Olink®

NPX™ Signature

Software User Manual

1606, v2.2, 2025-11-07

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Part 1: Introduction

1. About this manual

This user manual provides you with the instructions needed for processing the raw data and performing quality control on the data from Olink® panels with the NPX™ Signature Software version 2.2 and later. For user manual for NPX Signature Software v1.x, please contact *support@olink.com*.

The NPX Signature Software is a standalone Windows® software application that enables qualification and normalization of Olink mid and low plex data, which can be used in further downstream analysis revealing protein discoveries and signatures. The software enables this through data normalization in NPX, quantitative data and a fit-for purpose quality controls together with result exports.

1.1 Intended use

The NPX Signature Software is a data analysis software that is designed to use with the qPCR-based kits from Olink, including Olink® Target 48, Olink® Target 96, Olink® Focus as well as for Olink® Flex panels. It allows for importing run data, validating data quality, and normalizing Olink data for subsequent statistical analysis.

The kits can be used with the Olink® Signature Q100 qPCR device. NPX Signature Software lets you import data from Olink Signature Q100, validate its quality, and normalize it. The data is not required to be annotated or processed before importing it into the NPX Signature software. The software can also process Fluidigm® Biomark™ data after annotation.

This product is for Research Use Only. Not for use in diagnostic procedures.

1.2 Intended target group

NPX Signature Software is intended to be used by trained users of the Olink® Target 48, Olink® Target 96, Olink® Focus, and Olink® Flex panels. Quality control should be performed by trained users that determine whether data from a run can be approved for further analysis.

1.3 Definition of alert levels

The following alert levels are used in this user manual:

IMPORTANT: *Indicates an important action that may impair the results if not performed correctly.*

NOTE: *Contains information that can make it easier to understand or perform a certain task.*

2. List of abbreviations

%CV	Coefficient of Variation
IFC	Integrated Fluidic Circuit (also called "chip" in this document)
IPC	Inter-Plate Control
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LOQ	Limit of Quantification
LQL	Lowest Quantifiable Level
MAD	Median Absolute Deviation
NPX	Normalized Protein eXpression
PCR	Polymerase Chain Reaction
QC	Quality Control
ULOQ	Upper Limit of Quantification

3. Associated documentation

Manuals

- Olink® Signature Q100 User Manual, doc no 1172
- Olink® Target 48 User Manual, doc no 1141 or 1553
- Olink® Target 48 using Formulatrix F.A.S.T. User Manual, doc no 1414 or 2554
- Olink® Target 96 User Manual, doc no 0935 or 1546
- Olink® Target 96 using Formulatrix F.A.S.T. User Manual, doc no 1415 or 1552
- Olink® Flex User Manual, doc no 1314 or 1555
- Olink® Flex using Formulatrix F.A.S.T. User Manual, doc no 1421 or 1556
- Olink® Focus User Manual, doc no 1231 or 1535

Documents with double document numbers refers to manuals with the old or the new version of PCR Polymerese.

White paper

- "Pre-analytical variation in protein biomarker research", doc no 1095

All relevant Olink documentation is available from the Olink website: *olink.com/knowledge/documents*.

4. Technical support

For questions, guidance, and support, contact Olink Proteomics at *support@olink.com.*

4.1 Log files

The log file registers everything that happens in a project. When contacting the Olink Support, you might need to share the log files. They can be found on the local drive where NPX Signature was installed: `%LocalAppData%\Olink\NPX-Signature\logs`.

Do not move or in any other way manipulate the log files.

5.Software and file requirements

Components	Minimum	Recommended
Operating System	Windows® 11	
Processor	Intel® Core™ i5	
Memory	16 GB RAM	32 GB RAM
Disk Space	500 MB	
Display scale of computer screen		Scale 100 % * 14" screen

* The scale resolution might affect different functions and features in the NPX Signature software, and different scale resolutions might be needed for different displays. Please, go to the computer settings to change to your preferred settings.

6.Installation

6.1 License agreement

You are required to accept the end-user license agreement when installing the NPX Signature Software or before using an updated version for the first time.

6.2 Install software

The installer performs a first-time installation or updates the existing installation if it is already installed on the local computer.

1. Download the latest version of NPX Signature Software to your computer from *https://olink.com/software/download.* The password can be found in the release notes sent by email.
2. Double-click the installer (.msi) file.
3. Follow the on-screen instructions.

A desktop shortcut will be created for future use. To open the application, double-click on the icon.

6.3 Uninstall software

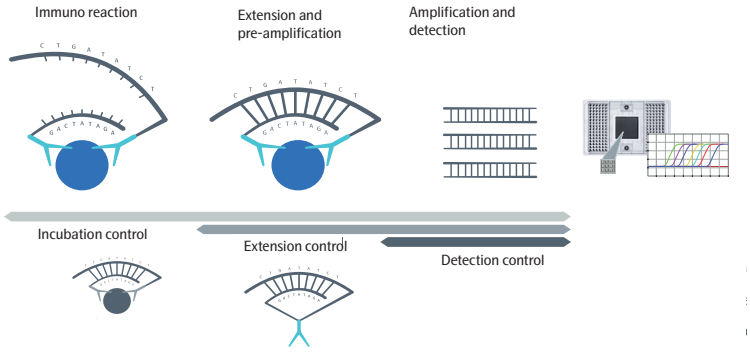
If you need to uninstall NPX Signature, use the **Programs → Uninstall a program** in the Control panel. Select NPX Signature from the program list and click **Uninstall**.

