VALIDATION DATA

# 1. Introduction

Olink® Organ Damage is a reagent kit measuring 92 immune response related human protein biomarkers simultaneously. The analytical performance of the product has been carefully validated and the results are presented below.

## 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 respective target protein present in the sample. A PCR reporter sequence is formed by a proximity dependent DNA polymerization event, amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format without any need for washing steps, see Figure 1.

## 1.2 QUALITY CONTROLS

Internal and external controls have been developed by Olink for data normalization and quality control purposes. These controls have been designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, providing information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include two Immunoassay controls, one Extension control and one Detection control. The Immunoassay controls (two non-human proteins) monitor all three steps starting with the immunoreaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity independent of antigen binding) monitors the extension and readout steps and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) monitors the readout step. Samples for which one or more of the internal control values deviate from a pre-determined range will be flagged and may be removed before statistical analysis.

An external control, inter-plate control (IPC), is included on each plate and used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. Furthermore, the improves inter-assay precision and allows for optimal comparison of data derived from multiple runs. The term "Normalized Protein eXpression (NPX)" refers to normalized data as described above.

## 1.3 DATA ANALYSIS

Data analysis was performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control was substracted, thus normalizing for technical variation within one run. Normalization between runs is 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 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. Coefficient of variation (CV) calculations were performed on linearized values.

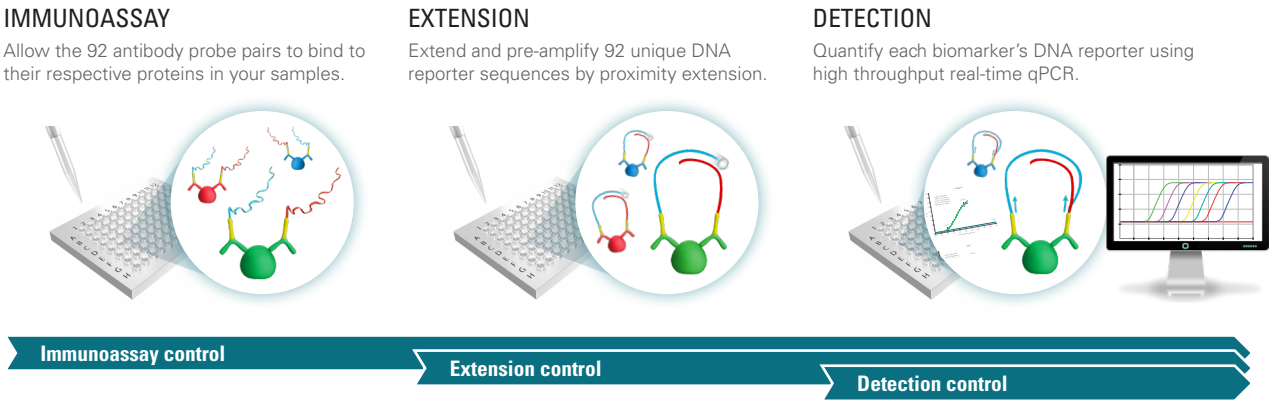


Fig 1. Olink assay procedure (above) and controls (below). The internal controls enables monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

# 2. Performance characteristics

## 2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Organ Damage by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Table 1 summarizes response values for 22 normal EDTA plasma samples expressed in NPX, as well as relative differences as compared to EDTA plasma. Variations observed between responses in heparin, citrate plasma and serum, as compared to EDTA plasma, were generally small, and all assays will therefore function without limitation in these sample types.

## 2.2 ANALYTICAL MEASUREMENT

### DETECTION LIMIT

Calibrator curves were determined for 90 out of 92 biomarkers simultaneously in a multiplex format. Two protein biomarkers (RASA1 and ATP6AP2) lacked accessible recombinant antigen. Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL for all assays where recombinant protein antigen was available, 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. In such cases, 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 for 91 out of 92 assays, 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. 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 assays with their analytical data are shown in Figure 2 and the distribution of measuring ranges of 90 assays and endogenous plasma levels are shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com.

