



VALIDATION DATA

# 1. Introduction

Olink<sup>®</sup> Cardiometabolic is a reagent kit measuring 92 cardiometabolic related human protein biomarkers simultaneously. The assays on this panel have been selected to target high-abundance proteins, and 1  $\mu$ L of a 1:2025 dilution of sample is used. 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<sup>1,2</sup>, 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.

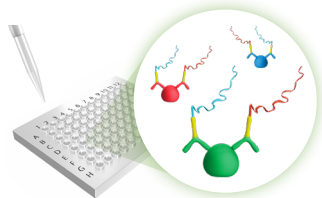
An external control, inter-plate control (IPC), is included on each plate and used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. Furthermore, the improves inter-assay precision 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 subtracted, thus normalizing for technical variation within one run. Normalization between runs is then performed for each assay by subtracting 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 log<sub>2</sub> 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.

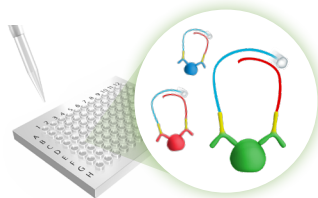
### IMMUNOASSAY

Allow the 92 antibody probe pairs to bind to their respective proteins in your samples.



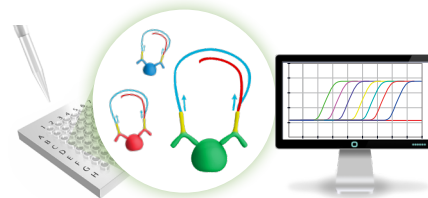
### EXTENSION

Extend and pre-amplify 92 unique DNA reporter sequences by proximity extension.



### DETECTION

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



Immunoassay control

Extension control

Detection control

**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<sup>®</sup> Biomark<sup>™</sup> or the Fluidigm<sup>®</sup> Biomark<sup>™</sup> HD system.

## 2. Performance characteristics

### 2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Cardiometabolic by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Response values observed between heparin, citrate plasma or serum, are expressed as relative differences (%) compared to EDTA plasma and shown in Table 1 for each sample type. To evaluate the measuring range of endogenous protein levels, response values levels were assessed in 22 normal EDTA plasma samples and reported in NPX, Table 1.

### 2.2 ANALYTICAL MEASUREMENT

#### DETECTION LIMIT

Calibrator curves were determined for 90 out of 92 biomarkers simultaneously in a multiplex format. 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. Please note that the Cardiometabolic panel uses a 1:2025 dilution of sample, whereas our technical validation assays are performed *in vitro* using recombinant antigens. The data presented in this document are based on these *in vitro* assays and a multiplication factor of 2025 should therefore be taken in consideration when comparing the addressable biological concentration to the *in vitro* validation data.

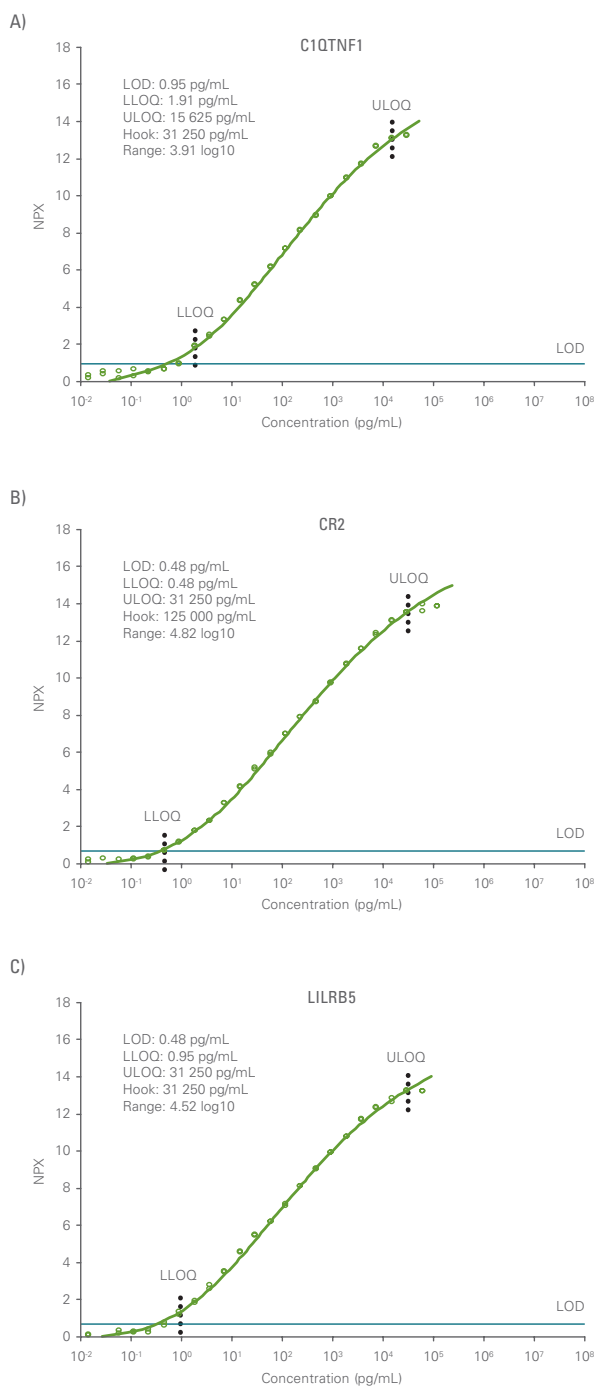
#### HIGH DOSE HOOK EFFECT

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 90 assays, see Table 1.

