
Midkine (MK)	P21741	57	130	55	78	44%	42%	60%	7.6	7.6	15625	125000	3.3	5%	20%
Monocyte chemotactic protein 1 (MCP-1)	P13500	642	609	534	787	105%	88%	129%	0.06	0.06	1953	3906	4.5	5%	17%
Myeloid differentiation primary response protein MyD88 (MYD88)	Q99836	1	1	1	1	50%	73%	106%	15	15	125000	250000	3.9	4%	23%
NF-kappa-B essential modulator (NEMO)	Q9Y6K9	7	13	8	14	55%	60%	109%	3.8	3.8	62500	250000	4.2	5%	25%
NT-3 growth factor receptor (NTRK3)	Q16288	72	108	92	117	67%	86%	109%	0.95	1.9	7812	31250	3.6	5%	23%
Ovarian cancer-related tumor marker CA 125 (CA-125) **	Q8WXI7	6	10	7	11	58%	78%	115%	0.04	0.04	625	1250	4.2	5%	22%
Parkinson disease protein 7 (PARK7)	Q99497	107	188	100	141	57%	53%	75%	1.9	1.9	31250	250000	4.2	4%	18%
Placenta growth factor (PlGF)	P49763	50	74	61	82	68%	83%	112%	0.24	0.24	15625	15625	4.8	5%	18%
Platelet endothelial cell adhesion molecule (PECAM-1)	P16284	52	70	60	75	75%	87%	108%	15	15	62500	125000	3.6	4%	17%
Platelet-derived growth factor subunit B (PDGF subunit B)	P01127	49	395	269	911	12%	68%	230%	7.6	61	62500	125000	3.0	5%	17%
Prolactin (PRL)	P01236	12	19	16	21	61%	82%	107%	1953	3906	1000000	2000000	2.4	4%	22%
Prostasin (PRSS8)	Q16651	287	304	277	358	94%	91%	118%	0.24	0.24	7812	62500	4.5	5%	19%
Receptor tyrosine-protein kinase erbB-2 (ErbB2/HER2)	P04626	337	349	329	395	97%	94%	113%	0.48	0.95	15625	31250	4.2	5%	21%
Receptor tyrosine-protein kinase erbB-3 (ErbB3/HER3)	P21860	279	327	282	375	85%	86%	115%	0.95	0.95	15625	62500	4.2	4%	20%
Receptor tyrosine-protein kinase erbB-4 (ErbB4/HER4)	Q15303	27	39	35	44	70%	90%	114%	3.8	3.8	15625	31250	3.6	4%	23%
Regenerating islet-derived protein 4 (REG-4)	Q9BYZ8	10	14	10	15	69%	73%	103%	488	488	500000	500000	3.0	5%	17%
Stem cell factor (SCF)	P21583	232	241	226	274	96%	94%	114%	1.9	1.9	31250	62500	4.2	5%	20%
Tartrate-resistant acid phosphatase type 5 (TR-AP)	P13686	23	41	28	43	56%	67%	104%	3.8	3.8	500000	500000	5.1	6%	15%
Thrombopoietin (THPO)	P40225	2	4	4	8	45%	90%	178%	122	122	1000000	1000000	3.9	5%	27%
Tissue factor (TF)	P13726	20	30	27	30	67%	89%	100%	0.12	0.12	3906	15625	4.5	5%	21%
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	O14763	6	10	9	11	68%	90%	113%	0.12	0.12	7812	15625	4.8	5%	15%
Transforming growth factor alpha (TGF-alpha)	P01135	3	5	4	17	66%	87%	362%	0.24	0.24	977	7812	3.6	5%	17%
Tumor necrosis factor (TNF)	P01375	1	2	1	2	68%	73%	106%	1.9	15	31250	31250	3.3	5%	19%
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	O43557	2	2	2	8	64%	100%	324%	3.8	3.8	15625	31250	3.6	5%	21%
Tumor necrosis factor receptor 1 (TNF-R1)	P19438	1949	2071	1980	2858	94%	96%	138%	0.95	1.9	62500	250000	4.5	6%	19%
Tumor necrosis factor receptor 2 (TNF-R2)	P20333	18	26	22	30	70%	83%	115%	244	244	500000	1000000	3.3	6%	15%
Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)	P43489	5	7	6	8	63%	88%	115%	0.48	0.48	7812	15625	4.2	4%	22%
Tumor necrosis factor receptor superfamily member 6 (FAS)	P25445	121	150	130	167	81%	87%	111%	3.8	3.8	250000	250000	4.8	5%	15%
Tyrosine-protein kinase Lyn (LYN)	P07948	1	2	1	2	59%	46%	70%	7.6	15	3906	7812	2.4	4%	20%
Tyrosine-protein phosphatase non-receptor type 22 (PTPN22)	Q9Y2R2	2	3	3	6	60%	107%	232%	977	977	8000000	8000000	3.9	4%	20%
Urokinase plasminogen activator surface receptor (U-PAR)	Q03405	451	526	502	704	86%	96%	134%	0.12	0.12	3906	15625	4.5	4%	17%
Vascular endothelial growth factor A (VEGF-A)	P15692	520	740	614	1180	70%	83%	159%	0.12	0.12	7812	15625	4.8	5%	18%
Vascular endothelial growth factor D (VEGF-D)	O43915	51	61	64	70	84%	106%	115%	31	31	62500	250000	3.3	5%	21%
Vascular endothelial growth factor receptor 2 (VEGFR-2)	P35968	108	140	129	156	77%	92%	111%	0.95	0.95	31250	62500	4.5	4%	22%
Vascular endothelial statin (VE-statin)	Q9UHF1	1	1	1	1	77%	63%	101%	1953	1953	1000000	1000000	2.7	4%	19%
Vimentin (VIM) *	P08670	2	4	10	16	45%	296%	463%						4%	21%

