Analysis Report

Olink® Target 48

PROJECT NAME	Target 48 Example 2 plates
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1. Project information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 48 Cytokine	80	2	Calibrator Normalized

1.1 Sample type

Plasma

2. Quality control

Three internal controls are added to each sample, the Incubation control, the Extension control and the Detection control. The Extension control is used for the data normalization of each sample but is not used as a quality control measure. The Incubation control and the Detection control are used to monitor the quality of assay performance, as well as the performance of individual samples. Three external controls are included in the kit and analyzed on each sample plate, Negative Control, Sample Control (pooled plasma samples with spike in antigens) and Calibrator (pooled plasma samples with spike in antigens). Negative controls are analyzed in duplicates and Sample control and Calibrator are analyzed in triplicates on each plate. The Calibrator is used for data normalization and both Calibrator and Sample Control are used to monitor the quality of assay performance.

Quality control of run and samples:

1. Each run (individual sample plate) is evaluated on the standard deviation of the NPX* values for Incubation- and Detection controls. This should be below 0.2 in samples and 0.5 in external controls. Only data from runs that pass this quality control (QC) will be reported.
2. The quality of each sample is assessed by evaluating the deviation of the Incubation- and Detection controls from the plate median for each of those two controls. Samples that deviate less than 0.3 NPX* from the plate median pass the quality control, samples that deviate more than 0.3 NPX get a QC Warning and results will be reported written in red text. Data from samples that do not pass QC should be treated with caution.

Data from samples that failed in the analysis are reported as "No data".

*NPX (Normalized Protein Expression) is Olink's arbitrary unit for relative quantification of proteins.

Quality control of assays:

1. The accuracy of the calculated mean concentration for the Sample Control for each assay is evaluated and must fall within +/- 30% of the known concentration.
2. The precision of the calculated concentration for the Sample Control is evaluated and must have an Intra-CV <30%.
3. Maximum one of the Calibrator replicates and/or the Sample control replicates can fall outside of limits of quantification (LOQ) (see section 5.3 for specification of LOQ).
4. The precision of the calculated concentration for the Calibrator is evaluated and should have an Intra-CV <30%.

Data from all samples that pass assay QC is included in the data output file.

Data from assays where the Sample Controls or Calibrators do not pass QC according to criteria 1-3 above is reported as "No data" in the output file.

Data from assays where the precision of the calibrator is out of specification (criteria 4 above) is included in the data output file but is indicated as "QC warning" in the row named "Assay warning" and results are shown in red numbers.Data from these assays should be treated with caution.

2.1 Summary of Quality Control of samples

Olink panel	No. of samples that passed QC / Tot no. of samples	Passed samples (%)
Target 48 Cytokine	78 / 80	98

2.2 Summary of Quality Control of assays

Olink panel	Plate	No. of assays that passed QC / Tot no. of assays	Passed assays (%)
Target 48 Cytokine	Target 48 Example plate 1	45 / 45	100
Target 48 Cytokine	Target 48 Example plate 2	45 / 45	100

2.3 Intra- and Inter-Assay Coefficient of Variance (%CV)

The Intra- and Inter-CVs reported below are based on the Sample controls that are analyzed on each plate in triplicate. Calculations are made using the calculated concentration values within limits of quantification in pg/mL. Average %CV for all assays on a panel is presented in section 2.3.1 The number of assays with CVs within defined intervals are presented in sections 2.3.2 and 2.3.3.

2.3.1 Average %CV

Olink panel	Intra-Assay %CV Reference intra CV <15%	Inter-Assay %CV Reference inter CV <25%
Target 48 Cytokine	5	9

2.3.2 Intra-Assay %CV Distribution

Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A*
Target 48 Cytokine	28	15	1	1	0

* Assays where CV is not possible to calculate

2.3.3 Inter-Assay %CV Distribution

Olink panel	<10%	≥10 - <20%	≥20 - <30%	≥30%	N/A*
Target 48 Cytokine	30	15	0	0	0

* Assays where CV is not possible to calculate

3. Protein quantification and detection results

3.1 Number of proteins detected within LOQ in >50% of the samples

Olink panel	No. of quantified proteins / Tot no. of proteins	Quantified proteins (%)
Target 48 Cytokine	41 / 45	91

3.2 Number of proteins detected above LOD in >75% of the samples

Olink panel	No. of detected proteins / Tot no. of proteins	Detected proteins (%)	Expected detectability in EDTA plasma* (%)
Target 48 Cytokine	42 / 45	93	N/A

*The expected detectability is based on EDTA plasma from healthy donors. These values are intended as guidelines only and protein levels may vary depending on different pathological conditions, sample matrices, or sample preparation methods.

3.3 Data output

Data is reported in standard units (pg/mL) as default and as Normalized Protein eXpression (NPX) values upon request. A four-parameter logistic (4PL) curve is generated for the standard curve during the product development. The 4PL-curve is used to calculate the concentration corresponding to the measured NPX values in analyzed samples in each run. Within limits of quantification (LOQ) the 4PL fitting describes the standard curve well with high precision and accuracy and the concentration can be correctly estimated. Outside LOQ the precision and accuracy of the 4PL fitting decreases. The lower and upper limits of quantification (LLOQ and ULOQ) for each assay are defined during the development of the panel. Limit of detection (LOD) for each plate is defined as three fixed standard deviations above average for the negative controls and is indicated as "Plate LOD" in the default results file. For more detailed information see panel specific Validation data and the Data Analysis User guide available at the Olink website (www.olink.com).

The data values are reported in a separate data file in standard concentration units (pg/mL). The data is reported as default in an Excel file as follows:

- Data between LQL* and ULOQ is reported as pg/mL value in white cells.
- Data >ULOQ is indicated as >ULOQ in red cells. Values above ULOQ are not reported in pg/mL due to high risk of misinterpreting hooking data.
- Data below LQL* is presented in pg/mL value in red cells. Values below LQL should be treated with caution due to decreased precision and accuracy in the lower range and should not be used for individual comparison to reference values.
- Data below lowest fitting parameter in the 4PL curve fit model cannot be calculated and is indicated as NaN in red cells.
- For samples and assays with QC warning, values are indicated as described above but marked in red text. Data from samples and assays that do not pass QC should be treated with caution.
- Failed data points (either because of assay failure, sample failure or chip failure) are indicated as No data in grey cells.
- For each plate and assay, values for LQL, LOD, LLOQ and ULOQ as well as "Assay warning" (with results for assay QC) are presented on separate rows below the data for the samples.
- Missing data frequency is reported for each assay and indicates the percentage of samples with values <LQL, >ULOQ and failed data.

* Lowest Quantifiable Level (LQL) is defined as the value used as lower limit, LLOQ (default) or plate LOD (when plate LOD > LLOQ)

Upon request, export of additional data will be presented in an Excel file as follows:

- NPX (Normalized Protein Expression) values
- Values below maximum plate LOD are indicated with red cells.
- Data for samples with QC warning are indicated with red text. Data from samples that do not pass QC should be treated with caution.
- Maximum plate LOD value for each assay is presented on a separate row below the data for the samples and is indicated as LOD.
- Missing data frequency is presented for each assay and indicates the percentage of samples with values below Maximum plate LOD.

4. Samples that did not pass QC

Sample ID	Target 48 Cytokine
Sample#4	x
Sample#5	x