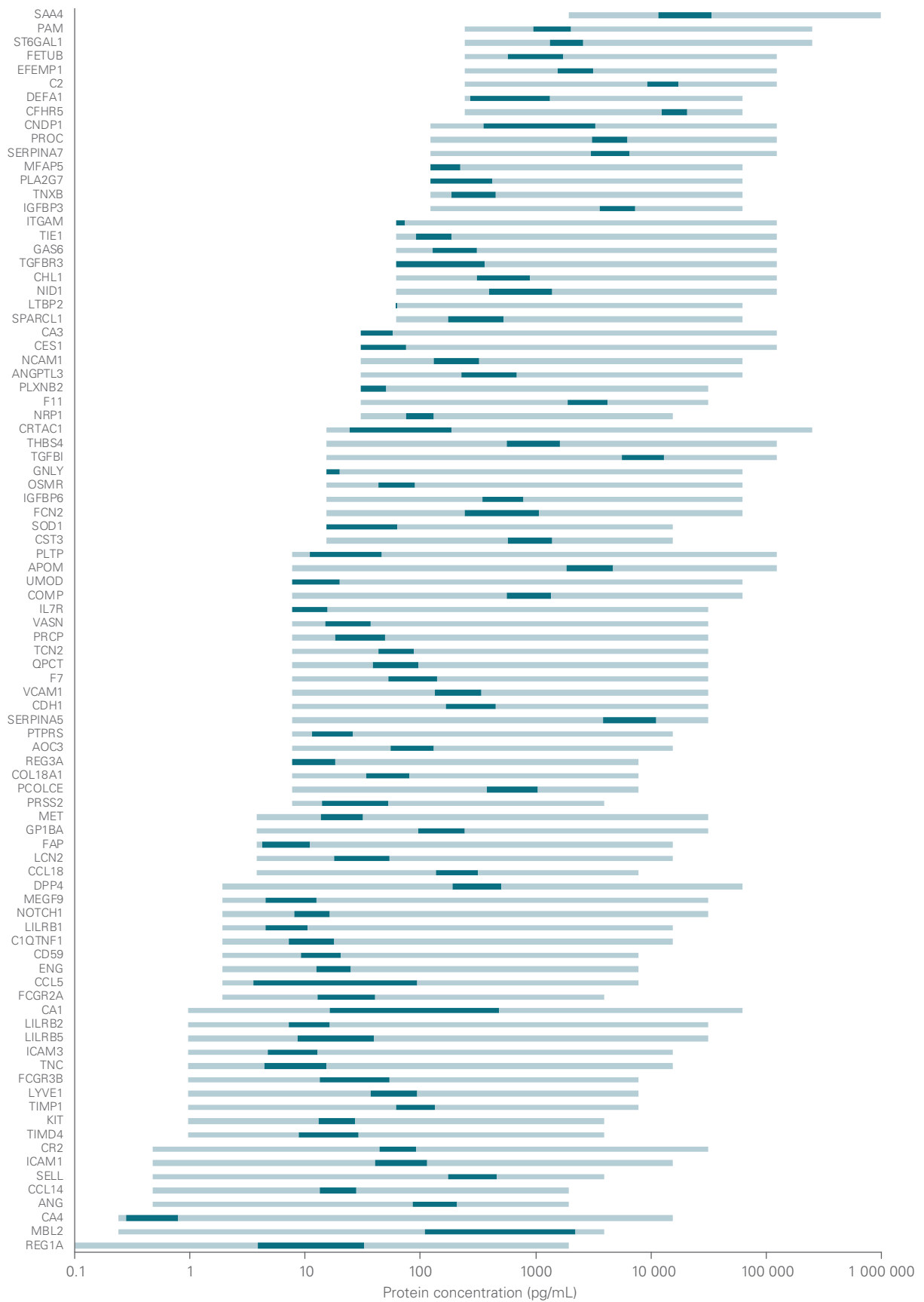
#### MEASURING RANGE

The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in order of log<sub>10</sub>, 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](http://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) in pg/mL. Normal plasma levels (dark green bars) are denoted for 90 analytes and here reported in pg/mL.

