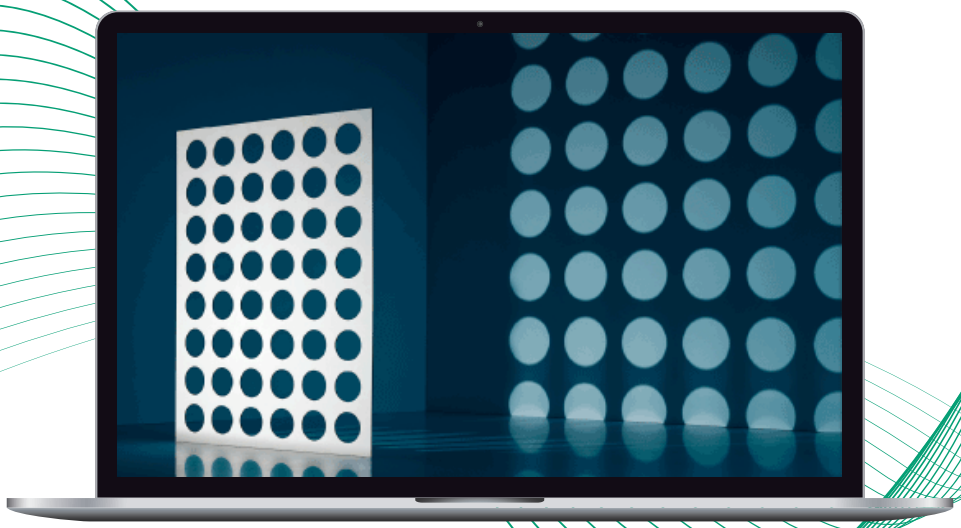
Protein Biomarker Detection with Olink® Proteomics: Example Data Reports



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Analysis Report

Olink® Target 48

STUDY NAME	30-000000001
ISSUE DATE	2/12/2024
CUSTOMER	NGS
	ngs@azenta.com
ANALYSIS LAB	Protein
	protein@azenta.com

1. Study information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 48 Cytokine	40	1	Calibrator Normalized

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.
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, additionally, if the data is exported in the Excel format results will be reported written in red text. Data from samples that do not pass QC should be treated with caution.

Data from samples and datapoints 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. A minimum of 2 Sample Control data points must be valid for each assay. I.e. maximum one of the Sample Control replicates can fall outside of limits of quantification (LOQ) (see section 3.3 for specification of LOQ), be manually failed or failed by the instrument.
4. 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 marked with Assay warning: Warning and if data is

exported in Excel the results from the affected assay/-s will be written in red text. Data from assays that do not pass QC should be treated with caution.

5. A minimum of 2 Calibrator data points must be valid for each assay. I.e. maximum one of the Calibrator replicates can fall outside of limits of quantification (LOQ) (see section 3.3 for specification of LOQ), be manually failed or failed by the instrument.

Data from assays where the Calibrators does not pass QC according to criteria 5 above is reported as "No data" in the output file and the assay is marked with Assay warning: Fail.

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	40 / 40	100

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	Plate1	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	4	N/A

2.3.2 Intra-Assay %CV Distribution

Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A*
Target 48 Cytokine	33	10	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	N/A	N/A	N/A	N/A	N/A

* 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	36 / 45	80

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	37 / 45	82	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 or sample failure) are indicated in grey cells.
- Failed data points (because of 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
-	

5. Observed deviations

Data for failed samples/assays is set to "No data" in the results output file. Assays with QC warning are marked with Warning in the row for Assay warning and data is marked in red text in the results output file.