All user data is retained where it was saved before the application was uninstalled, and your custom NPX Signature Software settings are retained on the system.

Part 2: Technology description

7.Overview

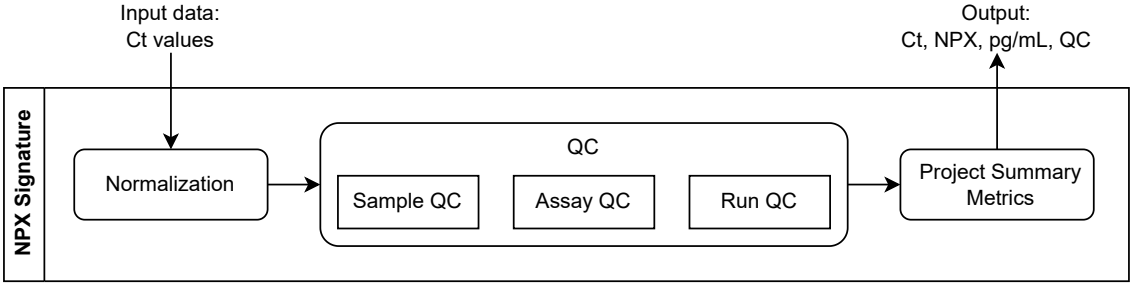
Olink has built-in quality controls in all multiplex panels. The internal controls allow for an in-process quality control designed to monitor different steps of the protocol: immuno reaction, extension and amplification/ detection. Each Olink panel also contains external controls that are added in triplicate or duplicate on each sample plate.
For numbers of assays and controls for the different panels, please refer to the product specific user manual.



- **The Incubation Control(s)** consists of a non-human antigen measured by specific PEA antibodies in the panel. This control monitors potential variation in all three steps of the reaction.
- **The Extension Control** is an antibody which contains two complementary DNA tags that are hence in close proximity. This control monitors the extension/pre-amplification and detection step and is used for normalization of the data.
- **The Detection Control** is a complete double stranded DNA amplicon which does not require any proximity binding or extension step. This control monitors the pre-amplification and detection step.

The Extension Control is used to calculate the NPX value, (refer to *8. Normalization methods*) and the other two are used in Quality Control as described below.

Normalization, Quality Control and calculation of project summary metrics are run automatically in the NPX Signature Software and build on each other.



8. Normalization methods

The raw data from qPCR are Ct values. The Ct refers to the number of cycles required to cross the detection threshold of the PCR amplification curve. Based on Ct values, the arbitrary, relative quantification unit NPX™ is calculated.

8.1 Plate control normalization

In this multi step normalization process, the protein expression values are normalized using the Extension Control, the plate control samples (Calibrators or IPCs) and a pre-determined correction factor. Each of these elements take a different source of potential run-to-run variation into account and reduce its effect.

Each sample is normalized with the Extension Control to reduce between sample variation:

$$dCt_{\text{Sample i, Assay j}} = Ct_{\text{Sample i, Assay j}} - Ct_{\text{Sample i, Ext ctrl}}$$

Each plate is normalized with the central value of its plate control samples (PCs) to reduce differences between runs within the same experiment and the pre-determined correction factor is used to limit technical differences between reagent batches:

$$NPX_{\text{Sample i, Assay j}} = \text{Correction factor}_{\text{Assay j}} - (dCt_{\text{Sample i, Assay j}} - \text{Central Value}(PC_{\text{Assay j}}))$$

The scale of the NPX unit is unique to each protein assay, meaning that even if two different proteins have the same NPX values, their concentrations in pg/mL may differ. It is not meaningful to compare NPX values between protein assays.

The correction factor is determined by Olink during the validation of the panels, and for each new reagent batch, a lot-specific correction factor is defined.

8.2 Calibrator normalization (Olink® Target 48, Olink® Flex, and Olink® Focus)

For products with absolute quantification units, the plate control samples are Calibrators (refer to respective user manual). Calibrator normalization is performed as described in *8.1 Plate control normalization* with the central value calculated for Olink® Target 48 and Olink® Flex as

$$\text{Central Value}(\text{Calibrator}_{\text{Assay j}}) = \text{median}(\text{Calibrator}_{\text{i, Assay j}})$$

While the central value in Olink® Focus is calculated as the mean over all median values per Calibrator type.

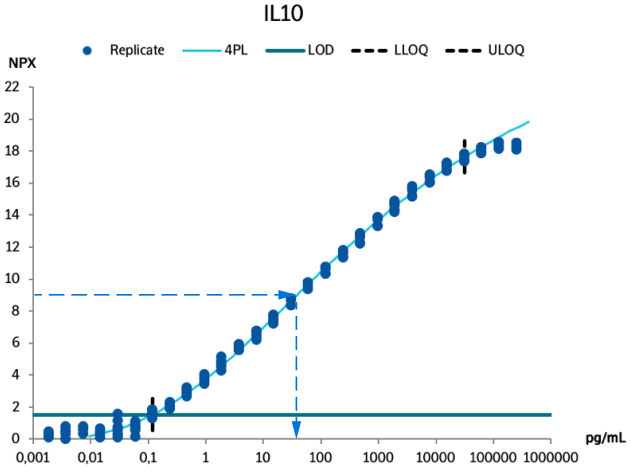
8.2.1 Results in absolute quantification units (pg/mL)

For products that provide output in absolute quantification units, the NPX results from Calibrator normalization can be used to calculate results in standard units, for example pg/mL.