**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. Please note: the Cardiometabolic panel uses a 1:2025 dilution which should be taken in consideration when comparing biological concentrations to the *in vitro* validation data.

| Target   | UniProt No | Sample types               |        |            | Endogenous interference     |         |       | Analytical measurement |       |      |        | Precision |       |       |       |
|--|------------|----------------------------|--------|------------|-----------------------------|---------|-------|------------------------|-------|------|--------|-----------|-------|-------|-------|
|  |            | Normal plasma levels (NPX) |        |            | Relative to EDTA plasma (%) |         |       | (mg/mL)                | pg/mL |      |        | log10     | % CV  |       |       |
|  |            | 10th %tile                 | Median | 90th %tile | ACD                         | Heparin | Serum | Hemolysate             | LOD   | LLOQ | ULOQ   | Hook      | Range | Intra | Inter |
| Angiogenin (ANG)   | P03950     | 7.2                        | 8.0    | 8.4        | 96                          | 75      | 90    | 15                     | 0.48  | 0.48 | 1953   | 3906      | 3.6   | 12    | 10    |
| Angiotensin-converting enzyme 2 (ACE2)                               | P15175     | 2.8                        | 3.6    | 4.3        | 101                         | 82      | 94    | 15                     | 15    | 31   | 62500  | 125000    | 3.3   | 12    | 8     |
| Angiopoietin-related protein 3 (ANGPTL3)                             | Q9Y5C1     | 2.8                        | 3.6    | 4.3        | 101                         | 82      | 94    | 15                     | 15    | 31   | 62500  | 125000    | 3.3   | 12    | 8     |
| Apolipoprotein M (APOM)  | O95445     | 6.5                        | 7.4    | 7.9        | 98                          | 91      | 101   | 15                     | 7.6   | 7.6  | 125000 | 500000    | 4.2   | 14    | 10    |
| Beta-Ala-His dipeptidase (CNDP1)                                     | Q96KN2     | 1.4                        | 2.6    | 3.8        | 366                         | 305     | 301   | 15                     | 122   | 122  | 125000 | 500000    | 3.0   | 12    | 10    |
| Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)             | P15907     | 3.2                        | 3.6    | 4.2        | 99                          | 96      | 131   | 15                     | 244   | 244  | 250000 | 500000    | 3.0   | 17    | 14    |
| Cadherin-1 (CDH1)  | P12830     | 4.2                        | 5.2    | 5.8        | 133                         | 124     | 132   | 15                     | 7.6   | 7.6  | 31250  | 62500     | 3.6   | 14    | 9     |
| Carbonic anhydrase 1 (CA1)   | P00915     | 2.5                        | 5.8    | 6.7        | 27                          | 17      | 86    | 0                      | 0.95  | 0.95 | 62500  | 125000    | 4.8   | 14    | 13    |
| Carbonic anhydrase 3 (CA3)   | P07451     | 0.6                        | 1.1    | 1.6        | 84                          | 69      | 95    | 0                      | 15    | 31   | 125000 | 500000    | 3.6   | 7     | 8     |
| Carbonic anhydrase 4 (CA4)   | P22748     | 1.5                        | 2.0    | 2.4        | 104                         | 94      | 105   | 15                     | 0.12  | 0.24 | 15625  | 62500     | 4.8   | 13    | 7     |
| Cartilage acidic protein 1 (CRTAC1)                                  | Q9NQ79     | 1.1                        | 2.1    | 3.2        | 136                         | 131     | 136   | 15                     | 15    | 15   | 250000 | 500000    | 4.2   | 12    | 10    |
| Cartilage oligomeric matrix protein (COMP)                           | P49747     | 7.0                        | 7.6    | 8.3        | 100                         | 93      | 98    | 15                     | 7.6   | 7.6  | 62500  | 125000    | 3.9   | 14    | 9     |
| C-C motif chemokine 14 (CCL14)                                       | Q16627     | 4.1                        | 4.7    | 5.2        | 91                          | 81      | 92    | 15                     | 0.48  | 0.48 | 1953   | 7812      | 3.6   | 14    | 7     |
| C-C motif chemokine 18 (CCL18)                                       | P55774     | 6.3                        | 7.1    | 7.9        | 98                          | 74      | 94    | 15                     | 3.8   | 3.8  | 7812   | 15625     | 3.3   | 16    | 10    |
| C-C motif chemokine 5 (CCL5)   | P13501     | 1.4                        | 2.8    | 5.2        | 22                          | 89      | 159   | 15                     | 1.9   | 1.9  | 7812   | 15625     | 3.6   | 13    | 8     |
| CD59 glycoprotein (CD59)   | P13987     | 2.0                        | 2.6    | 2.9        | 109                         | 116     | 138   | 3.8                    | 1.9   | 1.9  | 7812   | 7812      | 3.6   | 19    | 22    |
| Coagulation factor VII (F7)  | P08709     | 3.9                        | 4.9    | 5.3        | 97                          | 90      | 77    | 15                     | 1.9   | 7.6  | 31250  | 62500     | 3.6   | 15    | 10    |
| Coagulation factor XI (F11)  | P03951     | 6.8                        | 7.4    | 7.8        | 97                          | 89      | 95    | 15                     | 7.6   | 31   | 31250  | 62500     | 3.0   | 11    | 7     |
| Collagen alpha-1(XVIII) chain (COL18A1)                              | P39060     | 2.9                        | 3.5    | 4.0        | 102                         | 88      | 99    | 15                     | 7.6   | 7.6  | 7812   | 15625     | 3.0   | 12    | 7     |
| Complement C1q tumor necrosis factor-related protein 1 (C1QTNF1)     | Q9BXJ1     | 3.1                        | 3.7    | 4.3        | 86                          | 49      | 78    | 15                     | 0.95  | 1.9  | 15625  | 31250     | 3.9   | 16    | 10    |
| Complement C2 (C2)   | P06681     | 4.9                        | 5.3    | 5.8        | 99                          | 102     | 132   | 15                     | 122   | 244  | 125000 | 500000    | 2.7   | 13    | 8     |