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 Table 1.

Representative assays with their analytical data are exemplified in Figure 2 and the distribution of measuring ranges of 90 assays is shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com/products/proseek-multiplex/proseek-multiplex-oncology-i-v2.

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean CV for 6 individual samples run in triplicates, within each of 9 separate runs during the validation studies. Inter-assay variation (between-runs) was calculated as the mean CV, for the same 6 individual samples. Variation calculations were performed on linearized values for all 92 analytes, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 5% and 19%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

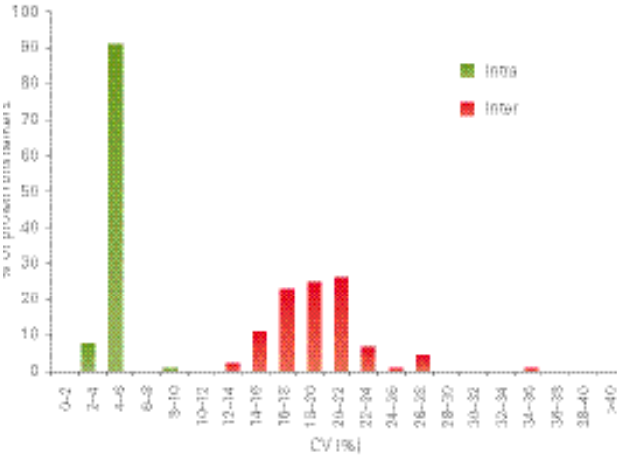

Fig 4. Distribution of intra-assay and inter-assay variations of Proseek Multiplex Oncology I v2$^{96\times96}$.

REPRODUCIBILITY

Inter-site variations (between-site) were investigated during the validation of previous panels (Proseek Multiplex CVD I$^{96\times96}$ and Proseek Multiplex Inflammation I$^{96\times96}$) in β-site studies to estimate the expected variations in values between different laboratories, with different operators and using different equipment. The β-site studies showed similar results and were therefore not performed for Proseek Multiplex Oncology I v2$^{96\times96}$.

The β-site studies followed the same protocol. Each site was instructed to perform the analysis of the 7 individual samples (distributed by Olink Biosciece) according to the same run design. Each site performed two independent runs.

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

Overall, both Proseek Multiplex Inflammation I$^{96\times96}$ and Proseek Multiplex CVD I$^{96\times96}$ showed very good reproducibility and repeatability. Here the result is shown for Proseek Multiplex Inflammation I$^{96\times96}$. For detailed information about Proseek Multiplex CVD I$^{96\times96}$, please download corresponding data package: www.olink.com/products/proseek-multiplex/downloads/data-packages.