Before the run (during product development at Olink):

1. A correction factor, utilized to ensure accuracy between production batches, is calculated.
2. A precise pre-defined standard curve is established for each protein.
3. A four parameter logistic (4PL) model fit is performed to define the standard curve mathematically within the measurement range for each protein in the panel.
4. The lower and upper limits of quantification (LLOQ and ULOQ) are defined during the development of the panel, see the panel specific Validation data document available on the Olink website (*www.olink.com*) for details.

The illustration below shows an example of a standard curve.



Standard curves for each assay can be found via the panel product page on *www.olink.com*.

After each run:

1. Calibrator normalization is used to normalize the current run to the reference runs used to establish the 4PL curve.
2. The standard curve model, is used to convert the measured NPX value to the protein concentration in pg/mL.

8.3 IPC normalization (Olink® Target 96)

For Olink® Target 96 the plate control samples are IPCs (refer to the Olink® Target 96 User Manual). IPC normalization is performed as described in *8.1 Plate control normalization* with the central value calculated as:

$$\text{Central Value}(IPC_{\text{Assay j}}) = \text{median}(IPC_{\text{i, Assay j}})$$

8.4 Intensity normalization (Olink® Target 96)

In Intensity normalization the data is adjusted so that the median NPX for a protein on each plate is equal across all plates of an experiment. This method relies on the assumption that the true median of each plate is the same. One way of ensuring this, is to randomize the samples across plates. If there is total randomization, this method typically outperforms other normalization methods. If there are specific types of samples that are only available on certain plates, this normalization method should not be used.

Intensity normalization subtracts the central value of all samples (excluding the control strip) for each assay per plate:

$$iNPX_{\text{Sample i, Assay j}} = NPX_{\text{Sample i, Assay j}} - \text{Central Value}(NPX_{\text{Assay j}})$$

> **NOTE:** *Internal Positive Controls (IPCs) are excluded from the normalization process when Intensity Normalization is applied. Despite this, IPCs remain part of the formal QC procedure, as they are included in the external controls provided with the Olink Target 96 kits. IPCs are expected to behave in a specific way. Deviations from expected behavior may indicate underlying issues with the run that are not apparent from customer sample data alone. When certain that the data is not affected, the warning can be ignored.*

QC levels and possible status:

Datapoint	Sample	Assay	Run
• Set by instrument • Can be set to fail manually	• Set as part of automatic QC • Can be set manually	• Set as part of automatic QC • Can be set manually	• Set as part of automatic QC
• Status: – Pass – Flag/Fail • Status other than Pass: – NA data	• Status: – Pass – Warning – Fail • Warning: – Data shown and exported • Fail: – Normalized data not shown or exported	• Status: – Pass – Warning – Fail – Undefined / NA • Warning: – Data shown and exported • Fail: – Normalized data not shown or exported	• Status: – Warning message • No consequences for data presentation

8.5 CV calculation

For evaluating the reproducibility and repeatability of data related to each plate in the project, as well as assessing the within- and between-plate variation in the project, two types of CV are calculated: within plate (intra CV) and between plates (inter CV) in Sample Controls. For the inter CV calculation, the mean of Sample Controls is used to represent the plate before CV calculation. These metrics are calculated for NPX and absolute quantification values using the following formulas:

NPX scale:

$$CV_{assay} = 100 * \sqrt{e^{(sln_{assay})^2} - 1}, \text{where } sln_{assay} = log_e(2) * SD(NPX_{assay})$$

Absolute quantification scale:

$$CV_{assay} = 100 * \frac{SD(QUANT_{assay})}{mean(QUANT_{assay})}$$

8.6 Sample QC

Each of the internal controls are spiked into all samples at a set concentration. The signals for these are therefore expected to be at comparable levels over the entire plate. Sample QC is performed using Detection Control and Incubation Control. If the observed value deviates more than 0.3 NPX from the plate wide median, or if the value is missing, the Sample QC is set to Warning. The Extension Control is used in the normalization step and in generation of NPX, and hence is not included in the Sample QC.

Deviating values for the internal controls can be caused by, for example, errors in pipetting or pre-analytical factors in the samples that affect the performance of the controls. For more information on troubleshooting samples that do not pass this QC, refer to *17. Deviating controls.*

8.7 Assay QC

Assay performance is assessed based on multiple criteria, taking into account availability of control data, variation of measured values and for certain products accuracy. Criteria checked against product specific reference values are listed in the table below. Recommended thresholds are shown in the software user interface.