| Complement factor H-related protein 5 (CFHR5)                        | Q9BXR6     | 8.1                        | 8.7    | 9.2        | 99                          | 89      | 95    | 15                     | 122   | 244  | 62500  | 125000    | 2.4   | 12    | 10    |
| Complement receptor type 2 (CR2)                                     | P20023     | 5.4                        | 6.1    | 6.5        | 98                          | 92      | 96    | 15                     | 0.48  | 0.48 | 31250  | 125000    | 4.8   | 13    | 8     |
| Cystatin-C (CST3)  | P01034     | 6.8                        | 7.6    | 8.4        | 95                          | 91      | 96    | 15                     | 15    | 15   | 15625  | 31250     | 3.0   | 17    | 11    |
| Dipeptidyl peptidase 4 (DPP4)  | P27487     | 4.9                        | 5.5    | 6.2        | 100                         | 93      | 104   | 15                     | 1.9   | 1.9  | 62500  | 125000    | 4.5   | 14    | 8     |
| EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1)  | Q12805     | 3.3                        | 3.8    | 4.4        | 108                         | 78      | 82    | 15                     | 244   | 244  | 125000 | 500000    | 2.7   | 14    | 9     |
| Endoglin (ENG)   | P17813     | 2.2                        | 2.5    | 2.9        | 101                         | 88      | 91    | 15                     | 1.9   | 1.9  | 7812   | 15625     | 3.6   | 12    | 9     |
| Fetuin-B (FETUB)   | Q9UGM5     | 1.4                        | 2.2    | 2.7        | 105                         | 95      | 102   | 15                     | 122   | 244  | 125000 | 125000    | 2.7   | 18    | 17    |
| Ficolin-2 (FCN2)   | Q15485     | 4.4                        | 5.7    | 6.6        | 122                         | 113     | 108   | 15                     | 7.6   | 15   | 62500  | 125000    | 3.6   | 14    | 8     |
| GlutaminyI-peptide cyclotransferase (QPCT)                           | Q16769     | 2.2                        | 2.7    | 3.4        | 105                         | 99      | 117   | 15                     | 7.6   | 7.6  | 31250  | 31250     | 3.6   | 14    | 23    |
| Granulysin (GNLY)  | P22749     | 0.3                        | 0.9    | 1.2        | 106                         | 94      | 100   | 15                     | 15    | 15   | 62500  | 125000    | 3.6   | 13    | 7     |
| Growth arrest-specific protein 6 (GAS6)                              | Q14393     | 3.5                        | 4.3    | 4.8        | 121                         | 111     | 120   | 15                     | 15    | 61   | 125000 | 250000    | 3.3   | 14    | 11    |
| Hepatocyte growth factor receptor (MET)                              | P08581     | 2.1                        | 2.6    | 2.9        | 97                          | 90      | 97    | 15                     | 3.8   | 3.8  | 31250  | 62500     | 3.9   | 12    | 7     |
| Ig lambda-2 chain C regions (IGLC2)                                  | P0CG05     | 6.2                        | 6.7    | 7.5        | 97                          | 91      | 99    | 15                     | NA    | NA   | NA     | NA        | NA    | 13    | 9     |
| Insulin-like growth factor-binding protein 3 (IGFBP3)                | P17936     | 4.4                        | 5.1    | 5.6        | 103                         | 98      | 106   | 15                     | 122   | 122  | 62500  | 125000    | 2.7   | 10    | 7     |
| Insulin-like growth factor-binding protein 6 (IGFBP6)                | P24592     | 4.0                        | 4.7    | 5.3        | 97                          | 94      | 103   | 15                     | 15    | 15   | 62500  | 125000    | 3.6   | 11    | 7     |
| Integrin alpha-M (ITGAM)   | P11215     | 0.5                        | 1.3    | 2.2        | 101                         | 83      | 91    | 15                     | 31    | 61   | 125000 | 125000    | 3.3   | 17    | 14    |
| Intercellular adhesion molecule 1 (ICAM1)                            | P05362     | 6.1                        | 6.7    | 7.5        | 96                          | 90      | 95    | 15                     | 0.48  | 0.48 | 15625  | 31250     | 4.5   | 13    | 7     |
| Intercellular adhesion molecule 3 (ICAM3)                            | P32942     | 2.5                        | 3.0    | 3.6        | 99                          | 90      | 99    | 15                     | 0.95  | 0.95 | 15625  | 31250     | 4.2   | 11    | 7     |
| Interleukin-7 receptor subunit alpha (IL7R)                          | P16871     | 1.1                        | 1.7    | 2.3        | 103                         | 89      | 101   | 15                     | 3.8   | 7.6  | 31250  | 125000    | 3.6   | 15    | 14    |
| Latent-transforming growth factor beta-binding protein 2 (LTBP2)     | Q14767     | NA                         | NA     | 0.8        | NA                          | NA      | NA    | 15                     | 61    | 61   | 62500  | 125000    | 3.0   | 11    | 9     |
| Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) | Q8NHL6     | 1.5                        | 1.9    | 2.2        | 102                         | 91      | 97    | 15                     | 1.9   | 1.9  | 15625  | 31250     | 3.9   | 10    | 8     |
| Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2) | Q8N423     | 2.8                        | 3.2    | 3.8        | 97                          | 85      | 97    | 15                     | 0.95  | 0.95 | 31250  | 31250     | 4.5   | 13    | 9     |
| Leukocyte immunoglobulin-like receptor subfamily B member 5 (LILRB5) | Q75023     | 3.5                        | 4.8    | 5.6        | 96                          | 90      | 98    | 15                     | 0.48  | 0.95 | 31250  | 31250     | 4.5   | 12    | 8     |
| Lithostathine-1-alpha (REG1A)  | P05451     | 5.7                        | 6.8    | 8.5        | 98                          | 92      | 101   | 15                     | 0.03  | 0.03 | 1953   | 31250     | 4.8   | 12    | 8     |