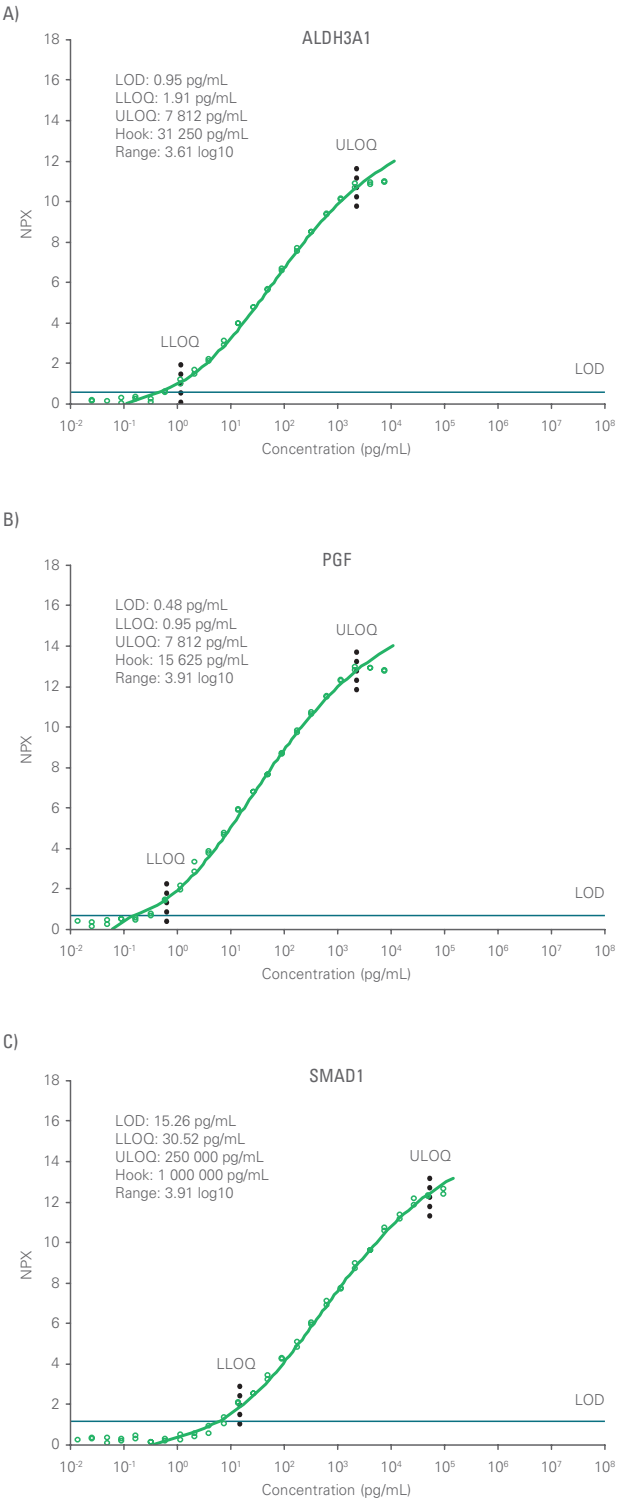


Fig 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

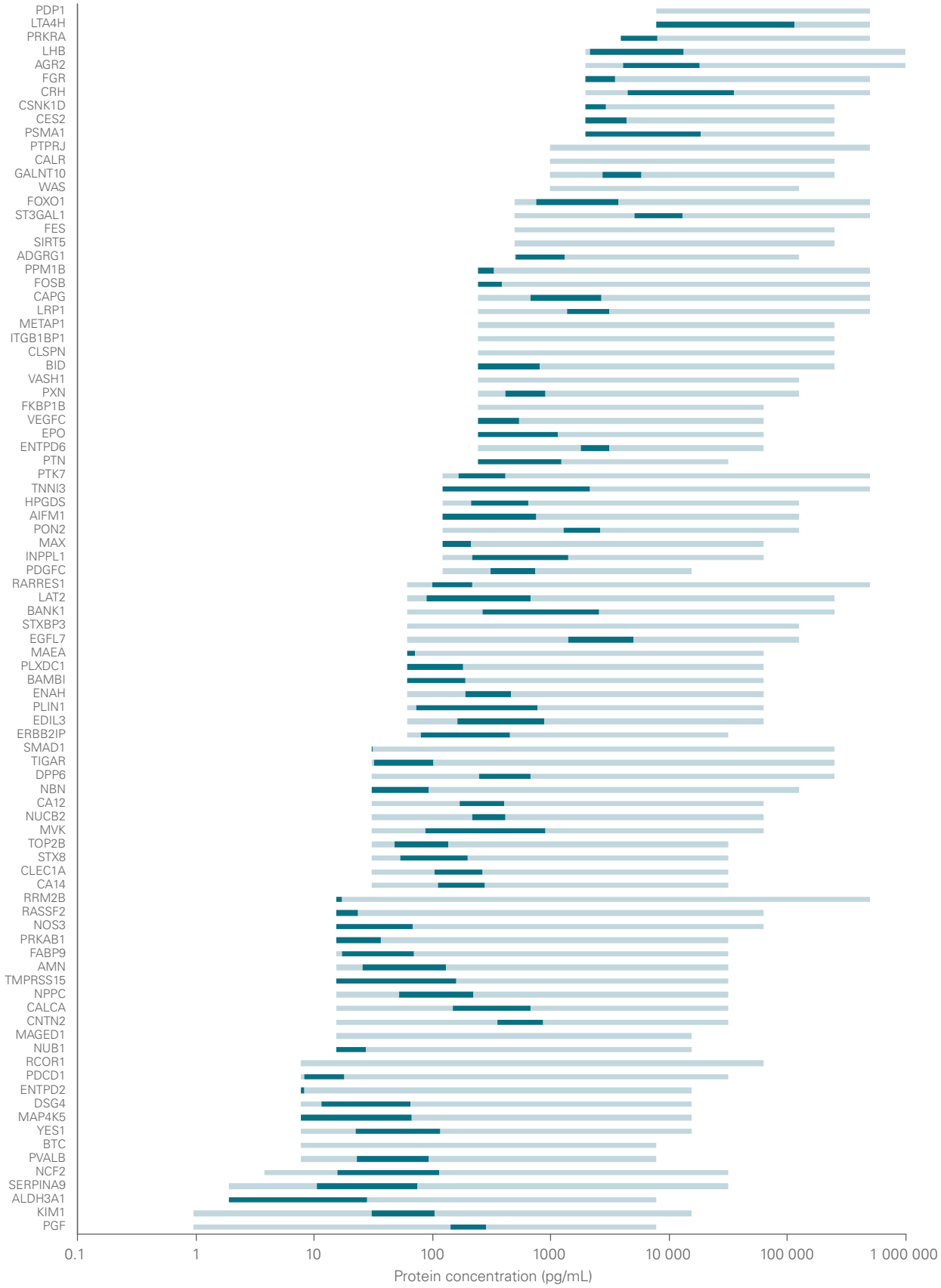


**Fig 3.** Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ). Normal plasma levels (dark green bars) are denoted for 90 out of 92 analytes.

**Table 1.** Sample Types; Normalized Protein eXpression (NPX), Endogenous Interference, Analytical Measurement; Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for 92 analytes. Not available, NA