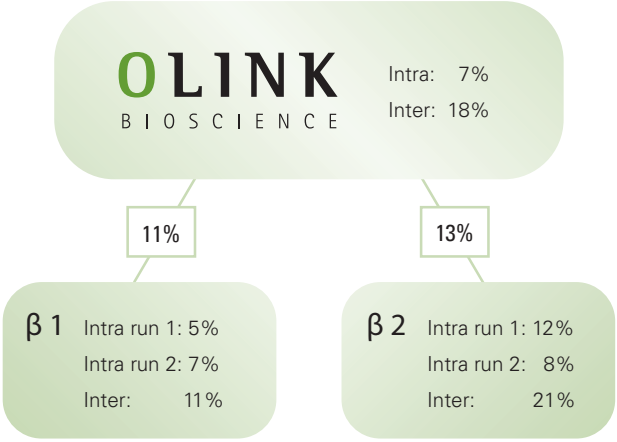

Fig 5. Validation of the Proseek Multiplex Inflammation I $^{96\times96}$ at 2 (β1-β2) different laboratories. Larger boxes show intra-assay and inter-assay variations for each site and small boxes represent the inter-site run variations in direct comparison to Olink Bioscience.

2.4 ANALYTICAL SPECIFICITY

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are known to cause problems in immunoassays. Evaluation of the potential impact of this specific interference has been performed previously (see www.olink.com/products/proseek-multiplex/downloads/data-packages) using a special "mismatch" system. The only way to generate a signal in this system 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. No interference interpreted as signal above LOD due to HAMA or RF disturbances could be detected for any of the samples in any of the previously tested panels, indicating sufficient blocking of these agents (data not shown).

Table 2. Performance characteristics. Endogenous interference was performed by addition of hemolysate (Hemo), lipids and bilirubin (Bili) in EDTA plasma matrix. Reported are the highest tested concentrations without impact on assay performance in either serum or EDTA plasma.