Sample Control precision	Olink Target 48 Olink Flex Olink Focus	• Use data in NPX units • Include all replicates that “Pass” Sample QC, independent of measured value.
Calibrator/IPC precision	All products	• Use data in NPX units • Include all replicates that “Pass” Sample QC, independent of measured value
Sample Control Accuracy	Products providing results in absolute quantification units	• Use data in absolute quantification units • Include all replicates that “Pass” Sample QC, independent on measured value. pg/mL values for NPX outside of the curve range is set to NA. • Median for point estimate
Minimum number of valid Sample Control replicates	Olink Target 48 Olink Flex Olink Focus	• 2 replicates with Sample Control QC “Pass” and no instrument flag required
Minimum number of valid Negative Control replicates	All products	• 2 replicates with Negative QC “Pass” required
Minimum number of valid Calibrator/IPC replicates	Olink Target 48 Olink Flex Olink Focus	• 2 replicates with Calibrator QC “Pass” and no instrument flag required
Consequences of Assay QC deviations	All products	• Minimum number of valid Calibrator/IPC replicates not reached: Assay Fail, only Ct data provided to user for this assay • All other Assay QC deviations: Assay Warning, all data types provided to user for this assay

8.8 Run QC

In Run QC the performance of the run as a whole is assessed. Warnings are triggered in case variation in internal controls is high, an excessive amount of samples or assays have QC deviations or if control data is missing (see below for details). Recommended thresholds are shown in the software user interface. Run QC supports the user to identify problematic runs, but Run QC deviations could also be triggered by for example mixing different Sample Types on the same plate.

If a too large variation is observed in either of the internal controls, review the data in the Plate Data view (refer to section *14.6 Plate Data*). For troubleshooting guidance, refer to section *17. Deviating controls*.

Run quality issues might also be causing systematic bias as measured by ANOVA tests. Go to the Metrics view for more information, refer to *14.2 Metrics.*

Median absolute deviation of internal controls	All products	• Use data in NPX units. • Separate for project samples and control samples. • The MAD should be less than 0.23 for samples and less than 0.25 for external controls.
Number of warned or failed samples	All products	• Only use project samples. • No more than 1/6 of customer samples are allowed to be warned or failed.
Number of warned or failed assays	All products	• Only use assays (excluding internal controls). • No more than 1/9 of the assays are allowed to be warned or failed.
Minimum number of valid Sample Control replicates	Olink Target 48 Olink Flex Olink Focus	• 2 replicates with Sample QC “Pass".
Minimum number of valid Negative Control replicates	All products	• 2 replicates with Negative Control QC “Pass” required.
Minimum number of valid Calibrator / IPC replicates	All products	• 2 replicates with Calibrator/IPC QC “Pass”.

8.9 QC criteria

Criteria	FAIL	WARN
Sample QC		
Incubation/Detection Control NPX deviation.	N/A	Deviation > 0.3 NPX from plate median
Extension Control	Data missing or >5 failed datapoints	N/A
Assay QC		
Calibrator replicates	< 2 valid replicates	N/A
Negative Control replicates	N/A	< 2 valid replicates
Calibrator precision	N/A	Too low (assay-dependent)
Sample Control replicates	N/A	< 2 valid replicates
Sample Control precision	N/A	Too low (assay-dependent)
Sample Control accuracy	N/A	Too low (assay-dependent; not applicable to panels without absolute quantification)
Intra-Assay %CV	> 15%	N/A
Inter-Assay %CV	> 25%	N/A

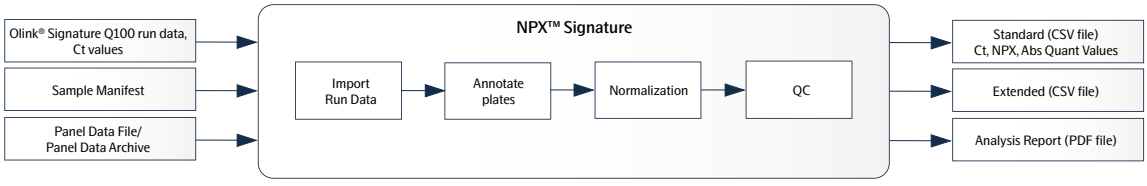
If a run fails to meet the acceptance criteria, or the QC is not accurate, please contact *support@olink.com*.

Part 3: Operation

9.Introduction

Input data for Olink NPX Signature Software are run files from Olink Signature Q100 in .q100 format, Signature Q100 or Biomark runs exported as csv files from Fluidigm realtime PCR analysis software, heat map format, NPX Signature Software (v2.x) .osp project files and Olink NPX Signature Software (v1.x) .npx study files. The output data are obtained in NPX values.

Further normalization against the Calibrators produces data in additional absolute quantification, provided in pg/ml, for the panels where absolute quantification is included.



10.Files and information needed for analysis

10.1 Panel Data Files and Panel Data Archive

The Panel Data Files or Panel Data Archive, depending on product, is needed to properly process and quality control your data. This file is unique to your panel version and reagent batch. The file is provided by Olink at the *olink.com/software* page.

Olink® Target 48 and Olink® Target 96

During a reagent batch release for Olink Target 96 or Olink Target 48, a new Panel Data Archive file will be issued. This file is a collection of all Olink Target Panel Data Files. It includes all previous Olink Target Panel Data Files along with the newly added Panel Data File. For how to import the file, refer to *11.2 Import Panel Data Files or Panel Data Archive.*

NOTE: *For the Olink Target Panels, it is sufficient to import the latest Panel Data Archive as this will include all previous released Panel Data Files.*

The Panel Data Archive file is an encrypted .dat file that is compatible with NPX™ Signature v2.0 and later. The file is not backward compatible.