| | | Sample types | | | | | | Endogenous Interference | Analytical measurement | | | | 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 | Hemolysate | LOD | LLOQ | ULOQ | Hook | Range | Intra | Inter |
| 5'-AMP-activated protein kinase subunit beta-1 (PRKAB1) | Q9Y478 | 0.4 | 0.8 | 1.7 | 103 | 100 | 102 | 15 | 15 | 15 | 31 250 | 1 000 000 | 3.3 | 5 | 16 |
| Adhesion G-protein coupled receptor G1 (ADGRG1) | Q9Y653 | 1.3 | 1.7 | 2.5 | 132 | 99 | 115 | 15 | 244 | 488 | 124 999 | 250 000 | 2.4 | 5 | 25 |
| Aldehyde dehydrogenase, dimeric NADP-preferring (ALDH3A1) | P30838 | 0.5 | 1.9 | 3.6 | 111 | 95 | 111 | 15 | 0.95 | 1.91 | 7812 | 31 250 | 3.6 | 4 | 18 |
| Anterior gradient protein 2 homolog (AGR2) | O95994 | 1.6 | 2.3 | 3.8 | 124 | 101 | 117 | 15 | 976 | 1953 | 1 000 000 | 1 000 000 | 2.7 | 6 | 26 |
| Apoptosis-inducing factor 1, mitochondrial (AIFM1) | O95831 | NA | 1.2 | 2.7 | 99 | 89 | 90 | 15 | 122 | 122 | 124 999 | 1 000 000 | 3.0 | 6 | 21 |
| B-cell scaffold protein with ankyrin repeats (BANK1) | Q8NDB2 | 2.5 | 4.2 | 6.0 | 34 | 108 | 34 | 15 | 61 | 61 | 250 000 | 499 999 | 3.6 | 6 | 22 |
| BH3-interacting domain death agonist (BID) | P55957 | NA | 1.0 | 1.6 | NA | NA | NA | 0.5 | 244 | 244 | 250 000 | 1 000 000 | 3.0 | 5 | 26 |
| BMP and activin membrane-bound inhibitor homolog (BAMBI) | Q13145 | 0.8 | 1.4 | 1.8 | 132 | 101 | 128 | 15 | 31 | 61 | 62 499 | 124 999 | 3.0 | 5 | 25 |
| Calcitonin (CALCA) | P01258 | 4.2 | 5.4 | 6.8 | 116 | 113 | 116 | 15 | 7.63 | 15 | 31 250 | 124 999 | 3.3 | 5 | 18 |
| Calreticulin (CALR) | P27797 | 0.4 | 1.0 | 1.3 | NA | NA | NA | 0.5 | 488 | 976 | 250 000 | 1 000 000 | 2.4 | 7 | 25 |
| Carbonic anhydrase 12 (CA12) | O43570 | 2.2 | 2.7 | 3.3 | 127 | 105 | 121 | 15 | 31 | 31 | 62 499 | 124 999 | 3.3 | 5 | 21 |
| Carbonic anhydrase 14 (CA14) | Q9ULX7 | 1.8 | 2.2 | 2.7 | 114 | 101 | 115 | 15 | 31 | 31 | 31 250 | 31 250 | 3.0 | 4 | 16 |
| Casein kinase I isoform delta (CSNK1D) | P48730 | NA | 0.6 | 1.0 | NA | NA | NA | 15 | 1953 | 1953 | 250 000 | 499 999 | 2.1 | 4 | 19 |
| Claspin (CLSPN) | Q9HAW4 | NA | NA | 0.6 | NA | NA | NA | 15 | 122 | 244 | 250 000 | 499 999 | 3.0 | 8 | 16 |
| CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1 (ST3GAL1) | Q11201 | 3.7 | 4.2 | 5.0 | 143 | 104 | 133 | 15 | 488 | 488 | 499 999 | 499 999 | 3.0 | 4 | 23 |
| Cocaine esterase (CES2) | O00748 | 0.5 | 1.2 | 1.8 | 107 | 93 | 96 | 15 | 1953 | 1953 | 250 000 | 1 000 000 | 2.1 | 5 | 18 |
| Contactin-2 (CNTN2) | Q02246 | 4.0 | 4.5 | 5.1 | 138 | 105 | 133 | 15 | 15 | 15 | 31 250 | 31 250 | 3.3 | 4 | 19 |
| Corticoliberin (CRH) | P06850 | 2.1 | 3.1 | 5.3 | 128 | 98 | 120 | 15 | 1953 | 1953 | 499 999 | 499 999 | 2.4 | 5 | 24 |
| C-type lectin domain family 1 member A (CLEC1A) | Q8NC01 | 2.6 | 3.2 | 3.9 | 152 | 107 | 144 | 15 | 31 | 31 | 31 250 | 124 999 | 3.0 | 5 | 21 |
| C-type natriuretic peptide (NPPC) | P23582 | 2.6 | 3.8 | 5.7 | 98 | 116 | 107 | 15 | 15 | 15 | 31 250 | 31 250 | 3.3 | 6 | 17 |
| Desmoglein-4 (DSG4) | Q86SJ6 | 2.3 | 3.5 | 4.3 | 128 | 103 | 134 | 15 | 3.81 | 7.63 | 15 624 | 31 250 | 3.3 | 5 | 13 |
| Dipeptidyl aminopeptidase-like protein 6 (DPP6) | P42658 | 3.0 | 3.7 | 4.2 | 126 | 103 | 124 | 15 | 31 | 31 | 250 000 | 1 000 000 | 3.9 | 6 | 24 |
| DNA topoisomerase 2-beta (TOP2B) | Q02880 | 1.3 | 1.7 | 2.5 | 200 | 101 | 227 | 15 | 15 | 31 | 31 250 | 31 250 | 3.0 | 6 | 16 |
| Ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2) | Q9Y5L3 | 0.9 | 1.3 | 1.6 | 114 | 103 | 103 | 15 | 1.91 | 7.63 | 15 624 | 31 250 | 3.3 | 6 | 13 |
| Ectonucleoside triphosphate diphosphohydrolase 6 (ENTPD6) | O75354 | 2.4 | 2.6 | 3.0 | 121 | 104 | 117 | 15 | 244 | 244 | 62 499 | 499 999 | 2.4 | 4 | 22 |
| EGF-like repeat and discoidin I-like domain-containing protein 3 (EDIL3) | O43854 | 1.5 | 2.1 | 3.2 | 113 | 100 | 100 | 1.9 | 61 | 61 | 62 499 | 1 000 000 | 3.0 | 5 | 19 |
| Enteropeptidase (TMPRSS15) | P98073 | 0.0 | 0.9 | 2.4 | 131 | 101 | 121 | 15 | 15 | 15 | 31 250 | 31 250 | 3.3 | 3 | 20 |
| Epidermal growth factor-like protein 7 (EGFL7) | Q9UHF1 | 4.2 | 4.9 | 6.0 | 133 | 108 | 135 | 15 | 61 | 61 | 124 999 | 250 000 | 3.3 | 5 | 19 |
| Erythropoietin (EPO) | P01588 | NA | 0.9 | 2.8 | 131 | 104 | 126 | 15 | 244 | 244 | 62 499 | 124 999 | 2.4 | 6 | 19 |
| Fatty acid-binding protein 9 (FABP9) | Q0Z7S8 | 1.1 | 1.8 | 2.4 | 118 | 102 | 117 | 15 | 15 | 15 | 31 250 | 124 999 | 3.3 | 5 | 22 |
| Forkhead box protein O1 (FOXO1) | Q12778 | 1.0 | 1.5 | 2.3 | 74 | 103 | 69 | 15 | 244 | 488 | 499 999 | 499 999 | 3.0 | 5 | 22 |
| Fructose-2,6-bisphosphatase TIGAR (TIGAR) | Q9NQ88 | 1.2 | 1.6 | 2.1 | 118 | 99 | 112 | 15 | 31 | 31 | 250 000 | 250 000 | 3.9 | 5 | 20 |
| Hematopoietic prostaglandin D synthase (HPGDS) | O60760 | 1.5 | 1.9 | 2.6 | 116 | 98 | 117 | 15 | 122 | 122 | 124 999 | 1 000 000 | 3.0 | 6 | 23 |
| Inactive tyrosine-protein kinase 7 (PTK7) | Q13308 | 1.5 | 1.9 | 2.3 | 114 | 96 | 107 | 15 | 122 | 122 | 499 999 | 499 999 | 3.6 | 6 | 25 |
| Integrin beta-1-binding protein 1 (ITGB1BP1) | O14713 | NA | NA | 0.8 | NA | NA | NA | 15 | 244 | 244 | 250 000 | 499 999 | 3.0 | 7 | 21 |
| Interferon-inducible double-stranded RNA-dependent protein kinase activator A (PRKRA) | O75569 | 0.5 | 0.9 | 1.5 | 100 | 95 | 98 | 15 | 1953 | 3906 | 499 999 | 499 999 | 2.1 | 5 | 18 |
| Kidney Injury Molecule (KIM1) | Q96D42 | 7.0 | 7.8 | 8.9 | 128 | 114 | 127 | 15 | 0.48 | 0.95 | 15 624 | 31 250 | 4.2 | 5 | 14 |
| Leukotriene A-4 hydrolase (LTA4H) | P09960 | NA | 1.4 | 3.4 | NA | NA | NA | 15 | 7812 | 7812 | 499 999 | 499 999 | 1.8 | 4 | 30 |
| Linker for activation of T-cells family member 2 (LAT2) | Q9GZY6 | 1.6 | 2.8 | 3.8 | 57 | 86 | 51 | 15 | 31 | 61 | 250 000 | 499 999 | 3.6 | 7 | 20 |
| Lutropin subunit beta (LHB) | P01229 | 1.2 | 2.2 | 4.2 | 114 | 99 | 110 | 15 | 976 | 1953 | 1 000 000 | 1 000 000 | 2.7 | 5 | 24 |
| Macrophage erythroblast attacher (MAEA) | Q7L5Y9 | 0.3 | 0.9 | 1.6 | NA | NA | NA | 3.8 | 61 | 61 | 62 499 | 124 999 | 3.0 | 6 | 18 |
| Macrophage-capping protein (CAPG) | P40121 | 2.2 | 2.8 | 3.8 | 147 | 96 | 142 | 15 | 244 | 244 | 499 999 | 499 999 | 3.3 | 6 | 18 |
| Melanoma-associated antigen D1 (MAGED1) | Q9Y5V3 | NA | 0.4 | 1.1 | NA | NA | NA | 15 | 15 | 15 | 15 624 | 31 250 | 3.0 | 5 | 13 |
| Methionine aminopeptidase 1 (METAP1) | P53582 | NA | NA | 0.8 | NA | NA | NA | 15 | 244 | 244 | 250 000 | 499 999 | 3.0 | 4 | 21 |
| Mevalonate kinase (MVK) | Q03426 | 1.8 | 2.7 | 4.6 | 143 | 110 | 137 | 15 | 15 | 31 | 62 499 | 250 000 | 3.3 | 5 | 19 |
| Mitogen-activated protein kinase kinase kinase kinase 5 (MAP4K5) | Q9Y4K4 | 1.1 | 2.3 | 4.3 | 31 | 104 | 30 | 0.5 | 7.63 | 7.63 | 15 624 | 31 250 | 3.3 | 5 | 20 |

