

Appendix 1

PEA QC and Normalization

1. Study information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 96 Cardiometabolic	352	4	Intensity Normalized (v.2)
Target 96 Development	352	4	Intensity Normalized (v.2)
Target 96 Inflammation	352	4	Intensity Normalized (v.2)
Target 96 Neurology	352	4	Intensity Normalized (v.2)
Target 96 Organ Damage	352	4	Intensity Normalized (v.2)

1.1 Sample type

Serum

2. Quality control

Four internal controls are added to each sample to monitor the quality of assay performance, as well as the quality of individual samples. The quality control (QC) is performed in two steps:

1. Each sample plate is evaluated on the standard deviation of the internal controls. This should be below 0.2 NPX. Only data from sample plate that pass this quality control will be reported.
2. The quality of each sample is assessed by evaluating the deviation from the median value of the controls for each individual sample. Samples that deviate less than 0.3 NPX from the median pass the quality control.

Data from all samples is included in the data output file. Samples that did not pass the QC are indicated in columns named "QC Warning". Data points from samples that do not pass QC should be treated with caution. [See 4]

2.1 Summary of Quality Control

Olink panel	No. of samples that passed QC / Tot no. of samples	Normalization Method
Target 96 Cardiometabolic	351/352	Intensity Normalized (v.2)
Target 96 Development	348/352	Intensity Normalized (v.2)
Target 96 Inflammation	347/352	Intensity Normalized (v.2)
Target 96 Neurology	346/352	Intensity Normalized (v.2)
Target 96 Organ Damage	344/352	Intensity Normalized (v.2)

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

Intra and inter CVs are based on control samples (pooled plasma samples) included on each plate. Calculations are made using linear NPX-values. The number of assays with CVs within defined intervals are presented.

2.2.1 Average %CV

Olink panel	Intra-Assay %CV Reference intra CV <15%	Inter-Assay %CV Reference inter CV <25%
Target 96 Cardiometabolic	8	11
Target 96 Development	7	20
Target 96 Inflammation	7	10
Target 96 Neurology	4	12
Target 96 Organ Damage	7	9

2.2.2 Intra-Assay %CV Distribution

Olink panel	No. of proteins with %CV within defined intervals				
	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A
Target 96 Cardiometabolic	5	68	13	1	5
Target 96 Development	31	35	11	4	11
Target 96 Inflammation	19	50	9	1	13
Target 96 Neurology	68	15	2	3	4
Target 96 Organ Damage	20	41	10	0	21

2.2.3 Inter-Assay %CV Distribution

Olink panel	No. of proteins with %CV within defined intervals				
	<10%	≥10 - <20%	≥20 - <30%	≥30%	N/A
Target 96 Cardiometabolic	34	51	2	0	5
Target 96 Development	6	34	38	6	8
Target 96 Inflammation	46	33	3	0	10
Target 96 Neurology	30	57	1	0	4
Target 96 Organ Damage	48	26	1	0	17

3. Protein detection results

3.1 Number of proteins detected in >75% of the samples

Olink panel	No. of detected proteins / Tot no. of proteins	Detected proteins (%)	Expected detectability in EDTA plasma* (%)
Target 96 Cardiometabolic	87/92	95	>90
Target 96 Development	86/92	93	>90
Target 96 Inflammation	78/92	85	>75
Target 96 Neurology	90/92	98	>90
Target 96 Organ Damage	80/92	87	>70

*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.

3.2 Data output

Data is presented as normalized protein expression (NPX) values, Olink Proteomics' arbitrary unit on log2 scale. [See 4]

The NPX values are presented in a separate data file. Data points for samples that did not pass QC are written in red text. Data values for measurements below limit of detection (LOD) are reported for all samples. Cells containing data values below LOD are indicated with a pink background. [See 4]

4. Further information

Collection of direct links to pages containing important information relating to Olink data generation and processing, as well as additional support content:

<https://www.olink.com/key-links/>

5. Samples that did not pass QC

	Target 96 Cardiometabolic	Target 96 Development	Target 96 Inflammation	Target 96 Neurology	Target 96 Organ Damage
Sample ID					
P60	X				
P43				X	
P14		X			
H66		X			
H64		X			
H54				X	X
H5					X
H35			X		X
H24		X			
C92			X	X	X
C69				X	
C49					X
C35			X	X	X
C34					X
C32			X		X
C145			X	X	

5.1 Observed deviations

Data for these samples/assays are set to "No data" in the results output file.

Datapoint	Plate	Reason
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H41_PRCP	Q-04242-010_SP230161_CAM	Datapoint failed
P31_IL-17A	Q-04242-013_SP230159_INFI	Datapoint failed
H15_IL-17A	Q-04242-014_SP230160_INFI	Datapoint failed
P70_IL-17A	Q-04242-014_SP230160_INFI	Datapoint failed
P24_IL-24	Q-04242-013_SP230159_INFI	Datapoint failed
C119_IL-24	Q-04242-013_SP230159_INFI	Datapoint failed
P2_TNNI3	Q-04242-020_SP230160_ODA	Datapoint failed
C82_TNNI3	Q-04242-020_SP230160_ODA	Datapoint failed