	Endogenous interference				Endogenous interference		
	mg/mL		µg/mL		mg/mL		µg/mL
Targets 1-46	Hemo	Lipids	Bili	Targets 47-92	Hemo	Lipids	Bili
Adrenomedullin (AM)	3.8	10	630	Interleukin-12 (IL-12)	15	20	630
Amphiregulin (AR)	15	20	630	Interleukin-17 receptor B (IL-17RB)	15	20	630
Angiopoietin-1 receptor (TIE2)	15	20	630	Kallikrein-11 (hK11)	15	20	630
B-cell activating factor (BAFF)	15	20	158	Kallikrein-6 (KLK6)	15	20	315
Cadherin-3 (CDH3)	15	20	630	Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1)	15	20	630
Carbonic anhydrase IX (CAIX)	15	20	630	Lipopolysaccharide-induced tumor necrosis factor-alpha factor (LITAF)	15	20	158
Carcinoembryonic antigen (CEA)	15	20	315	Macrophage colony-stimulating factor 1 (CSF-1)	15	20	630
Caspase-3 (CASP-3)	15	20	630	Matrix metalloproteinase-1 (MMP-1)	15	20	630
C-C motif chemokine 19 (CCL19)	15	20	158	Melanoma-derived growth regulatory protein (MIA)	15	20	630
CD40 ligand (CD40-L)	7.5	10	630	MHC class I polypeptide-related sequence A (MIC-A)	3.8	10	630
C-X-C motif chemokine 5 (CXCL5)	15	20	158	Midkine (MK)	15	20	630
C-X-C motif chemokine 9 (CXCL9)	15	20	315	Monocyte chemotactic protein 1 (MCP-1)	15	20	630
C-X-C motif chemokine 10 (CXCL10)	7.5	20	315	Myeloid differentiation primary response protein MyD88 (MYD88)	15	20	630
C-X-C motif chemokine 11 (CXCL11)	15	20	79	NF-kappa-B essential modulator (NEMO)	15	10	630
C-X-C motif chemokine 13 (CXCL13)	15	20	630	NT-3 growth factor receptor (NTRK3)	15	20	630
Cyclin-dependent kinase inhibitor 1 (CDKN1A)	15	20	630	Ovarian cancer-related tumor marker CA 125 (CA-125)	7.5	10	315
Cystatin-B (CSTB)	15	20	630	Parkinson disease protein 7 (PARK7)	15	20	630
Early activation antigen CD69 (CD69)	15	20	630	Placenta growth factor (PlGF)	15	20	630
Epidermal growth factor receptor (EGFR)	15	20	630	Platelet endothelial cell adhesion molecule (PECAM-1)	15	20	630
Epididymal secretory protein E4 (HE4)	15	20	630	Platelet-derived growth factor subunit B (PDGF subunit B)	15	20	630
Epithelial cell adhesion molecule (Ep-CAM)	15	20	158	Prolactin (PRL)	15	20	315
Erythropoietin (EPO)	7.5	10	158	Prostasin (PRSS8)	15	20	630
E-selectin (SELE)	7.5	10	630	Receptor tyrosine-protein kinase erbB-2 (ErbB2/HER2)	15	20	630
Eukaryotic translation initiation factor 4B (eIF-4B)	15	20	158	Receptor tyrosine-protein kinase erbB-3 (ErbB3/HER3)	15	20	630
Extracellular matrix metalloproteinase inducer (EMMPRIN)	15	20	630	Receptor tyrosine-protein kinase erbB-4 (ErbB4/HER4)	15	20	630
Ezrin (EZR)	15	20	630	Regenerating islet-derived protein 4 (REG-4)	15	20	630
Fas antigen ligand (FasL)	15	20	630	Stem cell factor (SCF)	15	20	630
FAS-associated death domain protein (FADD)	15	20	630	Tartrate-resistant acid phosphatase type 5 (TR-AP)	7.5	10	630
Fms-related tyrosine kinase 3 ligand (Flt3L)	15	20	630	Thrombopoietin (THPO)	15	20	630
Folate receptor alpha (FR-alpha)	15	20	630	Tissue factor (TF)	15	20	630
Follistatin (FS)	15	20	630	TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	15	20	630
Furin (FUR)	15	20	315	Transforming growth factor alpha (TGF-alpha)	15	20	79
Growth hormone (GH)	3.8	5	158	Tumor necrosis factor (TNF)	15	20	630
Growth/differentiation factor 15 (GDF-15)	15	20	630	Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	15	20	315
Heparin-binding EGF-like growth factor (HB-EGF)	15	20	158	Tumor necrosis factor receptor 1 (TNF-R1)	15	20	630
Hepatocyte growth factor (HGF)	15	20	158	Tumor necrosis factor receptor 2 (TNF-R2)	15	20	630
ICOS ligand (ICOSLG)	15	20	630	Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)	15	20	630
Immunoglobulin-like transcript 3 (ILT-3)	7.5	20	630	Tumor necrosis factor receptor superfamily member 6 (FAS)	15	20	630
Integrin alpha-1 (ITGA1)	15	20	630	Tyrosine-protein kinase Lyn (LYN)	15	20	630
Interferon gamma (IFN-gamma)	3.8	10	315	Tyrosine-protein phosphatase non-receptor type 22 (PTPN22)	7.5	10	315
Interleukin-1 receptor antagonist protein (IL-1ra)	15	20	158	Urokinase plasminogen activator surface receptor (U-PAR)	15	20	79
Interleukin-2 (IL-2)	15	20	630	Vascular endothelial growth factor A (VEGF-A)	15	20	630
Interleukin-6 (IL-6)	15	20	79	Vascular endothelial growth factor D (VEGF-D)	15	20	630
Interleukin-6 receptor subunit alpha (IL-6RA)	15	20	630	Vascular endothelial growth factor receptor 2 (VEGFR-2)	15	20	630
Interleukin-7 (IL-7)	15	20	630	Vascular endothelial statin (VE-statin)	15	20	630
Interleukin-8 (IL-8)	15	20	79	Vimentin (VIM)	15	20	630

The potential impact of certain known interfering serum and components was evaluated by using serial dilutions of hemolysate, lipids and bilirubin in EDTA plasma and serum, as shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. Table 2 shows the highest concentration of each interfering substance without impact on assay performance. In 12 out of 92 assays altered expression was observed by the addition of hemolysate. The reason is most likely due to actual analyte leaking out of the disrupted blood cells. A concentration of 15 g/L of hemolysate represents 10% hemolysis of a sample. In 91 out of 92 assays lipids could be added to a high concentration (≥10 mg/mL) without affecting assay performance. In only 1 assay interference was observed by addition of lipids ≥5 mg/mL. This relatively low concentration corresponds to very high serum triglyceride levels[3]. 66 out of 92 assays were unaffected by addition of extremely high levels of bilirubin, ≥630 µg/mL. Addition of bilirubin at ≥79 µg/mL altered only 5 out of 92 assays, which is more than 4 times the normal total bilirubin levels[4].