| | | Sample types | | | | | | Endogenous Interference | Analytical measurement | | | | | 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 | Hemolysate | LOD | LLOQ | ULOQ | Hook | Range | Intra | Inter |
| Mothers against decapentaplegic homolog 1 (SMAD1) | Q15797 | NA | 0.9 | 1.7 | NA | 105 | NA | 15 | 15 | 31 | 250 000 | 1 000 000 | 3.9 | 6 | 16 |
| NAD-dependent protein deacylase sirtuin-5, mitochondrial (SIRT5) | Q9NXA8 | NA | 0.2 | 1.1 | NA | NA | NA | 15 | 488 | 488 | 250 000 | 499 999 | 2.7 | 6 | 16 |
| NEDD8 ultimate buster 1 (NUB1) | Q9Y5A7 | NA | 0.9 | 1.8 | 95 | 99 | NA | 0.5 | 15 | 15 | 15 624 | 31 250 | 3.0 | 5 | 15 |
| Neutrophil cytosol factor 2 (NCF2) | P19878 | 2.7 | 4.1 | 5.6 | 577 | 105 | 581 | 15 | 1.91 | 3.81 | 31 250 | 31 250 | 3.9 | 5 | 21 |
| Nibrin (NBN) | O60934 | 0.3 | 1.3 | 1.9 | 181 | 104 | 175 | 15 | 31 | 31 | 124 999 | 499 999 | 3.6 | 7 | 16 |
| Nitric oxide synthase, endothelial (NOS3) | P29474 | 0.7 | 1.8 | 2.6 | 65 | 90 | NA | 15 | 7.63 | 15 | 62 499 | 124 999 | 3.6 | 5 | 16 |
| Nucleobindin-2 (NUCB2) | P80303 | 3.1 | 3.6 | 4.3 | 173 | 107 | 170 | 15 | 31 | 31 | 62 499 | 124 999 | 3.3 | 5 | 24 |
| Parvalbumin alpha (PVALB) | P20472 | 3.4 | 4.3 | 5.6 | 144 | 110 | 145 | 15 | 3.81 | 7.63 | 7812 | 31 250 | 3.0 | 4 | 18 |
| Paxillin (PXN) | P49023 | 2.4 | 2.9 | 3.7 | 208 | 100 | 193 | 3.8 | 122 | 244 | 124 999 | 250 000 | 2.7 | 6 | 24 |
| Peptidyl-prolyl cis-trans isomerase FKBP1B (FKBP1B) | P68106 | NA | 0.9 | 1.5 | NA | NA | NA | 15 | 122 | 244 | 62 499 | 124 999 | 2.4 | 5 | 19 |
| Perilipin-1 (PLIN1) | O60240 | 1.7 | 2.8 | 4.2 | 163 | 107 | 157 | 15 | 31 | 61 | 62 499 | 250 000 | 3.0 | 5 | 19 |
| Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2 (INPPL1) | O15357 | 1.3 | 2.1 | 3.6 | NA | 99 | NA | 3.8 | 122 | 122 | 62 499 | 499 999 | 2.7 | 5 | 18 |
| Placenta growth factor (PGF) | P49763 | 8.0 | 8.5 | 9.0 | 133 | 111 | 130 | 15 | 0.48 | 0.95 | 7812 | 15 624 | 3.9 | 5 | 10 |
| Platelet-derived growth factor C (PDGFC) | Q9NRA1 | 2.7 | 3.5 | 4.0 | 101 | 104 | 99 | 15 | 61 | 122 | 15 624 | 124 999 | 2.1 | 4 | 18 |
| Pleiotrophin (PTN) | P21246 | 0.7 | 1.3 | 2.6 | NA | NA | NA | 15 | 244 | 244 | 31 250 | 31 250 | 2.1 | 7 | 15 |
| Plexin domain-containing protein 1 (PLXDC1) | Q8IUK5 | 0.4 | 1.4 | 1.8 | 108 | 106 | NA | 15 | 61 | 61 | 62 499 | 124 999 | 3.0 | 4 | 20 |
| Polypeptide N-acetylgalactosaminyltransferase 10 (GALNT10) | Q86SR1 | 1.9 | 2.2 | 2.6 | 128 | 101 | 123 | 15 | 976 | 976 | 250 000 | 499 999 | 2.4 | 4 | 23 |
| Probetacellulin (BTC) | P35070 | 0.1 | 0.9 | 1.5 | 127 | NA | 119 | 15 | 0.95 | 7.63 | 7812 | 15 624 | 3.0 | 6 | 10 |
| Programmed cell death protein 1 (PDCD1) | Q15116 | 1.1 | 1.3 | 1.6 | NA | 96 | 100 | 15 | 7.63 | 7.63 | 31 250 | 31 250 | 3.6 | 4 | 21 |
| Prolow-density lipoprotein receptor-related protein 1 (LRP1) | Q07954 | 1.8 | 2.2 | 2.7 | NA | 98 | NA | 15 | 244 | 244 | 499 999 | 1 000 000 | 3.3 | 6 | 29 |
| Proteasome subunit alpha type-1 (PSMA1) | P25786 | NA | 0.9 | 3.6 | 108 | NA | NA | 3.8 | 488 | 1953 | 250 000 | 499 999 | 2.1 | 4 | 18 |
| Protein amnionless (AMN) | Q9BXJ7 | 2.3 | 3.4 | 4.3 | 128 | 105 | 122 | 15 | 3.81 | 15 | 31 250 | 124 999 | 3.3 | 4 | 26 |
