Humanizing Phenomics 120
psomagen

Raw data Report

2025-01-14

Dr. John Doe

Olink Target 48 Cytokine Panel

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1. Project Information

Customer	Dr. John Doe
Institute	Example University
Project ID	AB00000123
Olink Assay	Target 48
Panel	Cytokines
Species	*Homo sapiens*
Target Proteins	45
Number of Samples	40
Number of Plates	1
Normalization Method	Intensity

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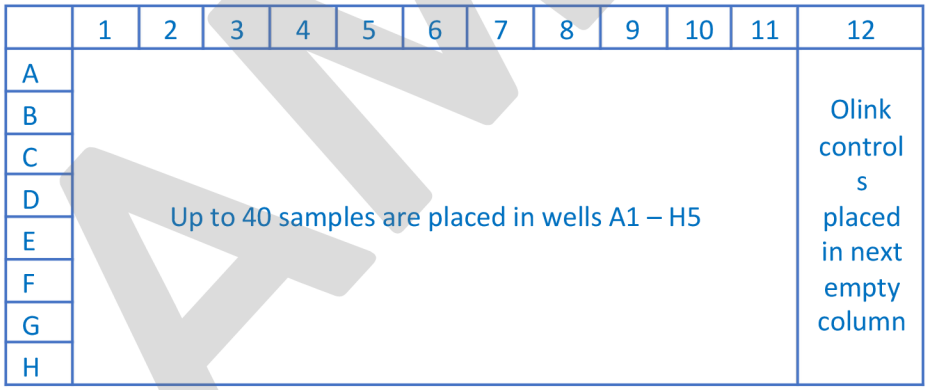
2. Assay Description

The Olink Target 48 assay is designed to measure circulating human protein levels using the Olink Proteomics Proximity Extension Assay technology. The Target 48 Cytokines panel is a manually designed and curated panel of 45 cytokine and chemokine assays selected for broad coverage of inflammatory pathways. The assay is available on a single 96-well plate. Data are presented in both absolute quantification with protein concentrations in pg/ml and normalized protein expression (NPX) values. Results are available for Research Use Only (RUO).

Target 48 Cytokines assays				
CCL2	CSF-2	HGF	IL-15	MMP12
CCL3	CSF-3	IFN-γ	IL-17A	OLR1
CCL4	CXCL8	IL-1β	IL-17C	OSM
CCL7	CXCL9	IL-2	IL-17F	TGFα
CCL8	CXCL10	IL-4	IL-18	TNF
CCL11	CXCL11	IL-6	IL-27	TNFSF10
CCL13	CXCL12	IL-7	IL-33	TNFSF12
CCL19	EGF	IL-10	LTA	TSLP
CSF-1	FLT3LG	IL-13	MMP1	VEGFA



3. List of Acronyms:

NPX – normalized protein expression

PEA – proximity extension assay

IC – incubation (or immuno) control

EC – extension control

AC – amplification (or detection) control

NC – negative control

SC – sample control

4. Workflow Summary

4.1 Sample Reception and Exclusion:

A total of 40 samples were received for this project. Upon inspection, 0 samples were excluded from testing. Therefore, a total of 40 samples were analyzed using the Olink Target 48 Cytokine/Immuno-Surveillance panel.

4.2 Randomization Process:

Total randomization may be applied by either the customer or internally at Psomagen. For Psomagen randomized samples, randomization was conducted using an internally developed randomization tool, placing control and treatment samples on each plate. The randomized samples were then divided into incubation batches of 40 samples each and registered in Psomagen's Laboratory Information Management System (LIMS) under 1 distinct order number(s) for further processing.

4.3 Plate Naming:

Each Olink Target 48 panel processes 40 samples, leading to the generation of 1 unique plate name(s). The plates following a naming convention of [ProjectID]-#, where "#" indicates the plate number in sequential order (1 to n).

4.4 Experimental Procedure:

1. Sample Incubation: The samples were incubated with paired antibodies linked to DNA oligonucleotides for a fixed duration of 18 hours. These antibodies specifically bind to target proteins in the sample.
2. Proximity Extension Assay (PEA): After binding, the oligonucleotides come into proximity, allowing them to hybridize and form new DNA sequences through enzymatic extension.
3. Amplification and Quantification: The new DNA sequences are amplified and quantified using the Q100 instrument.

4.5 Quality Control and Data Analysis:

After all experiments were completed, each plate underwent individual quality control (QC). Upon successful completion of QC, the data were analyzed and consolidated into both Quant.csv and NPX.csv files, which are provided as the final assay results.

5. Data Access and Downloading

5.1 Download Links:

File name	File size	md5sum
AB00000XYZ	xxx	xxxx

Report.zip - This is a zip file of analysis results.

md5sum - In order to verify the integrity of files, md5sum is used. If the values of md5sum are the same, there is no forgery, modification or omission.

Your data will be retained in our server for 3 months. Should you wish to extend the retention period, please contact us.