The file name will have the following format:

```
OlinkTarget_PanelDataArchive_[DATE]_NPXSv2.dat
```

Example:

```
OlinkTarget_PanelDataArchive_2025MAY22_NPXSv2.dat
```

Olink® Flex and Olink® Focus

For NPX Signature v2.0 and later, the Panel Data File will have the file format NPXSv2. The file is not backward compatibe. For how to import the file, refer to *11.2 Import Panel Data Files or Panel Data Archive.*

For Olink Flex, the file name will have the following format:

```
OlinkFlex_PanelDataFile_[panelfilename]_NPXSv2.xml
```

Example:

```
OlinkFlex_PanelDataFile_FXXX-XXXX_NPXSv2.xml
```

For Olink Focus, the file name will have the following format:

```
OlinkFocus_PanelDataFile_[NAME]_NPXSv2.xml
```

Example:

```
OlinkFocus_PanelDataFile_CustomPanelName_NPXSv2.xml
```

10.2 Olink® Signature Q100 Run Data

Olink Signature Q100 run data can be imported directly into NPX™ Signature without any need for annotation or processing in advance. The following file types and formats are supported for import and analysis:

- Olink Signature Q100 export files:
  - Accepted formats include .q100, .csv, .csvx, and .zip
- Fluidigm realtime PCR analysis software exported heat map or table files in .csv format. A csv file is needed to be able to analyze Biomark runs in NPX™ Signature.

10.3 Sample Manifest file

The Sample Manifest is a structured table with four required columns and one row per well of a plate. This document is mandatory for importing individual runs into NPX™ Signature. Product specific templates for the Sample Manifest are available in Microsoft Excel (.xlsx) format and can be downloaded from the Project/Sample Manifest view, refer to *14.1.2 Sample Manifest,* or from the Create Project dialog, refer to *11.3 Create a new project.*

The Sample Manifest can include up to 40 plates.

Using the Sample Manifest file

The Sample Manifest file provides a standardized layout for sample metadata. Although the format is standardized, the sequence of data entries can be rearranged to align with specific workflow requirements:

1. Rearrange the row order as needed to match the preferred layout.
2. Save the customized file for future use.
3. Upload the customized file during project setup.

Required columns

The Sample Manifest must include the following columns:

- WellID:
  - Unique identifier of the well (for example A1–H12).
- SampleID:
  - Unique identifier for the sample.
  - Maximum length: 100 characters.
  - Must include at least one character which is not space.
  - May be reused across different plates for samples classified as External Control or NOT_USED, but must be unique within each individual plate.
  - Must be unique within the entire Sample Manifest for samples classified as Customer Samples.
  - Empty or unused wells do not require a SampleID; the system will assign an internal identifier automatically for processing purposes.
- PlateID:
  - Unique identifier for the plate. This is the ID that will be included in the data export file.
  - Every row for a plate must have the same PlateID.
  - Maximum length: 100 characters.
  - Must include at least one character which is not space.
  - Up to 40 plates can be specified in a single Sample Manifest. If multiple plates are specified, the PlateID for each plate must be different.

- SampleType:
  - Type of sample.
    Refer to *14.1.2 Sample Manifest* for more information about the different sample types.

Sample Manifests used in NPX Signature Software v1.x can be updated to be used in NPX Signature v2.x, if the requirements above are fulfilled.

The following sample types can be defined in the Sample Manifest file:

Sample Type	Olink® Target 96	Olink® Target 48	Olink® Flex	Olink® Focus
NOT_USED	√	√	√	√
SAMPLE	√	√	√	√
NEGATIVE_CONTROL	√	√	√	√
SAMPLE_CONTROL	√	√	√	
SAMPLE_CONTROL_1				√
SAMPLE_CONTROL_2				√
SAMPLE_CONTROL_3				√
SAMPLE_CONTROL_4				√
CALIBRATOR		√	√	
CALIBRATOR_A				√
CALIBRATOR_B				√
CALIBRATOR_C				√
INTERPLATE_CONTROL	√			

10.4 NPX™ Signature Study File (.npx)

Study files previously created in NPX Signature v1.x can be migrated into NPX Signature v2.x. Refer to *11.5 Migration of NPX™ Signature Software v1.x projects* for more information.

11.Operating workflow

This section describes how you analyze data step-by-step in NPX™ Signature Software. The following steps are included in the standard operating workflow:

1. Download Panel Data Files or Panel Data Archive.
2. Import Panel Data Files or Panel Data Archive.
3. Create a new project.
   - Select Panel and Panel Data File version
   - Import Run data
   - Import Sample Manifest
4. Perform quality control.
5. Export result files.
6. Finalize the project.

11.1 Download Panel Data Files or Panel Data Archive

If running an Olink Target 48 or Olink Target 96 project, a panel data archive must be downloaded. Go to *Olink.com/software/download* and download the Panel Data Archive. This Panel Data Archive contains all Olink Target Panel Data Archive.

For Olink Flex and Olink Focus projects, the Panel Data Files are provided by Olink.