| Protein enabled homolog (ENAH) | Q8N8S7 | 1.7 | 2.3 | 3.1 | 127 | 109 | 117 | 15 | 61 | 61 | 62 499 | 62 499 | 3.0 | 5 | 17 |
| Protein fosB (FOSB) | P53539 | NA | 0.8 | 2.4 | NA | NA | NA | 15 | 61 | 244 | 499 999 | 499 999 | 3.3 | 8 | 17 |
| Protein LAP2 (ERBB2IP) | Q96RT1 | 0.7 | 1.9 | 3.3 | NA | 96 | NA | 0.5 | 61 | 61 | 31 250 | 499 999 | 2.7 | 4 | 22 |
| Protein max (MAX) | P61244 | NA | 0.6 | 1.6 | NA | NA | NA | 15 | 122 | 122 | 62 499 | 124 999 | 2.7 | 5 | 18 |
| Protein phosphatase 1B (PPM1B) | O75688 | NA | 0.4 | 0.9 | NA | NA | NA | 7.5 | 244 | 244 | 499 999 | 499 999 | 3.3 | 5 | 17 |
| Pyruvate dehydrogenase [acetyl-transferring]-phosphatase 1, mitochondrial (PDP1) | Q9P0J1 | NA | NA | NA | NA | NA | NA | 15 | 7812 | 7812 | 499 999 | 1 000 000 | 1.8 | 9 | 33 |
| Ras association domain-containing protein 2 (RASSF2) | P50749 | 0.6 | 1.0 | 2.0 | 163 | 97 | 157 | 15 | 7.63 | 15 | 62 499 | 124 999 | 3.6 | 4 | 17 |
| Ras GTPase-activating protein 1 (RASA1) | P20936 | NA | NA | 1.4 | NA | NA | NA | 15 | NA | NA | NA | NA | NA | 8 | 22 |
| Receptor-type tyrosine-protein phosphatase eta (PTPRJ) | Q12913 | NA | NA | 1.2 | 127 | 92 | 127 | 15 | 488 | 976 | 499 999 | 1 000 000 | 2.7 | 6 | 23 |
| Renin receptor (ATP6AP2) | O75787 | 0.7 | 1.0 | 1.4 | 139 | NA | 124 | 15 | NA | NA | NA | NA | NA | 10 | 23 |
| REST corepressor 1 (RCOR1) | Q9UKL0 | 0.8 | 1.0 | 1.5 | 127 | NA | 112 | 15 | 3.81 | 7.63 | 62 499 | 124 999 | 3.9 | 6 | 11 |
| Retinoic acid receptor responder protein 1 (RARRES1) | P49788 | 1.7 | 2.1 | 2.5 | 92 | 98 | NA | 15 | 61 | 61 | 499 999 | 1 000 000 | 3.9 | 5 | 20 |
| Ribonucleoside-diphosphate reductase subunit M2 B (RRM2B) | Q7LG56 | 0.3 | 0.9 | 1.3 | 142 | 102 | 136 | 15 | 7.63 | 15 | 499 999 | 1 000 000 | 4.5 | 12 | 14 |
| Serpin A9 (SERPINA9) | Q86WD7 | 2.0 | 3.1 | 4.3 | 135 | 116 | 146 | 7.5 | 1.91 | 1.91 | 31 250 | 31 250 | 4.2 | 5 | 17 |
| Serum paraoxonase/arylesterase 2 (PON2) | Q15165 | 2.5 | 2.9 | 3.6 | 82 | 102 | 74 | 15 | 122 | 122 | 124 999 | 499 999 | 3.0 | 9 | 23 |
| Syntaxin-8 (STX8) | Q9UNK0 | 1.2 | 1.9 | 2.5 | 124 | 97 | 118 | 0.5 | 31 | 31 | 31 250 | 124 999 | 3.0 | 4 | 18 |
| Syntaxin-binding protein 3 (STXBP3) | O00186 | NA | NA | 1.2 | NA | NA | NA | 0.9 | 61 | 61 | 124 999 | 250 000 | 3.3 | 5 | 20 |
| Troponin I, cardiac muscle (TNNI3) | P19429 | NA | 1.5 | 5.4 | NA | 105 | NA | 15 | 61 | 122 | 499 999 | 499 999 | 3.6 | 10 | 19 |
| Tyrosine-protein kinase Fes/Fps (FES) | P07332 | NA | NA | 0.7 | NA | NA | NA | 15 | 61 | 488 | 250 000 | 1 000 000 | 2.7 | 6 | 20 |
| Tyrosine-protein kinase Fgr (FGR) | P09769 | 1.3 | 2.1 | 2.9 | 148 | 95 | 133 | 0.9 | 976 | 1953 | 499 999 | 499 999 | 2.4 | 5 | 22 |
| Tyrosine-protein kinase Yes (YES1) | P07947 | 2.8 | 4.1 | 5.5 | 62 | 101 | 59 | 0.2 | 3.81 | 7.63 | 15 624 | 31 250 | 3.3 | 5 | 18 |
| Vascular endothelial growth factor C (VEGFC) | P49767 | 1.3 | 1.7 | 2.7 | 310 | NA | 271 | 15 | 122 | 244 | 62 499 | 124 999 | 2.4 | 5 | 18 |
| Vasohibin-1 (VASH1) | Q7L8A9 | NA | NA | NA | NA | NA | NA | 15 | 122 | 244 | 124 999 | 250 000 | 2.7 | 6 | 18 |
| Wiskott-Aldrich syndrome protein (WAS) | P42768 | NA | NA | 1.4 | NA | NA | NA | 15 | 976 | 976 | 124 999 | 1 000 000 | 2.1 | 6 | 27 |