5.2 Download Instructions:

Data has been transferred via sFTP/Globus/hard drive. Log-in instructions are provided below:

5.3 Folder and Data Structure:

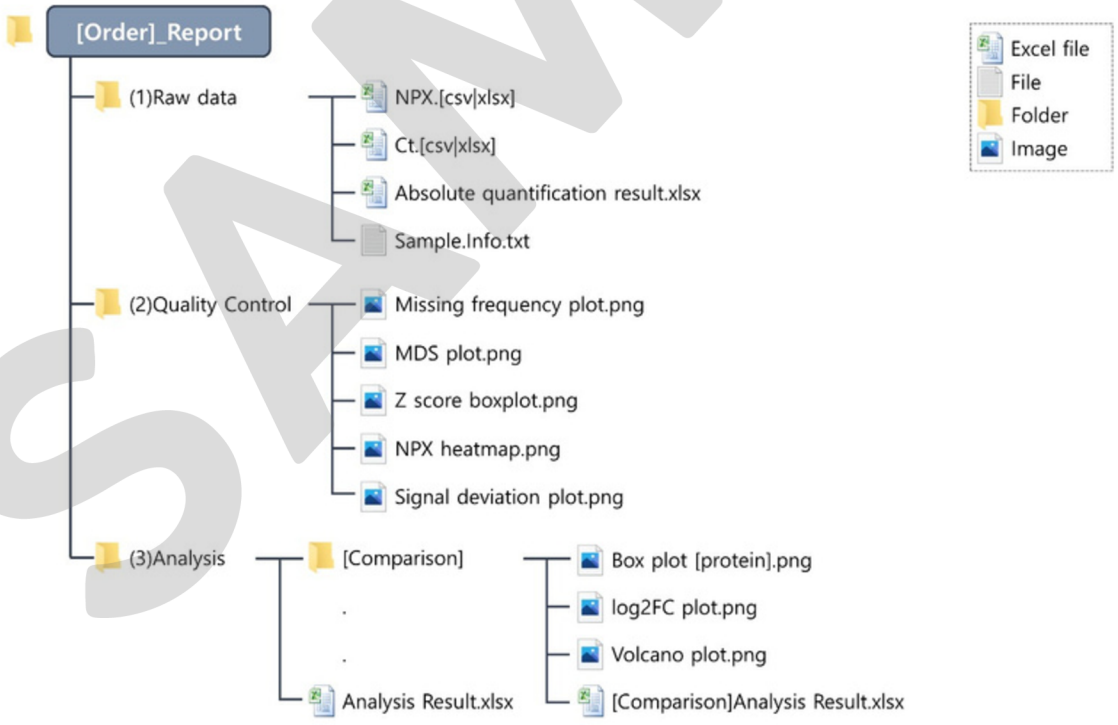


Figure 1: Example data organization.

5.4 Provided Files:

1. Normalized data:
    a. Target_48_quant.csv
    b. Target_48_npx.csv
    c. Sample.Info.csv

2. Quality Control:
    a. MDS plot(multidimensional scaling plot) PlateID.png
    b. MDS plot(multidimensional scaling plot) PlateID.txt
    c. MDS plot(multidimensional scaling plot) total.png
    d. MDS plot(multidimensional scaling plot) total.txt
    e. Missing_frequency_count.txt
    f. Missing_frequency_prop
    g. Signal deviation plot (Plate 1).png
    h. Signal deviation plot (Plate 1).txt
    i. Total NPX heatmap clustering.png
    j. zscore_boxplot.png

3. Results:
    a. DEP_ttest_results.txt
    b. DEP_volcano.png
    c. DEP_samples_heatmap.png
    d. GSEA_results.txt
    e. GSEA_heatmap.png
    f. Survival_KM_plot.png
    g.

6. Explanation of Quality Controls and Assay Normalization

6.1 Internal Controls:

- **Incubation/Immuno Controls 1 and 2 (IC)** – non-human antigens (GFP) which monitor potential technical variation in all three steps of the reaction.
- **Extension Control (EC)** – an antibody coupled to a unique pair of DNA oligo tags used for data normalization and NPX value generation. The EC monitors variation in the extension, amplification, and sequencing step for each sample.
- **Amplification/Detection Control (AC)** – a synthetic double stranded DNA which monitors the amplification and sequencing steps and does not require any proximity binding or extension steps.

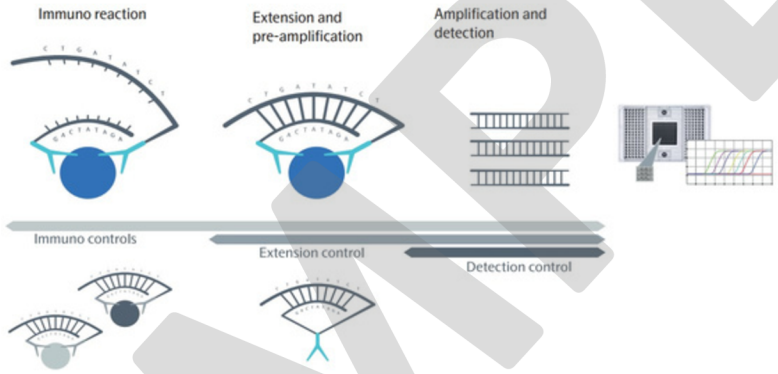


Figure 2: Assay and control design.

6.2 External Controls:

- **Negative Control (NC)** – empty buffer used for monitoring (1) contamination, (2) background noise generated by close-proximity DNA tags without binding the target protein first, and (3) calculating the Limit of Detection (LOD) for each plate.
- **Calibrator** – pooled human plasma samples from healthy donors with spiked-in recombinant antigens for proteins with low endogenous levels to ensure that all 45 proteins are detected within LOQ. The calibrator is used to adjust the predefined standard curve along the y-axis during development at Olink. The calibrator is also included in triplicate on each sample plate and used to normalize all samples. See the Quantified data section below for details. Calibrator replaces plate controls and is present in triplicate on each plate.
- **Sample Control (SC)** – pooled human plasma samples from healthy donors run in triplicate and used to calculate inter and intra Coefficient of Variance.

6.3 Calculation of Normalized Protein Expression (NPX):

The raw output for Olink Target panel is qPCR, where these raw data counts are converted into NPX values for use in further analysis.