For information about the Panel Data Files, refer to *10.1 Panel Data Files and Panel Data Archive.*

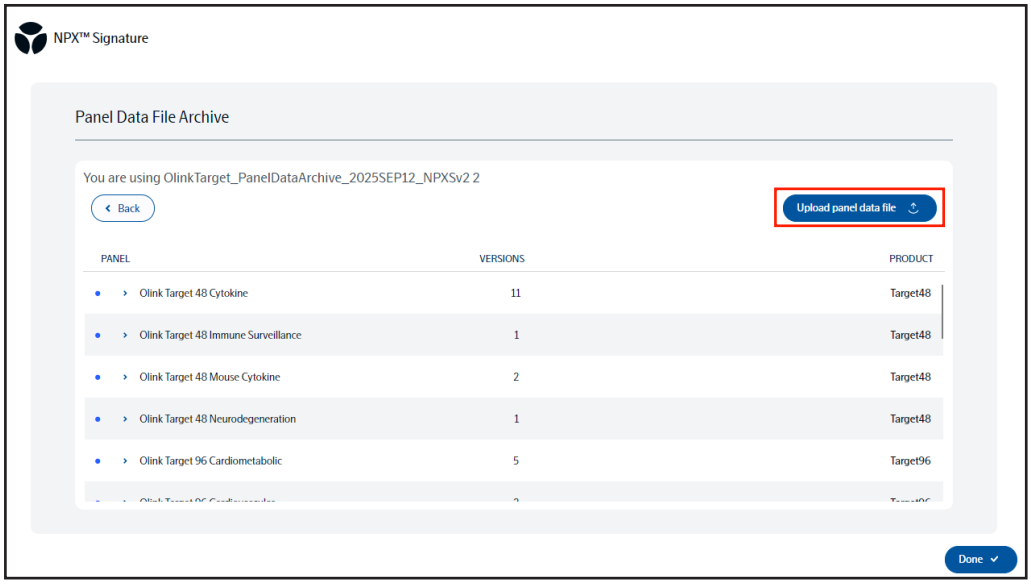
11.2 Import Panel Data Files or Panel Data Archive

Before creating a project, corresponding Panel Data Files must be imported into the NPX Signature Software.

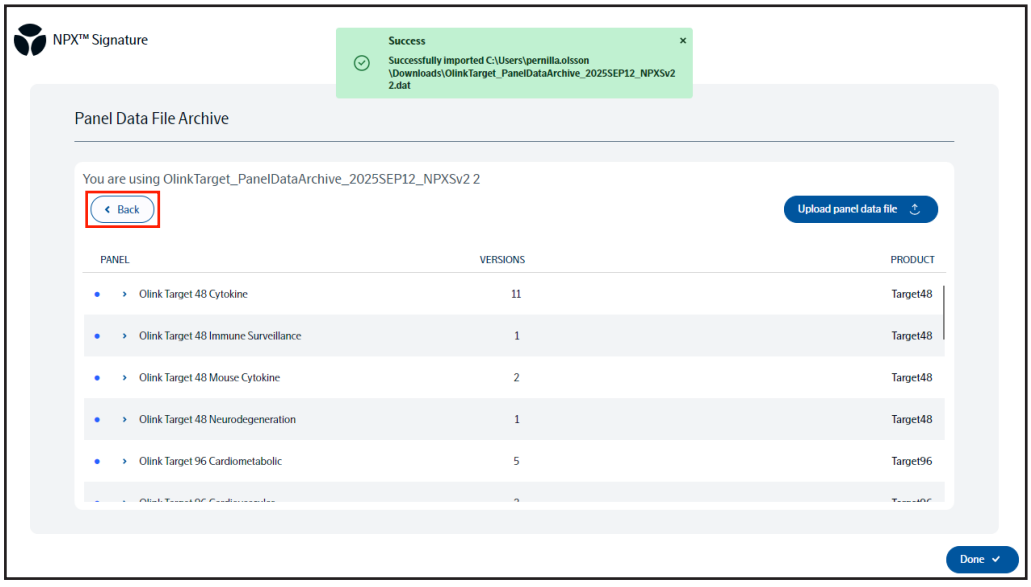
The following steps must be repeated when new panel versions are needed and have been downloaded from the Olink web page or provided by Olink.

1. On the start page, click **Manage panel data files.**

2. The view lists all Panel Data Files uploaded to the current version of NPX Signature Software.
   Click **Upload panel data file**.