| Target  | UniProt No | Sample types               |        |            | Endogenous interference     |         |       | Analytical measurement |       |      |         | Precision |       |       |       |
|---|------------|----------------------------|--------|------------|-----------------------------|---------|-------|------------------------|-------|------|---------|-----------|-------|-------|-------|
|   |            | Normal plasma levels (NPX) |        |            | Relative to EDTA plasma (%) |         |       | (mg/mL)                | pg/mL |      |         | log10     | % CV  |       |       |
|   |            | 10th %tile                 | Median | 90th %tile | ACD                         | Heparin | Serum | Hemolysate             | LOD   | LLOQ | ULOQ    | Hook      | Range | Intra | Inter |
| Liver carboxylesterase 1 (CES1)                                     | P23141     | 1.6                        | 2.2    | 3.1        | 143                         | 459     | 107   | 15                     | 15    | 31   | 125000  | 125000    | 3.6   | 17    | 12    |
| Low affinity immunoglobulin gamma Fc region receptor II-a (FCGR2A)  | P12318     | 2.9                        | 3.5    | 4.4        | 98                          | 93      | 101   | 15                     | 1.9   | 1.9  | 3906    | 15625     | 3.3   | 12    | 9     |
| Low affinity immunoglobulin gamma Fc region receptor III-B (FCGR3B) | Q75015     | 3.5                        | 4.4    | 5.4        | 95                          | 90      | 97    | 15                     | 0.95  | 0.95 | 7812    | 31250     | 3.9   | 12    | 8     |
| L-selectin (SELL)   | P14151     | 7.7                        | 8.5    | 9.0        | 98                          | 94      | 97    | 15                     | 0.48  | 0.48 | 3906    | 15625     | 3.9   | 13    | 7     |
| Lymphatic vessel endothelial hyaluronan acid receptor 1 (LYVE1)     | Q9Y5Y7     | 4.8                        | 5.4    | 6.1        | 99                          | 94      | 100   | 15                     | 0.95  | 0.95 | 7812    | 15625     | 3.9   | 12    | 9     |
| Lysosomal Pro-X carboxypeptidase (PRCP)                             | P42785     | 0.6                        | 1.8    | 2.2        | NA                          | NA      | NA    | 15                     | 7.6   | 7.6  | 31250   | 62500     | 3.6   | 13    | 14    |
| Mannose-binding protein C (MBL2)                                    | P11226     | 7.8                        | 9.9    | 11         | 98                          | 90      | 92    | 15                     | 0.24  | 0.24 | 3906    | 7812      | 4.2   | 12    | 10    |
| Mast/stem cell growth factor receptor Kit (KIT)                     | P10721     | 4.5                        | 4.9    | 5.4        | 97                          | 92      | 101   | 15                     | 0.95  | 0.95 | 3906    | 7812      | 3.6   | 11    | 9     |
| Membrane cofactor protein (CD46)                                    | P15529     | 2.3                        | 2.8    | 3.2        | 98                          | 91      | 104   | 15                     | NA    | NA   | NA      | NA        | NA    | 16    | 11    |
| Membrane primary amine oxidase (AOC3)                               | Q16853     | 2.9                        | 3.6    | 4.0        | 97                          | 91      | 97    | 15                     | 3.8   | 7.6  | 15625   | 15625     | 3.3   | 10    | 5     |
| Metalloproteinase inhibitor 1 (TIMP1)                               | P01033     | 5.0                        | 5.7    | 6.2        | 86                          | 104     | 167   | 15                     | 0.95  | 0.95 | 7812    | 15625     | 3.9   | 13    | 8     |
| Microfibrillar-associated protein 5 (MFAP5)                         | Q13361     | 1.5                        | 2.0    | 2.5        | 96                          | 82      | 89    | 15                     | 61    | 122  | 62500   | 125000    | 2.7   | 13    | 10    |
| Multiple epidermal growth factor-like domains protein 9 (MEGF9)     | Q9H1U4     | 2.3                        | 3.0    | 3.4        | 97                          | 89      | 92    | 15                     | 0.95  | 1.9  | 31250   | 62500     | 4.2   | 12    | 9     |
| Neural cell adhesion molecule 1 (NCAM1)                             | P13591     | 2.8                        | 3.4    | 3.9        | 101                         | 93      | 99    | 15                     | 15    | 31   | 62500   | 125000    | 3.3   | 12    | 9     |