*U/µl

## 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 6 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 of three operators' %CV. Variation calculations were performed on linearized values for 91 analytes for which response levels could be measured in serum and normal plasma, see Table 1.

Across all 91 assays, the mean intra-assay and inter-assay variations were observed to be 6.0% and 20.2%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

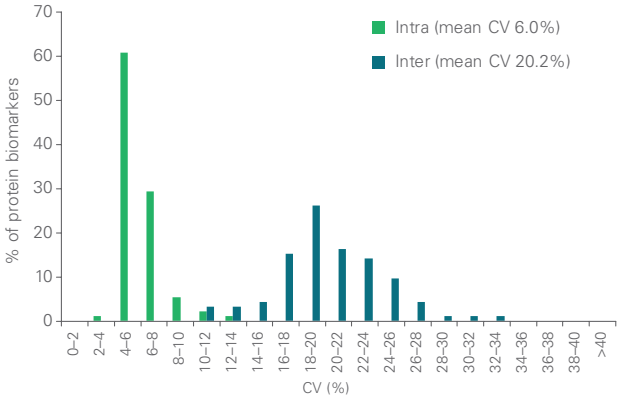


Fig 4. Distribution of intra-assay and inter-assay variations of Olink Organ Damage.

### REPRODUCIBILITY

Inter-site variations (between-site) were investigated during the validation of previous panels in beta-site studies to estimate the expected variations in values between different laboratories, with different operators and using different equipment. The beta-site studies have previously shown reproducibility and repeatability in line with Olink Proteomics results. For information on performed beta-site studies, download our Data Validation documents or contact support@olink.com.