Datapoint	Plate	Reason
1-4_dil_9 / IL4	Plate1	Datapoint failed
1-4_dil_3 / IL4	Plate1	Datapoint failed
1-4_dil_11 / IL4	Plate1	Datapoint failed
No_dil_19 / IL4	Plate1	Datapoint failed
1-4_dil_13 / IL4	Plate1	Datapoint failed
No_dil_13 / IL4	Plate1	Datapoint failed
No_dil_8 / IL4	Plate1	Datapoint failed
No_dil_9 / IL2	Plate1	Datapoint failed
1-4_dil_13 / IL2	Plate1	Datapoint failed

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Analysis Report

Olink® Target 96

STUDY NAME	30-000000001
ISSUE DATE	2/12/2024
CUSTOMER	NGS
	ngs@azenta.com
ANALYSIS LAB	Protein
	protein@azenta.com

1. Study information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Target 96 Oncology II	88	1	IPC Normalized

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	Passed samples (%)
Target 96 Oncology II	88 / 88	100

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 Oncology II	3	N/A

2.2.2 Intra-Assay %CV Distribution

	No. of proteins with %CV within defined intervals				
Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A
Target 96 Oncology II	74	10	3	2	3

2.2.3 Inter-Assay %CV Distribution

Not applicable.

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 Oncology II	31 / 92	34	>90

*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/

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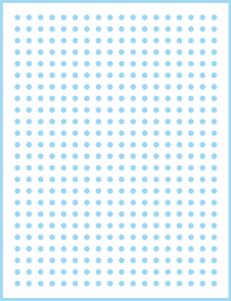
Analysis Report



Olink® Explore

PROJECT NAME	Demo_project
ISSUE DATE	2024-01-31
CONTACT	N/A
	N/A
BUSINESS DEVELOPMENT MANAGER	N/A
	N/A
ANALYSIS LAB	Azenta
	protein@azenta.com

1. Project information

No. of samples	Normalization method
88	Intensity normalization

1.1 Sample matrix

N/A

1.2 Project specific comments

Please note that data for PNLIPRP2 has been plate control normalized because this assay shows a natural bimodal distribution. For more info see https://www.olink.com/faq-category/data/

2. Quality control

Three internal controls are added to each sample, the Incubation control, the Extension Control and the Amplification control. The Extension Control is used for the generation of the NPX values. The Incubation Control and the Amplification Control are used to monitor the quality of assay performance, as well as the quality of individual samples.

Three external controls are included in each run, the Plate Control (healthy pooled plasma), Sample Control (healthy pooled plasma) and Negative Control. The Plate Control is used for data normalization, the Sample Control is used to assess potential variation between runs and plates, and the Negative Control is used to calculate Limit of Detection for each assay and to assess potential contamination of assays.

The following parameters are evaluated in the Quality Control (QC):

1. The average matched counts[1] for each sample. To pass QC, there should be at least 500 counts, otherwise the sample receives a QC warning status.

2. The deviation of the median value of the Negative Controls from a predefined value set for each assay. To pass QC, the deviation of the median of the Negative Controls must be less or equal to 5 standard deviations from the set predefined value, otherwise the assay will receive a warning status.

All samples included in the project are presented in the data output file. Samples that do not pass the QC are indicated with WARN in the column named QC_warning. Data points from samples that do not pass QC should be treated with caution. Manual QC warnings are indicated with MANUAL_WARN in the column named QC_warning. Section 2.1 reports the fraction of samples that pass QC for all assays per panel and the fraction of data points passing QC per panel. Samples with manual QC warning are counted as not passed QC. Assays that do not pass the QC are indicated with WARN in the column named Assay_warning. Data points from assays that do not pass QC should be treated with caution.

[1] The number of reads for each specific combination of sample and assay.

2.1 QC summary

Olink Panel	Samples passed QC	Samples passed QC (%)	Datapoints passed QC	Datapoints passed QC (%)
Explore 384 Cardiometabolic	85 / 88	97	32222 / 32472	99
Explore 384 Inflammation	88 / 88	100	32384 / 32384	100

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

Intra- and inter-CVs are based on the Sample Controls (pooled plasma samples) included on each sample plate. Calculations are made for each assay using NPX-values. Average % CV for all assays on a panel is presented in section 2.2.1. The number of assays with CVs within defined intervals are presented in sections 2.2.2 and 2.2.3.