NPX values are calculated by first determining the cycle threshold (Ct) value of the qPCR reaction, defined as the number of cycles needed for signal to surpass the fluorescent signal threshold line.

1. $Ct_{(Analyte)} - Ct_{(EC)} = \Delta Ct_{(Analyte)}$

   -Extension Control (EC)
   -Per sample, decreases technical variation

2. $\Delta Ct_{(Analyte)} - \Delta Ct_{(Inter\text{-}PC)} = \Delta\Delta Ct_{(Analyte)}$

   -Normalize by median of interplate control (PC)
   -Per assay, improves inter-plate variation

3. Correction Factor - $\Delta\Delta Ct_{(Analyte)} = NPX_{(Analyte)}$

   -Per assay, calculated during validation of the panels

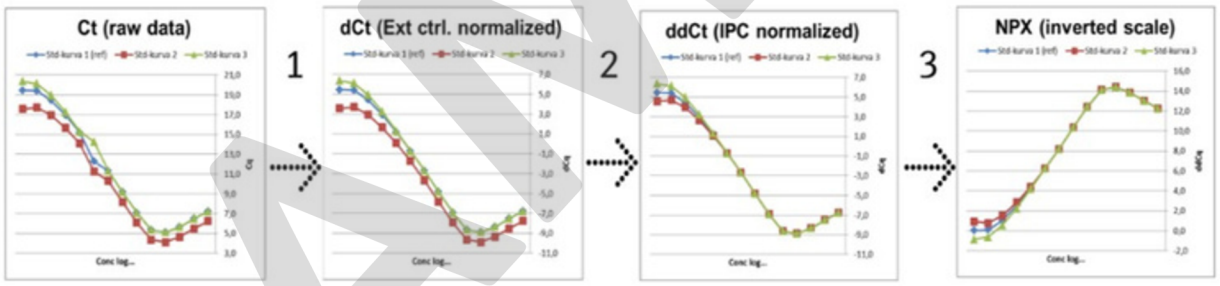


Figure 3: Calculation of NPX values.

6.4 Absolute Quantification Calculation

1. Olink has previously developed a 24-point standard curve for a Four Parameter Logistic regression (4PL) curve fitting for each protein assay within the Target 48 panel.
2. A single Calibrator point is measured in triplicate on each sample plate. The median value on this Calibrator point is used to re-adjust the pre-defined calibration curves on each run.
3. Measured sample values are compared to the adjusted standard curve model, translating the measured value to pg/mL.
4. Accuracy and precision of the quantification values are further validated by the known protein concentrations in the triplicate Sample Controls.

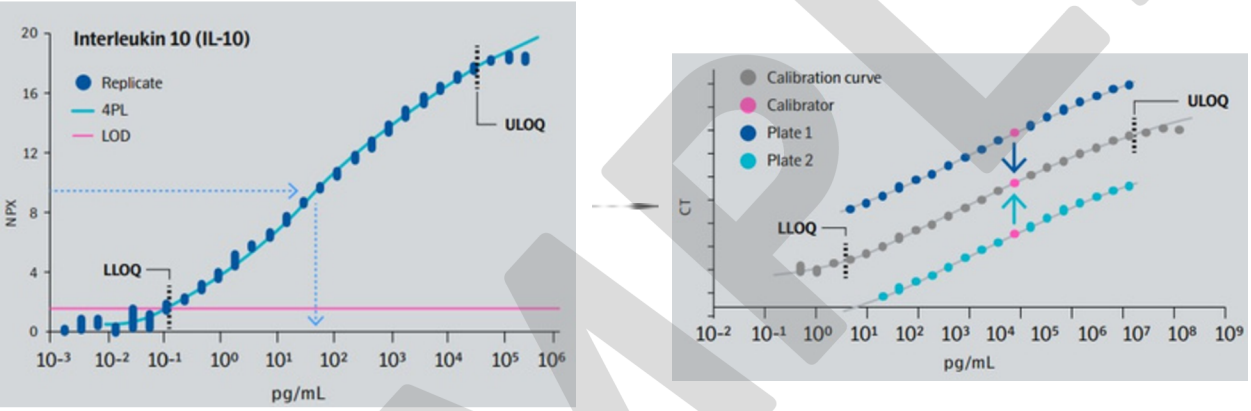


Figure 4: Calculation of absolute quantification values.

6.5 QC Passing:

1. Each plate is evaluated on the number of counts, both total and internal control.
2. Sample quality is determined by the deviation of the ICs and ACs from the plate median for each of those two controls. Samples within ± 0.3 NPX from the plate median pass QC.

6.6 Control QC Warning or Failure:

1. Controls will fail if total assay counts are too low, while samples will be given a warning flag if internal controls have low counts within a block.
2. Plate Controls fail if their internal controls deviate more than ± 30% of the known concentration.
3. Negative Controls fail if assay counts are higher than expected relative to internal controls.
4. A minimum of 2 Sample Control data points should pass for each assay.
5. The precision of the calculated concentration for the Calibrator is evaluated and should have an Intra-CV <30%. If any of the four criteria above is not fulfilled, the assay will be reported as Assay warning: “Warning. Data from assays that do not pass QC should be treated with caution.”

6. The precision of the calculated concentration for the Calibrator is evaluated and should have an Intra-CV <30%. If any of the four criteria above is not fulfilled, the assay will be reported as Assay warning: Warning. Data from assays that do not pass QC should be treated with caution.