## 2.4 ANALYTICAL SPECIFICITY

### ASSAY SPECIFICITY

The antibodies used in Olink Organ Damage were all specific for their respective targets. 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 in Olink, see Figure 5.

### ENDOGENOUS INTERFERENCE

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

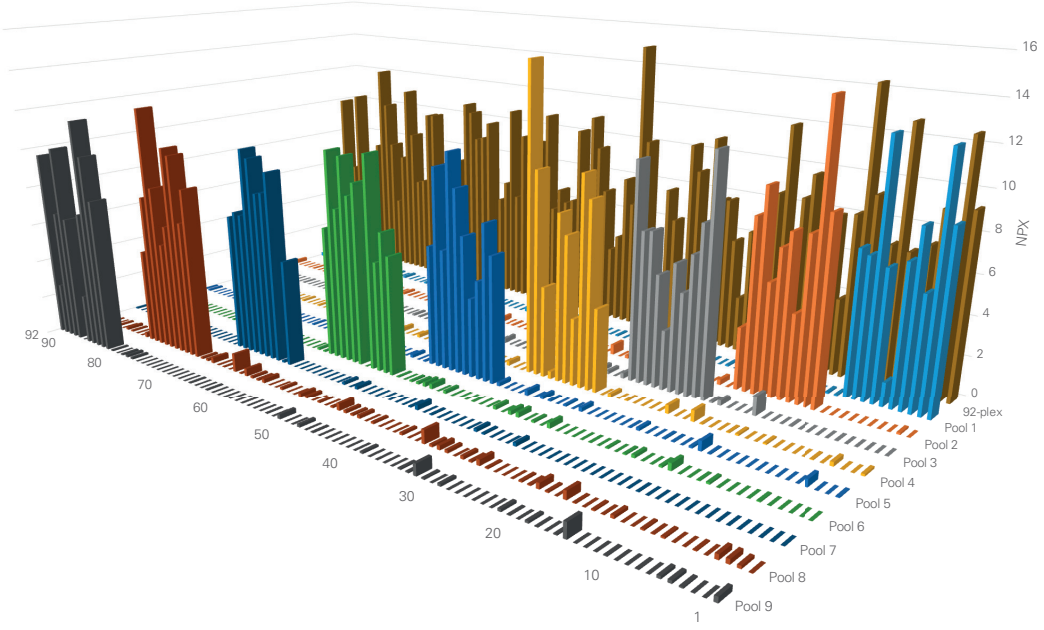


Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

impact of this specific interference was investigated during the validation of previous panels. No interference due to HAMA or RF could be detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown).

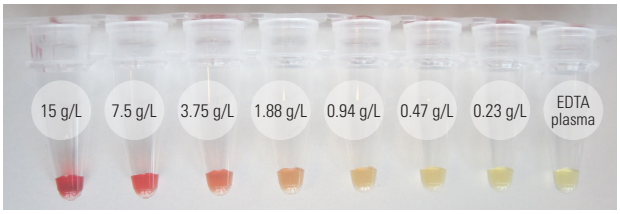


**Fig 6.** Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of bilirubin, lipids and hemolysate, known interfering plasma and serum components, were evaluted at different added concentrations. An example of hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. Interferens by bilirubin and lipids has previously been evaluated, and disturbance has only been observed at extrem levels corresponding to 8 or 10 times normal [3, 4] values and therefore not performed for Olink Organ Damage. In 16 out of 92 assays, altered signal 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. Table 1 reports the highest concentration of hemolysate that does not have an impact on assay performance.

## 2.5 SCALABILITY

Assay performance was further evaluated with regard to scalability, meaning the capability of the Olink technology to maintain the same quality of performance irrespective of multiplex 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 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.

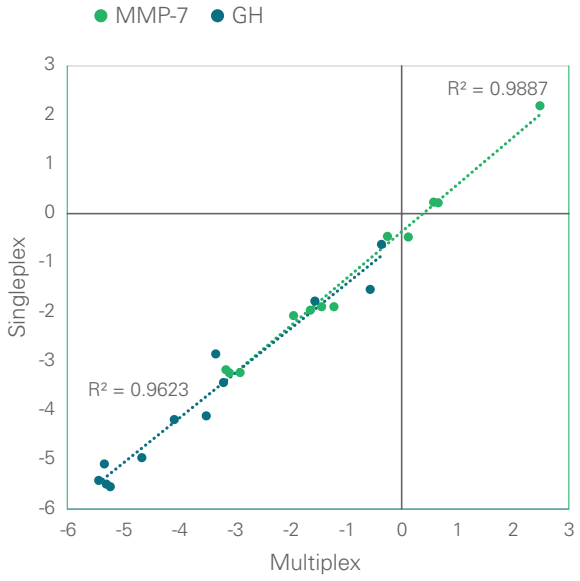


**Fig 7.** Scalability of the Olink technology platform. The experiment was performed using the Olink CVD II $^{96\times96}$ panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The observed dCq (log2) values were plotted, and the correlation coefficient $R^2$ value was generated by linear regression.

# 3. References


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## TECHNICAL SUPPORT

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