2.2.1 Average %CV

Olink Panel	Intra-assay %CV	Inter-assay %CV
Explore 384 Cardiometabolic	12	12
Explore 384 Inflammation	27	27

2.2.2 Intra-assay %CV distribution

Olink Panel	≤5%	>5 - 10%	>10 - 15%	>15%	N/A*
Explore 384 Cardiometabolic	101	110	54	74	30
Explore 384 Inflammation	89	27	10	178	64

* Assays where CV is not possible to calculate

2.2.3 Inter-assay %CV distribution

Olink Panel	≤10%	>10 - 20%	>20 - 30%	>30%	N/A*
Explore 384 Cardiometabolic	211	80	20	28	30
Explore 384 Inflammation	116	25	20	143	64

* Assays where CV is not possible to calculate

3. Protein detection results

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

Olink Panel	No. of detected proteins / Total no. of proteins	Detected proteins (%)
Explore 384 Cardiometabolic	343 / 369	93
Explore 384 Inflammation	325 / 368	88

3.2 Data output

Data is presented as NPX values. NPX is Olink's relative protein quantification unit on log2 scale. NPX values are calculated from the number of matched counts, using NGS (Next Generation Sequencing) as readout. The NPX values are presented in a separate results file. Data values for measurements below limit of detection (LOD) are reported for all samples.

4. Software version information

The results presented in this document have been calculated using version 3.6.1 of the Explore calculation module.

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Analysis Report

Olink® Flex

STUDY NAME	30-000000001
ISSUE DATE	2/12/2024
CUSTOMER	NGS
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ANALYSIS LAB	Protein
	protein@azenta.com

1. Study information

Olink panel	No. of Samples	No. of Plates	Normalization Method
Flex Panel (FFRD-XJFP)	40	1	Calibrator Normalized

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.
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, additionally, if the data is exported in the Excel format results will be reported written in red text. Data from samples that do not pass QC should be treated with caution.

Data from samples and datapoints 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. A minimum of 2 Sample Control data points must be valid for each assay. I.e. maximum one of the Sample Control replicates can fall outside of limits of quantification (LOQ) (see section 3.3 for specification of LOQ), be manually failed or failed by the instrument.
4. 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 marked with Assay warning: Warning and if data is

exported in Excel the results from the affected assay/-s will be written in red text. Data from assays that do not pass QC should be treated with caution.

5. A minimum of 2 Calibrator data points must be valid for each assay. I.e. maximum one of the Calibrator replicates can fall outside of limits of quantification (LOQ) (see section 3.3 for specification of LOQ), be manually failed or failed by the instrument.

Data from assays where the Calibrators does not pass QC according to criteria 5 above is reported as "No data" in the output file and the assay is marked with Assay warning: Fail.

2.1 Summary of Quality Control of samples

Olink panel	No. of samples that passed QC / Tot no. of samples	Passed samples (%)
Flex Panel (FFRD-XJFP)	40 / 40	100

2.2 Summary of Quality Control of assays

Olink panel	Plate	No. of assays that passed QC / Tot no. of assays	Passed assays (%)
Flex Panel (FFRD-XJFP)	1243153098_Flex	20 / 21	95

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%
Flex Panel (FFRD-XJFP)	6	N/A

2.3.2 Intra-Assay %CV Distribution

Olink panel	<5%	≥5 - <10%	≥10 - <15%	≥15%	N/A*
Flex Panel (FFRD-XJFP)	6	15	0	0	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*
Flex Panel (FFRD-XJFP)	N/A	N/A	N/A	N/A	N/A

* 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 (%)
Flex Panel (FFRD-XJFP)	13 / 21	62

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 (%)
Flex Panel (FFRD-XJFP)	13 / 21	62

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 or sample failure) are indicated in grey cells.
- Failed data points (because of 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	Flex Panel (FFRD-XJFP)

-

5. Observed deviations

Data for failed samples/assays is set to "No data" in the results output file. Assays with QC warning are marked with Warning in the row for Assay warning and data is marked in red text in the results output file.

Assay	Plate	Reason
IL5	1243153098_Flex	Sample control accuracy out of specifications: 93.28 ±30%

Datapoint	Plate	Reason
SFJ00048-L1 / IL2	1243153098_Flex	Datapoint failed