| Neural cell adhesion molecule L1-like protein (CHL1)                | O00533     | 3.3                        | 4.0    | 4.7        | 100                         | 93      | 99    | 15                     | 61    | 61   | 125000  | 125000    | 3.3   | 12    | 8     |
| Neurogenic locus notch homolog protein 1 (NOTCH1)                   | P46531     | 2.9                        | 3.4    | 3.8        | 101                         | 97      | 100   | 15                     | 0.95  | 1.9  | 31250   | 31250     | 4.2   | 11    | 7     |
| Neuropilin-1 (NRP1)   | O14786     | 1.8                        | 2.1    | 2.5        | 100                         | 91      | 99    | 15                     | 15    | 31   | 15625   | 31250     | 2.7   | 9     | 7     |
| Neutrophil defensin 1 (DEFA1)                                       | P59665     | 0.5                        | 1.1    | 2.1        | 118                         | 101     | 166   | 15                     | 244   | 244  | 62500   | 125000    | 2.4   | 19    | 28    |
| Neutrophil gelatinase-associated lipocalin (LCN2)                   | P80188     | 1.6                        | 2.4    | 3.0        | NA                          | NA      | NA    | 15                     | 3.8   | 3.8  | 15625   | 62500     | 3.6   | 18    | 11    |
| Nidogen-1 (NID1)  | P14543     | 3.1                        | 3.7    | 4.8        | 75                          | 92      | 102   | 15                     | 31    | 61   | 125000  | 125000    | 3.3   | 12    | 9     |
| Oncostatin-M-specific receptor subunit beta (OSMR)                  | Q99650     | 1.6                        | 2.0    | 2.4        | 98                          | 88      | 97    | 15                     | 7.6   | 15   | 62500   | 62500     | 3.6   | 10    | 7     |
| Peptidyl-glycine alpha-amidating monooxygenase (PAM)                | P19021     | 2.1                        | 2.6    | 3.1        | 109                         | 97      | 106   | 15                     | 244   | 244  | 250000  | 500000    | 3.0   | 11    | 8     |
| Phospholipid transfer protein (PLTP)                                | P55058     | 1.2                        | 1.7    | 2.3        | 94                          | 86      | 98    | 15                     | 7.6   | 7.6  | 125000  | 500000    | 4.2   | 15    | 12    |
| Plasma serine protease inhibitor (SERPINA5)                         | P05154     | 8.0                        | 8.7    | 9.3        | 97                          | 155     | 171   | 15                     | 7.6   | 7.6  | 31250   | 62500     | 3.6   | 11    | 7     |
| Platelet glycoprotein Ib alpha chain (GP1BA)                        | P07359     | 4.1                        | 4.9    | 5.4        | 98                          | 99      | 126   | 15                     | 1.9   | 3.8  | 31250   | 125000    | 3.9   | 14    | 9     |
| Platelet-activating factor acetylhydrolase (PLA2G7)                 | Q13093     | 0.2                        | 1.5    | 1.9        | 101                         | 88      | 98    | 15                     | 122   | 122  | 62500   | 500000    | 2.7   | 13    | 8     |
| Plexin-B2 (PLXNB2)  | O15031     | 1.3                        | 1.7    | 2.0        | 104                         | 97      | 101   | 15                     | 15    | 31   | 31250   | 62500     | 3.0   | 11    | 9     |
| Procollagen C-endopeptidase enhancer 1 (PCOLCE)                     | Q15113     | 5.1                        | 6.2    | 6.6        | 96                          | 74      | 91    | 15                     | 7.6   | 7.6  | 7812    | 31250     | 3.0   | 15    | 8     |
| Prolyl endopeptidase FAP (FAP)                                      | Q12884     | 1.3                        | 1.8    | 2.2        | 97                          | 88      | 94    | 15                     | 1.9   | 3.8  | 15625   | 31250     | 3.6   | 11    | 8     |
| Receptor-type tyrosine-protein phosphatase S (PTPRS)                | Q13332     | 1.3                        | 1.6    | 1.9        | 102                         | 90      | 95    | 15                     | 3.8   | 7.6  | 15625   | 62500     | 3.3   | 10    | 7     |