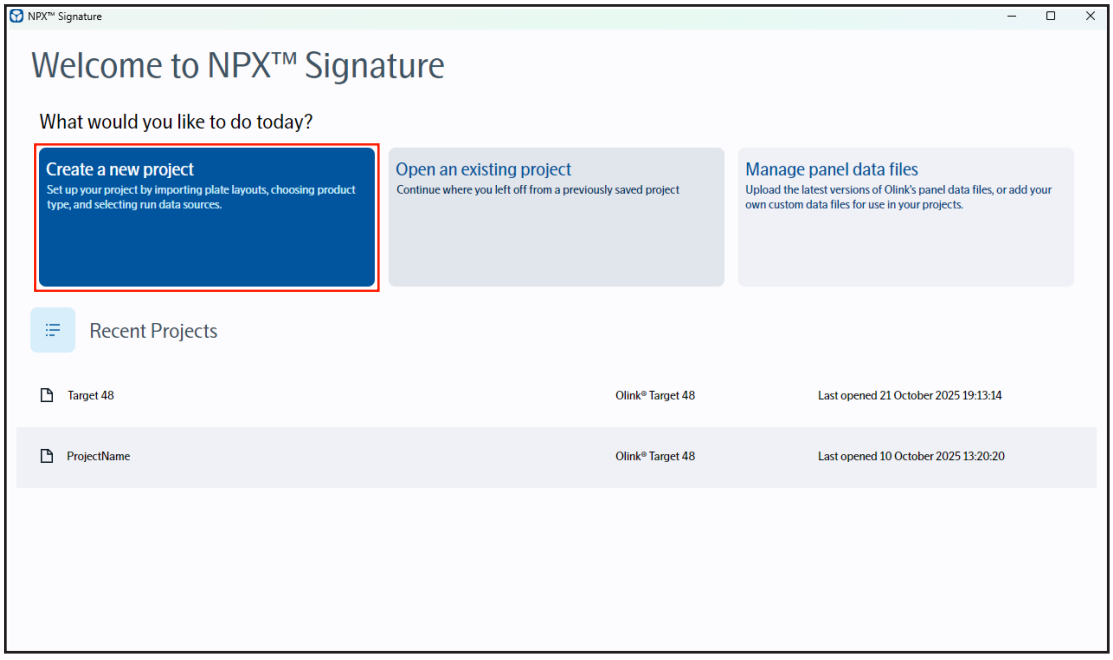


3. Browse for files to upload. Click **Open**.
   The uploaded Panel Data Files will be listed.
   Click **Back** to go back to the start page.

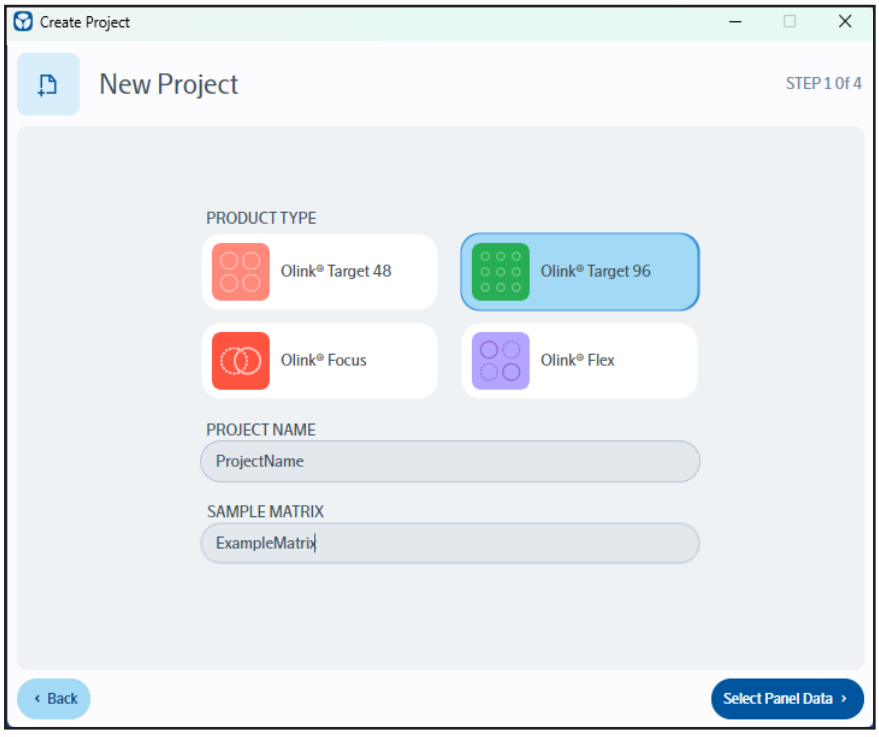


11.3 Create a new project

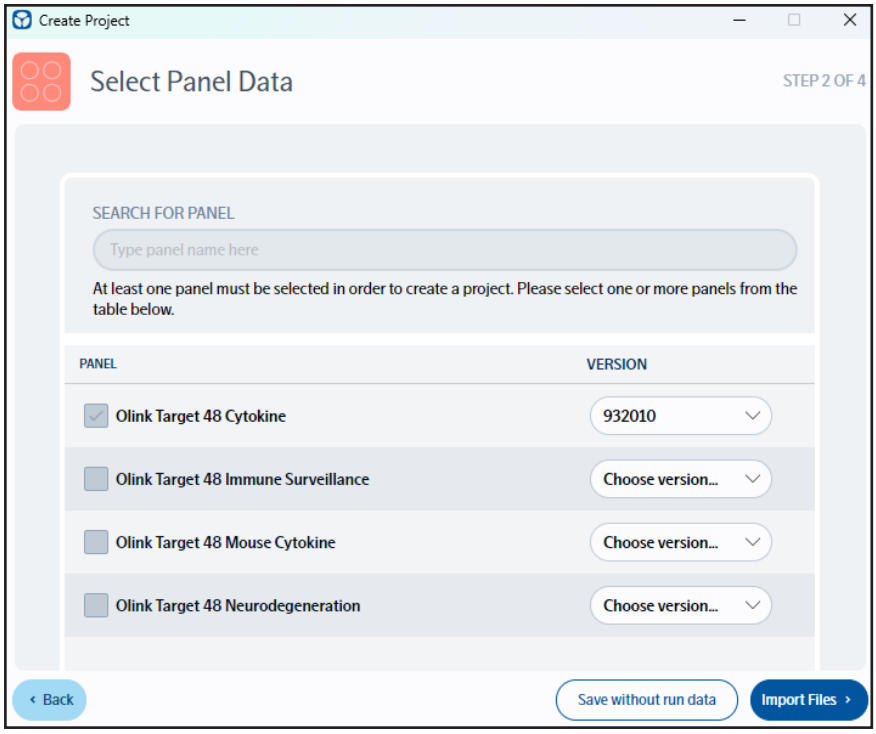
1. Click **Create a new project**.