7. A minimum of 2 Calibrator data points must be valid for each assay – i.e., only one SC replicate may fall outside the LOD or be failed by the instrument or by the user. Data from assays where the Calibrators do not pass QC according to criteria 5 above is reported as "No data" in the output file and the assay is reported as Assay warning: Fail.

6.7 Sample QC Warning or Failure:

1. Samples with NPX value deviating more than ± 0.3 from the plate median receive a warning.
2. If total assay counts are too low, while samples will be given a warning flag if internal controls have low counts within a block.

In sample data file (ProjectID_npx.csv), two columns, SampleQC and AssayQC, indicate the QC status of samples and assays per plate and block (PASS, WARN, FAIL, or NA). “NA” refers to excluded assays, internal controls, or assays where QC cannot be performed.

7. Project QC Results

7.1 QC Summary:

	Total samples (n)	Samples passed QC (n)	Passed samples (%)
Negative Controls	2	2	100
Calibrator	3	3	100
Sample Controls	3	3	100
Samples	40	38	95

7.2 Assay QC Summary:

Plate	Total assays (n):	Assays passed QC (n):	Passed assays (%):
ProjectID_plate1	45	42	93.3

7.3 Project Percent Coefficient of Variance (%CV) Changes

CV measures sample dispersion, or variation, around the mean within a dataset, with intra-%CV measuring variation within a plate and inter-%CV measuring variation between plates. Note that inter-%CV is omitted in projects with only one plate.

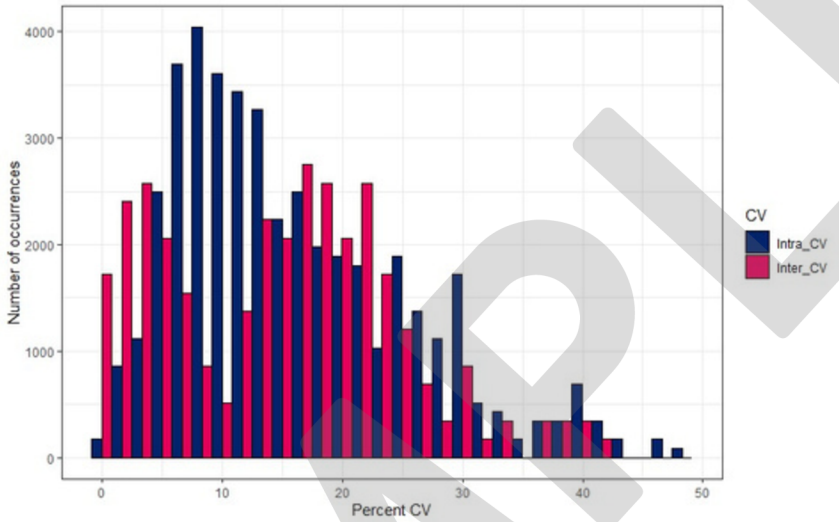


Figure 5: Project Intra- and Inter-%CV.

7.4 Signal deviation from median

Incubation and Amplification Controls are calculated per sample by deviation from the median value of each control. It is possible for individual samples to fall outside the proscribed range of ± 0.3 NPX from the median and still pass QC. This is determined by the fraction of samples within and outside the range. Red colored sample indicates flag (QC warning) sample and grey colored sample indicates the sample that does not have any NPX value.

Calculation of standard deviations for Incubation and Amplification Controls should be within the pre-determined quality threshold (< 0.3). The Signal deviation plots are provided as individual files for each plate run to assess the quality evaluation of both the run and the sample. In this report, only one plot is shown as an example:

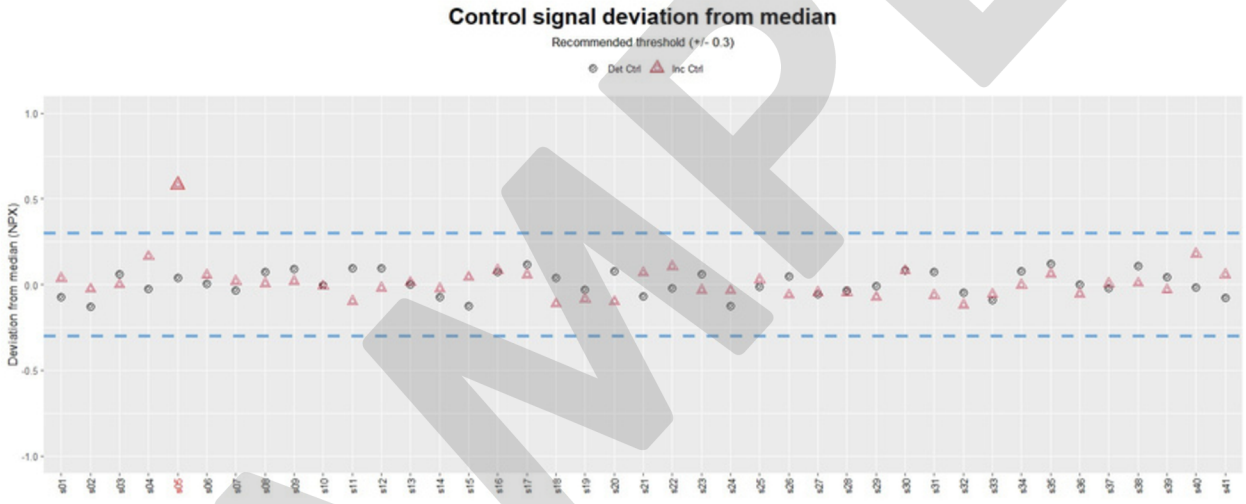


Figure 6: Example signal deviation plot

7.5 Missing frequency plot

Missing frequencies indicate the level of detectability of each target protein based on the limit of detection (LOD) of the target protein. Olink recommends that assays with a large proportion of samples below LOD are excluded from the analysis. The limit for exclusion should be decided on a study basis and consider design including sample size and experimental variables. Suitable exclusion limits may be in the range of less than 25-50% of samples above LOD.