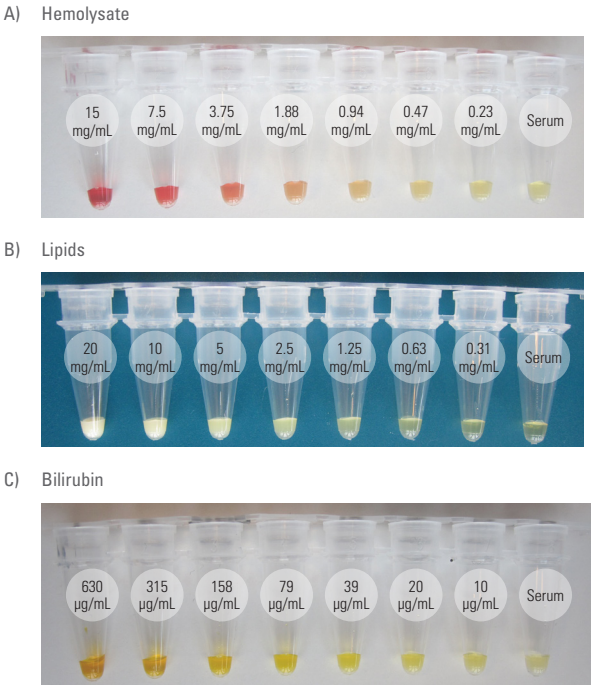

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23 - 15 mg/mL hemoglobin, lipids 0.3 - 20 mg/mL and bilirubin 10 - 630 µg/mL. The highest hemolysate concentration translates to about 10% hemolysis.

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 level. 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 square of the correlation coefficient R^2 value generated by linear regression

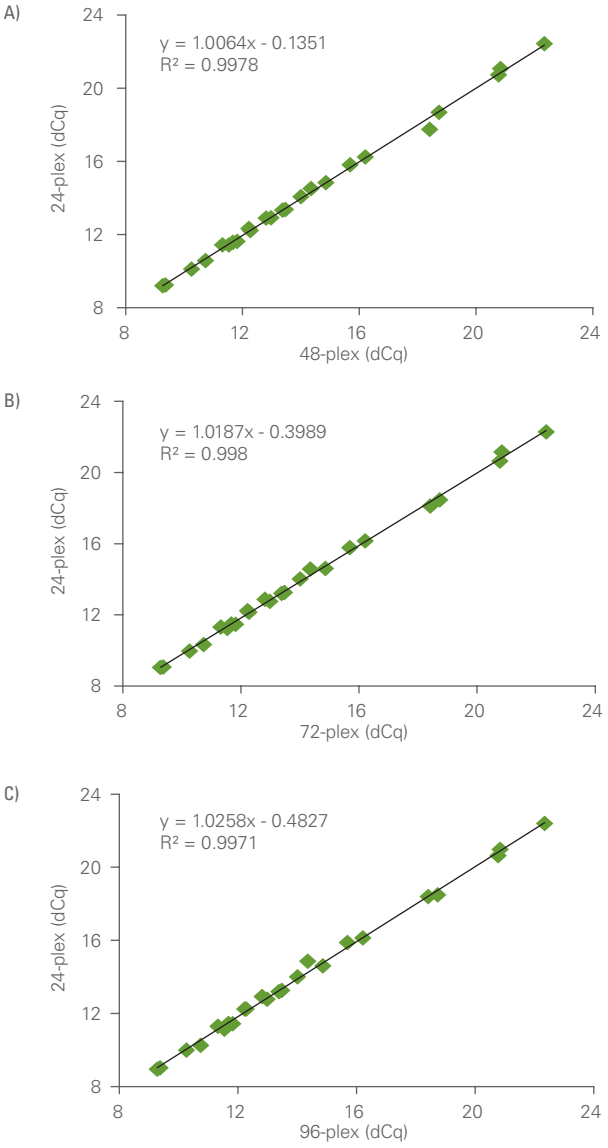

Fig 7. Scalability of the Proseek Multiplex technology platform. This experiment was performed using the Proseek Multiplex Oncology I $^{96\times96}$ panel. Human serum samples were analyzed with a 24-plex, 48-plex and 72-plex assay and the complete Proseek Mutliplex Oncology I $^{96\times96}$ panel.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

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.

3. References

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