| Regenerating islet-derived protein 3-alpha (REG3A)                  | Q06141     | NA                         | 0.7    | 1.2        | 96                          | 89      | 87    | 15                     | 7.6   | 7.6  | 7812    | 15625     | 3.0   | 10    | 6     |
| Serum amyloid A-4 protein (SAA4)                                    | P35542     | 3.6                        | 4.5    | 5.3        | 86                          | 88      | 97    | 15                     | 1953  | 1953 | 1000000 | 1000000   | 2.7   | 21    | 11    |
| SPARC-like protein 1 (SPARCL1)                                      | Q14515     | 2.0                        | 2.6    | 3.2        | 102                         | 80      | 83    | 15                     | 31    | 61   | 62500   | 125000    | 3.0   | 12    | 9     |
| Superoxide dismutase [Cu-Zn] (SOD1)                                 | P00441     | NA                         | NA     | 1.5        | NA                          | NA      | NA    | 0                      | 15    | 15   | 15625   | 125000    | 3.0   | 10    | 7     |
| T-cell immunoglobulin and mucin domain-containing protein 4 (TIMD4) | Q96H15     | 3.5                        | 4.4    | 5.1        | 99                          | 92      | 98    | 15                     | 0.95  | 0.95 | 3906    | 7812      | 3.6   | 12    | 9     |
| Tenascin (TNC)  | P24821     | 2.2                        | 2.8    | 3.6        | 96                          | 81      | 98    | 15                     | 0.95  | 0.95 | 15625   | 31250     | 4.2   | 12    | 8     |
| Tenascin-X (TNXB)   | P22105     | 1.0                        | 1.3    | 1.7        | 99                          | 88      | 100   | 15                     | 122   | 122  | 62500   | 125000    | 2.7   | 9     | 6     |
| Thrombospondin-4 (THBS4)  | P35443     | 4.2                        | 5.2    | 5.9        | 104                         | 87      | 96    | 15                     | 15    | 15   | 125000  | 250000    | 3.9   | 12    | 9     |
| Thyroxine-binding globulin (SERPINA7)                               | P05543     | 4.6                        | 5.3    | 5.8        | 96                          | 92      | 97    | 15                     | 122   | 122  | 125000  | 500000    | 3.0   | 12    | 8     |
| Transcobalamin-2 (TCN2)   | P20062     | 3.7                        | 4.2    | 4.7        | 100                         | 89      | 96    | 15                     | 1.9   | 7.6  | 31250   | 62500     | 3.6   | 13    | 12    |
| Transforming growth factor beta receptor type 3 (TGFB3)             | Q03167     | 1.4                        | 3.2    | 3.6        | 96                          | 51      | 51    | 15                     | 61    | 61   | 125000  | 125000    | 3.3   | 14    | 11    |
| Transforming growth factor-beta-induced protein ig-h3 (TGFB1)       | Q15582     | 7.7                        | 8.5    | 9.2        | 94                          | 85      | 91    | 15                     | 15    | 15   | 125000  | 125000    | 3.9   | 14    | 8     |
| Trypsin-2 (PRSS2)   | P07478     | 1.9                        | 2.9    | 3.7        | 100                         | 90      | 95    | 15                     | 7.6   | 7.6  | 3906    | 31250     | 2.7   | 12    | 8     |
| Tyrosine-protein kinase receptor Tie-1 (TIE1)                       | P35590     | 1.6                        | 2.0    | 2.3        | 99                          | 90      | 96    | 15                     | 31    | 61   | 125000  | 125000    | 3.3   | 9     | 7     |
| Uromodulin (UMOD)   | P07911     | NA                         | 1.1    | 1.5        | 105                         | 91      | 100   | 15                     | 7.6   | 7.6  | 62500   | 125000    | 3.9   | 11    | 7     |
| Vascular cell adhesion protein 1 (VCAM1)                            | P19320     | 4.3                        | 4.9    | 5.5        | 99                          | 92      | 96    | 15                     | 7.6   | 7.6  | 31250   | 125000    | 3.6   | 12    | 8     |
| Vasorin (VASN)  | Q6EMK4     | 2.1                        | 2.7    | 3.1        | 97                          | 87      | 98    | 15                     | 3.8   | 7.6  | 31250   | 62500     | 3.6   | 12    | 9     |
| Vitamin K-dependent protein C (PROC)                                | P04070     | 4.3                        | 5.1    | 5.6        | 99                          | 93      | 97    | 15                     | 122   | 122  | 125000  | 125000    | 3.0   | 15    | 12    |