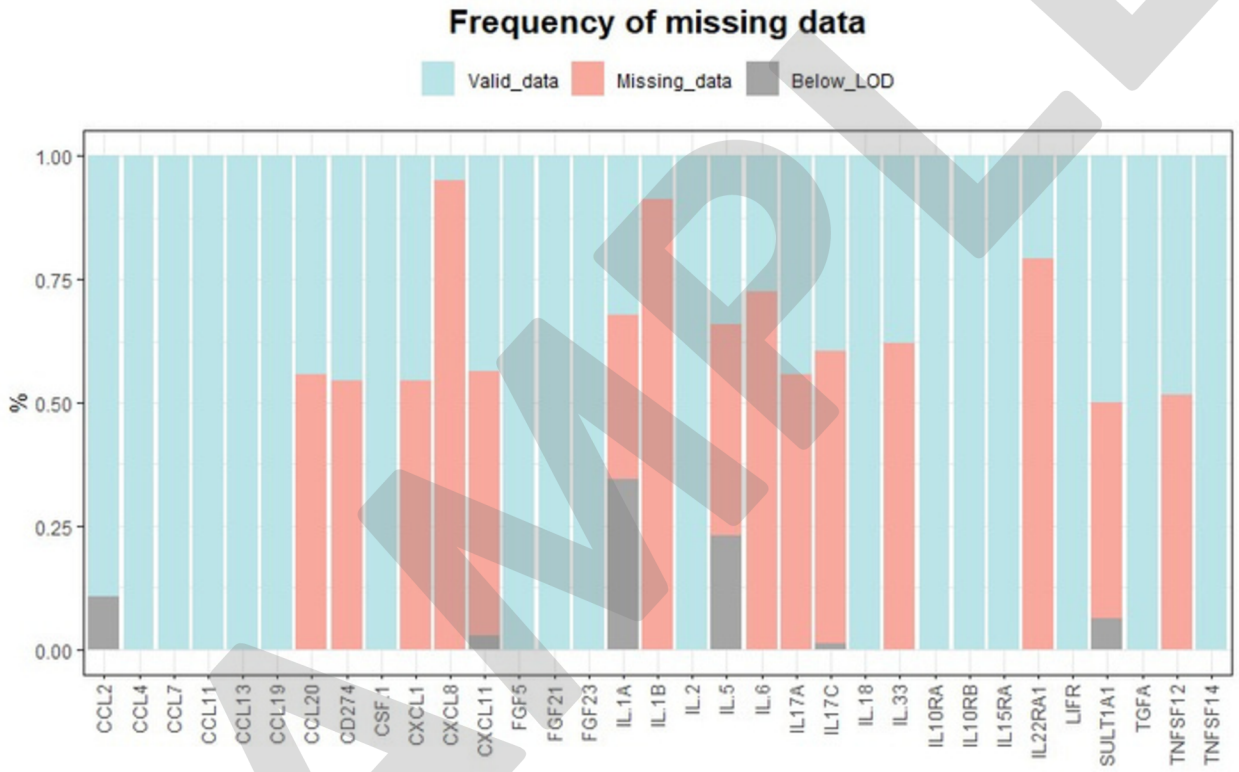


Figure 7: Frequency of missing data.

7.6 Z-score box plot

A visualization of the NPX value from every sample and plate. Each boxplot represents a separate plate where median and quartile values indicated by box edges and error bars are standard deviation.

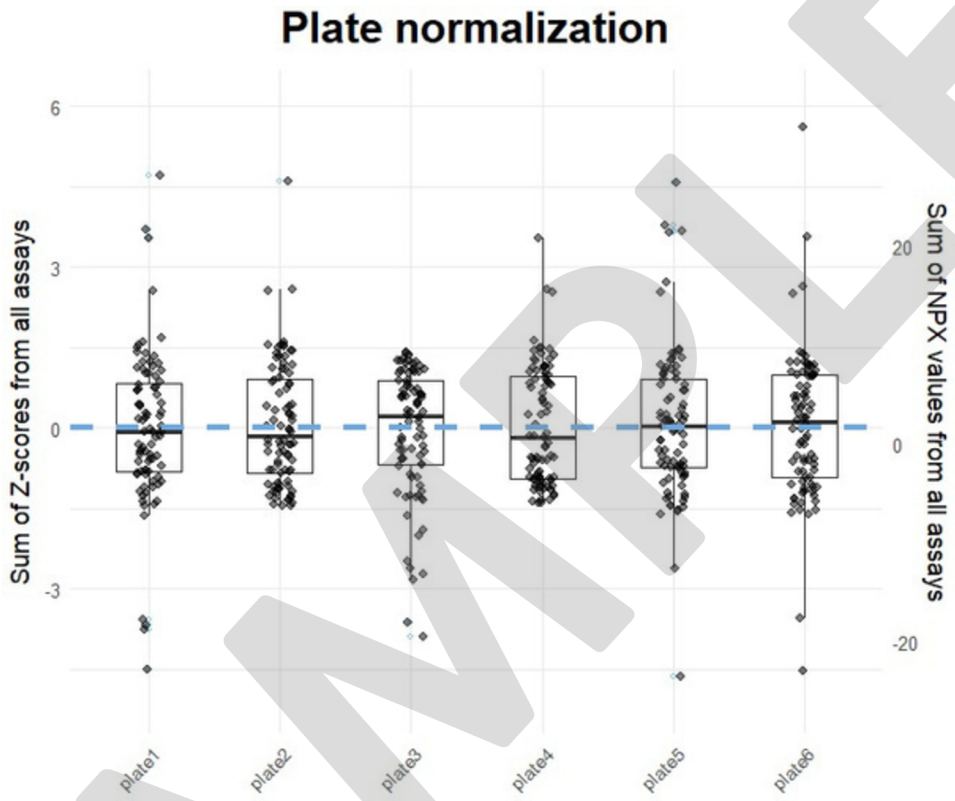


Figure 8: Project Z-score variation of NPX values.

7.7 Principal Component Analysis (PCA) of Controls and Samples

Multi-dimensional spatial groupings of controls and samples showing variation within the dataset.

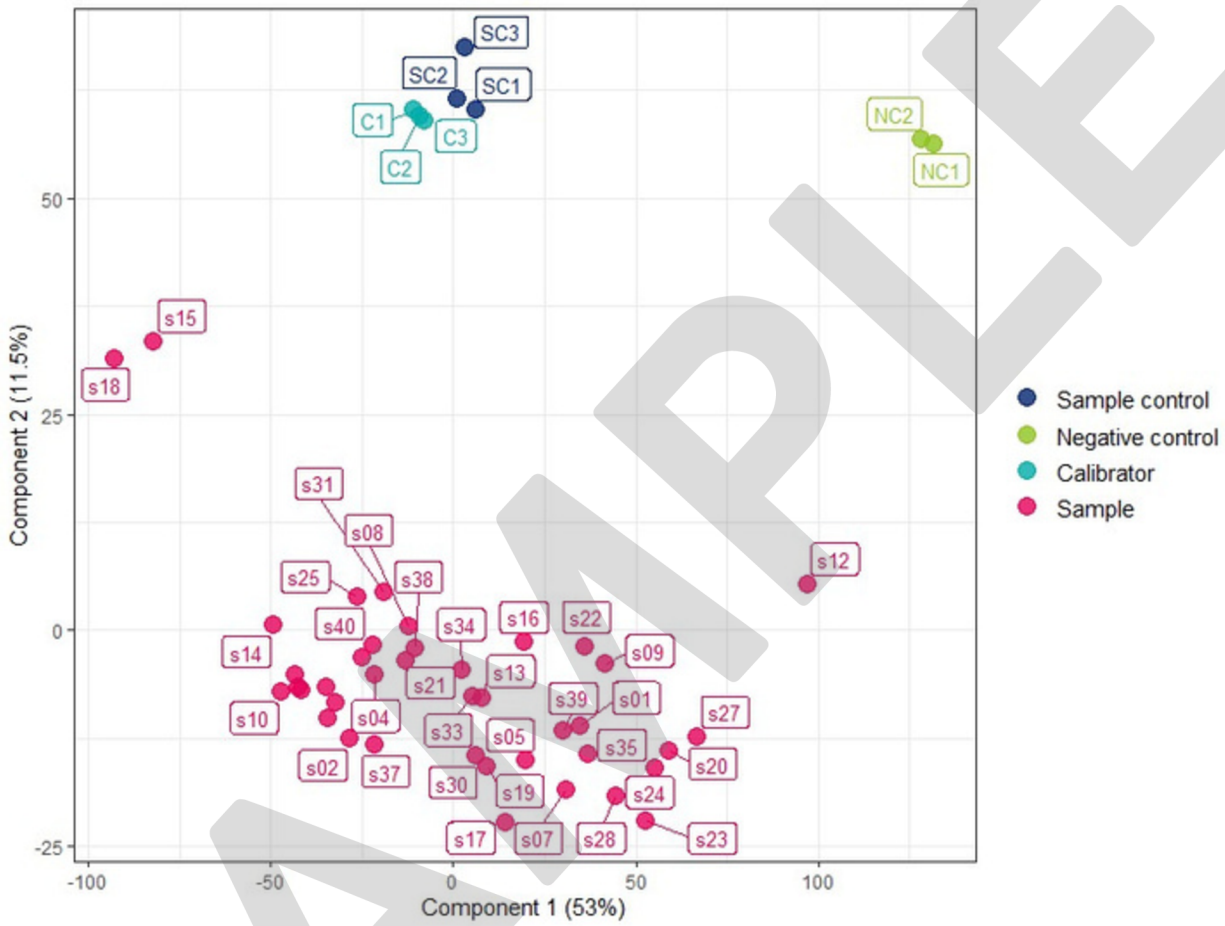


Figure 9: PCA plot of samples and controls.

The previous section included example QC output files of Olink panel. These primary analyses and output files are included free-of-charge for customers.

The below section is an example output of secondary data analysis available from Psomagen, at an additional cost.
Customers who may be interested in such analysis are encouraged to contact our bioinformatics department at "inquiry@psomagen.com" to discuss specific requirements or outputs, referring to Quote or Project ID number. Metadata or further information regarding experimental design may be required depending on analysis type(s) requested.