\*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 10 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 92 analytes for which response levels could be measured in serum and normal plasma, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 12.9% and 9.5%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

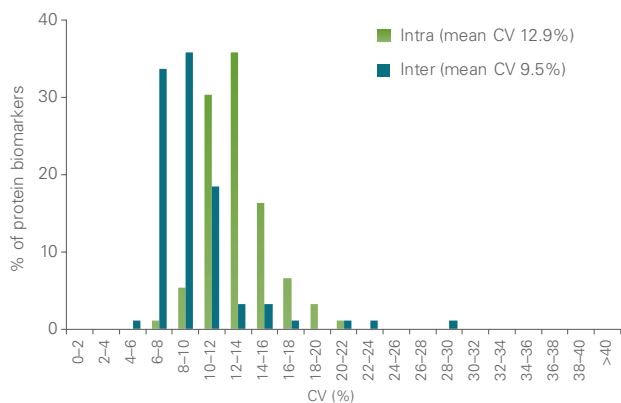


Fig 4. Distribution of intra-assay and inter-assay variations of Olink Cardiometabolic.

### REPRODUCIBILITY

Inter-site variations (between-site) was also investigated during the validation in a beta-site study to estimate the expected increase in variation values produced by introducing a 2025 fold pre-dilution step of samples prior to running the Olink Cardiometabolic assay protocol. Six individual samples were distributed to two laboratories together with Olink Cardiometabolic reagent kits. Each site was instructed to perform the analysis of the 6 individual samples according to the same run design and asked to perform two independent runs. In this document the results from beta-site 2 were excluded due to technical failure which was independent of the performance of the reagent kit. The intra-assay mean CV value results for beta-site 1 was 9.9% and the mean inter-assay CV was 5.4%. Overall, a very good reproducibility and repeatability was observed with an average global inter-site CV of 22.9%.

## 2.4 ANALYTICAL SPECIFICITY

### ASSAY SPECIFICITY

The antibodies used in Olink Cardiometabolic 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.

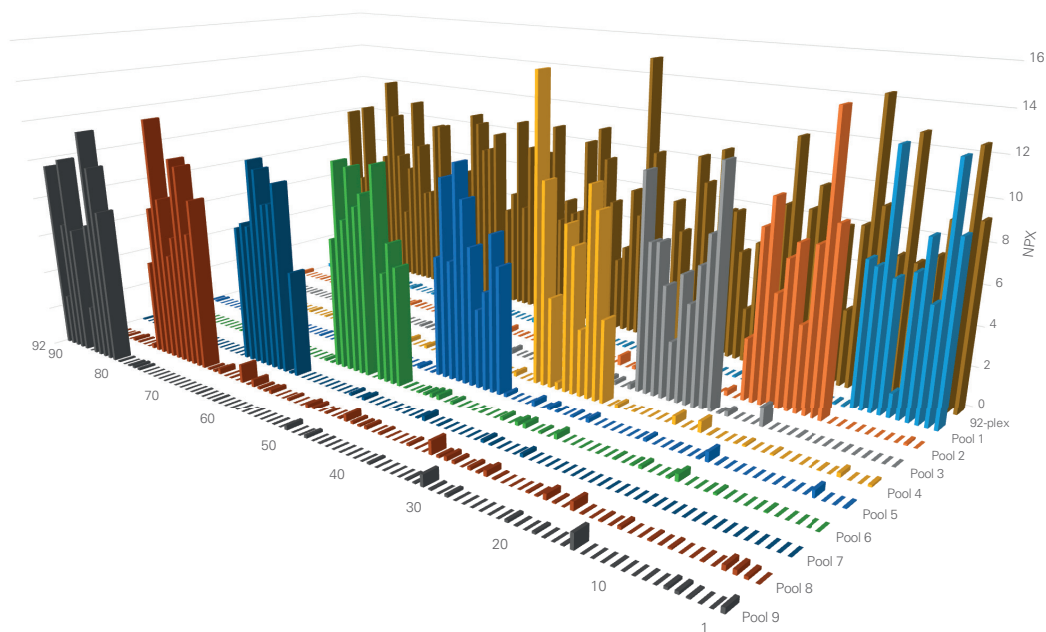
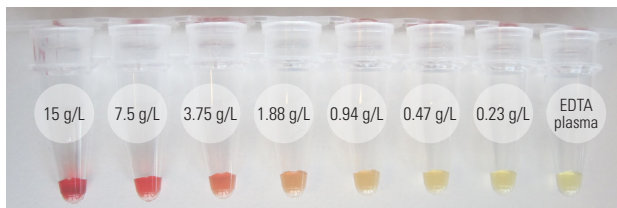


Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

## 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 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).

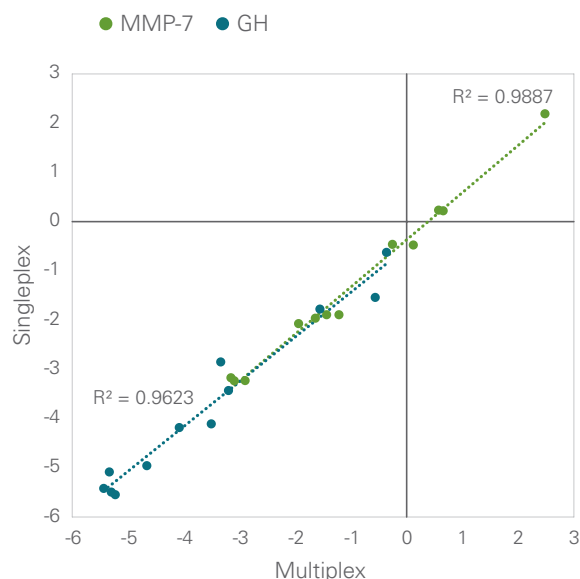


**Fig 6.** Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of bilirubin, lipids and hemolysate, known interfering plasma and serum components, were evaluated 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<sup>3, 4</sup> values and therefore not performed for Olink Cardiometabolic. In 4 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 96x96 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

For technical support, please contact us at [support@olink.com](mailto:support@olink.com) or +46 18 444 3970

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