8. Sample results overview

8.1 Differentially Expressed Proteins (DEPs)

Samples are tested for DEPs based on experimental design [treatment versus control]. The full expression table is provided in /Results/DEP_[ttest]_results.txt/

Protein	UniProt.ID	Treated	Untreated	p.value	adj.p.value	method	alternative
TRAIL	P50591	7.4	10	1.85E-06	0.000171	Sample t-test Welch Two	two.sided
SERPINA7	P05543	9.06	12.3	1.86E-06	0.000171	Sample t-test Welch Two	two.sided
CXCL11	O14625	5.62	3.9	2.96E-05	0.00181	Sample t-test Welch Two	two.sided
MMP-10	P09238	9.2	11.3	8.49E-05	0.0039	Sample t-test Welch Two	two.sided
CD6	Q8WWJ7	2.73	1.84	1.06E-04	0.0039	Sample t-test Welch Two	two.sided
Flt3L	P49771	6.13	4.17	1.57E-04	0.00481	Sample t-test Welch Two	two.sided
DPP4	P27487	4.23	6.22	2.37E-04	0.00622	Sample t-test Welch Two	two.sided
TWEAK	O43508	8.13	10.1	4.36E-04	0.01	Sample t-test Welch Two	two.sided
EFEMP1	Q12805	2.09	2.78	1.66E-03	0.0329	Sample t-test Welch Two	two.sided
REG3A	Q06141	7.23	9.49	2.00E-03	0.0329	Sample t-test Welch Two	two.sided
DEFA1	P59665	4.31	3.47	2.01E-03	0.0329	Sample t-test Welch Two	two.sided
ICAM1	P05362	2.5	3.09	2.15E-03	0.0329	Sample t-test Welch Two	two.sided
IL-22 RA1	Q8N6P7	8.99	11.5	2.72E-03	0.0385	Sample t-test Welch Two	two.sided
TCN2	P20062	5.54	8.46	3.49E-03	0.0459	Sample t-test	two.sided

DEPs were visualized by volcano plot with significantly overexpressed proteins labeled in pink and significantly downregulated proteins labeled in navy (NPX values).

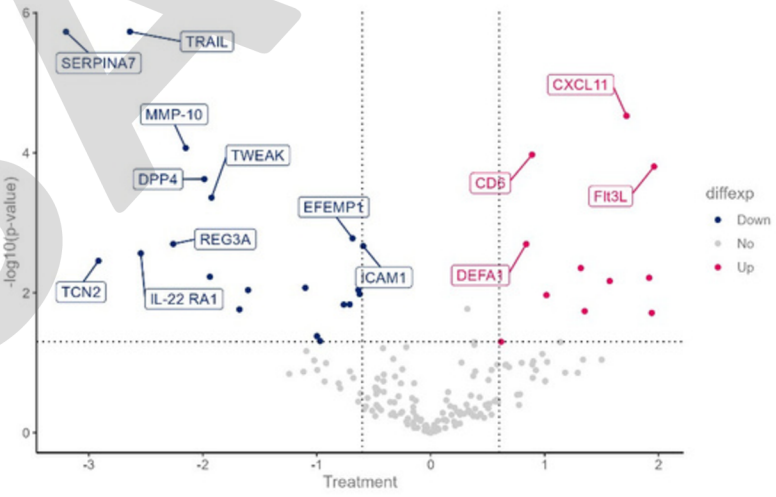


Figure 10: Volcano plot of DEPs.

DEPs were visualized by heatmap and classified by treatment group.

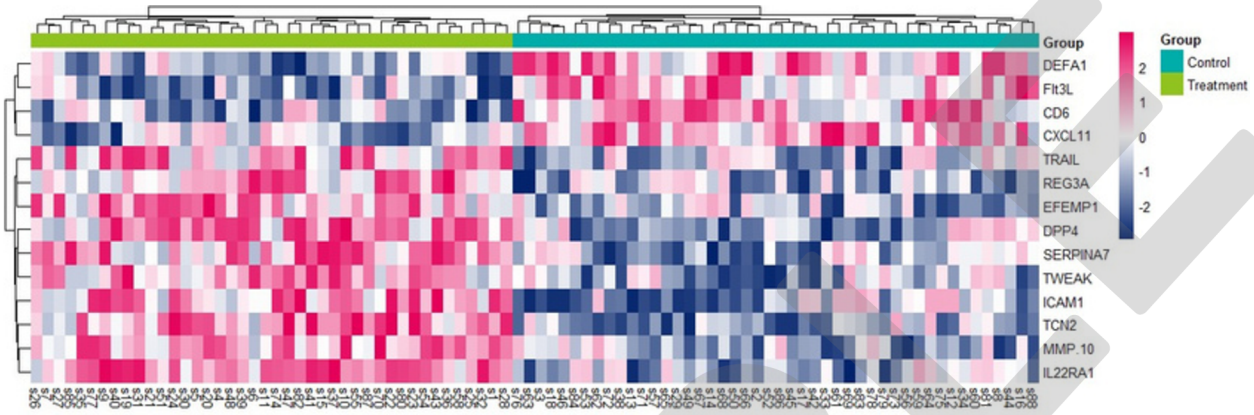


Figure 11: Significant differentially expressed proteins across samples.

8.2 GSEA Pathway Enrichment

DEPs were passed through GSEA for pathway enrichment scores. The top 10 differentially enriched pathways are provided below in table and heatmap format. The full table is provided in /Results/gsea_results.txt/

ID	Set Size	Enrichment Score	NES	P value	P adjust	Q value	rank
LEE_BMP2_TARGETS_UP	18	-0.73251	-1.93225	0.000401	0.224638	0.216615	28
WP_ALLOGRAFT_REJECTION	14	0.689654	1.914638	0.002998	0.29257	0.282121	8
GOBP_RESPONSE_TO_LIPID	26	0.592278	1.891845	0.002019	0.29257	0.282121	40
GOBP_RESPONSE_TO_MOLECULE_OF_BACTERIAL_ORIGIN	22	0.610721	1.87099	0.003738	0.29257	0.282121	30
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	14	0.668808	1.856767	0.005498	0.29257	0.282121	40
GOBP_NEGATIVE_REGULATION_OF_NUCLEOBASE_CONTAINING_COMPOUND_METABOLIC_PROCESS	10	0.721473	1.79663	0.00733	0.29257	0.282121	13
GOMF_G_PROTEIN_COUPLED_RECEPTOR_BINDING	19	0.602718	1.795635	0.006249	0.29257	0.282121	58
GOMF_CHEMOKINE_ACTIVITY	18	0.608482	1.773397	0.008276	0.29257	0.282121	58
GOMF_CHEMOKINE_RECEPTOR_BINDING	18	0.608482	1.773397	0.008276	0.29257	0.282121	58
KEGG_CHEMOKINE_SIGNALING_PATHWAY	18	0.608482	1.773397	0.008276	0.29257	0.282121	58

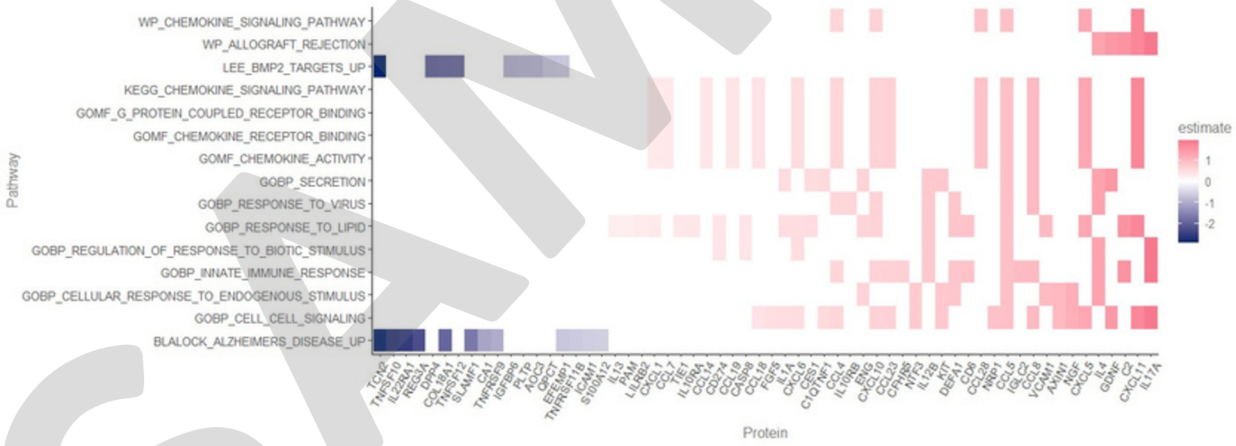


Figure 12: Differentially pathways after GSEA by assay.

8.3 Survival Analysis:

All differentially expressed proteins were checked for significance in survival status based on median NPX value for each protein. Example plots are provided below.

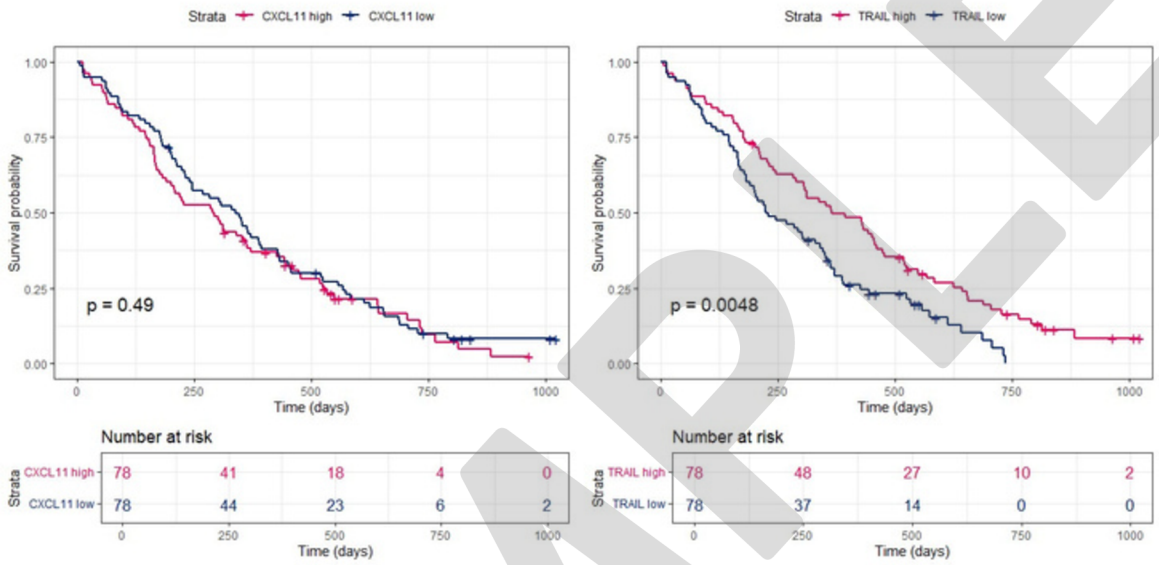


Figure 13: Representative Kaplan-Meier survival plots

8.4 Cox Regression:

Proteins significantly associated with survival were passed to a Cox multivariate regression model with provided metadata categories.

	estimate	std.error	statistic	p.value
TRAIL	-0.05782	0.025411	-2.27554	0.022874
age	0.017178	0.010452	1.643556	0.100268
sex	-0.53844	0.186449	-2.88787	0.003879
ph.ecog	0.377067	0.122942	3.067035	0.002162

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