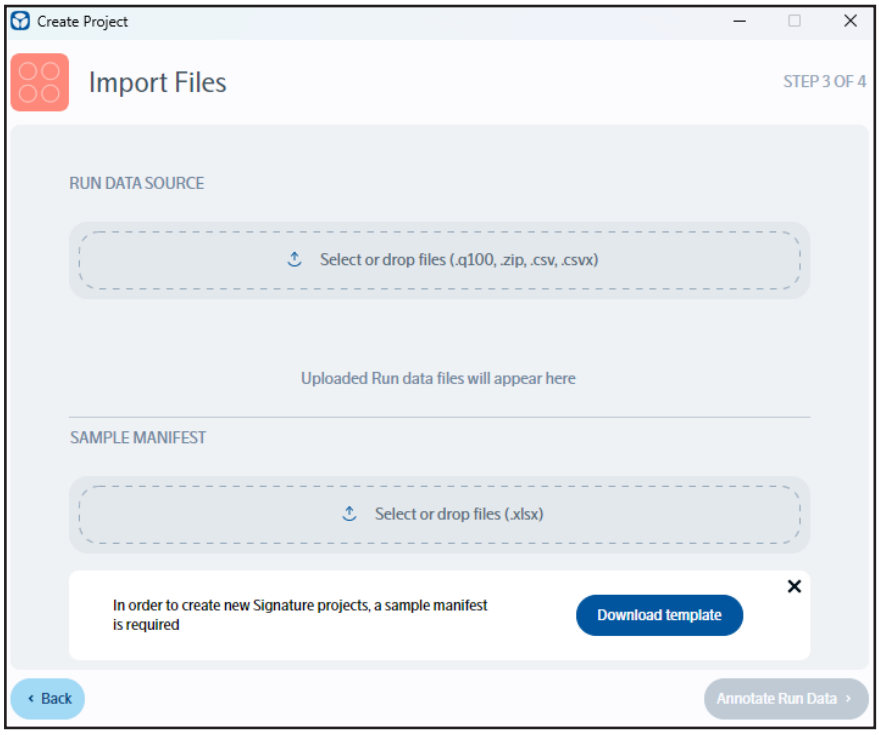
2. Select **Product Type**.
   Enter **Project name** and **Sample matrix** (optional).
   If Panel Data Files have not been added, the corresponding product types can not be selected and the corresponding icon will be blurred.
   Click **Select Panel Data**.



3. Select **Panel(s)**. Use the Version dropdown menu and select **Panel Version.** Panel Version depends on the used reagent batch and can be found on the Lot configuration sheet provided with the reagents.
   To deselect, uncheck the checkmark box next to the panel.
   Click **Import Files.**
   Alternatively, you can also select **Save** without run data to create the project in advance without adding runs directly.
   For Olink Focus, select either absolute or quantitative panels. It is not possible to combine them in one project.



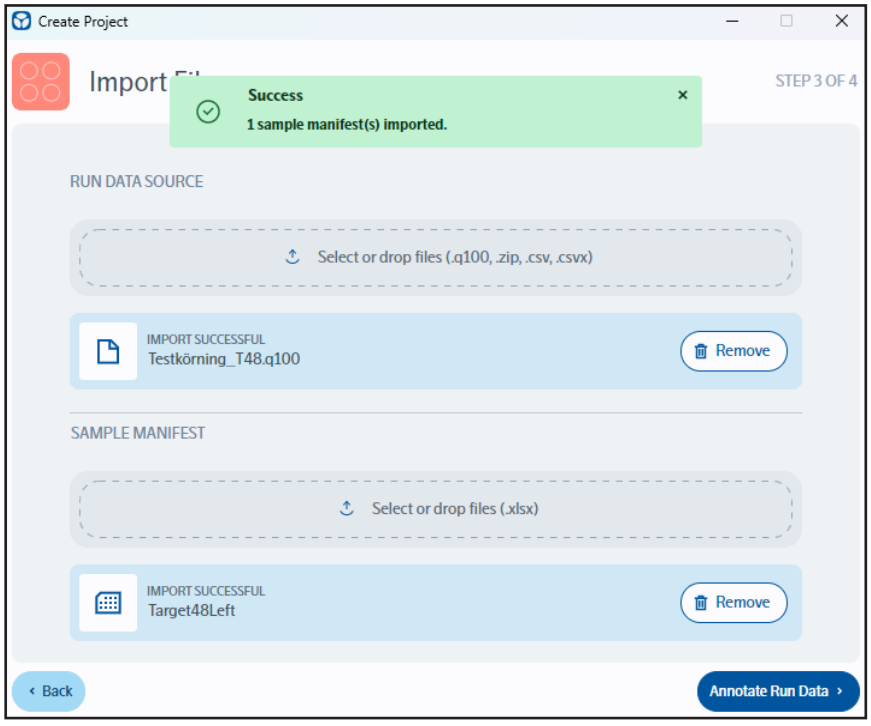
4. Upload **Run Data** and corresponding **Sample Manifest** files.
   Either drag and drop one or more files, or click on the dotted square and browse for preferred file(s).



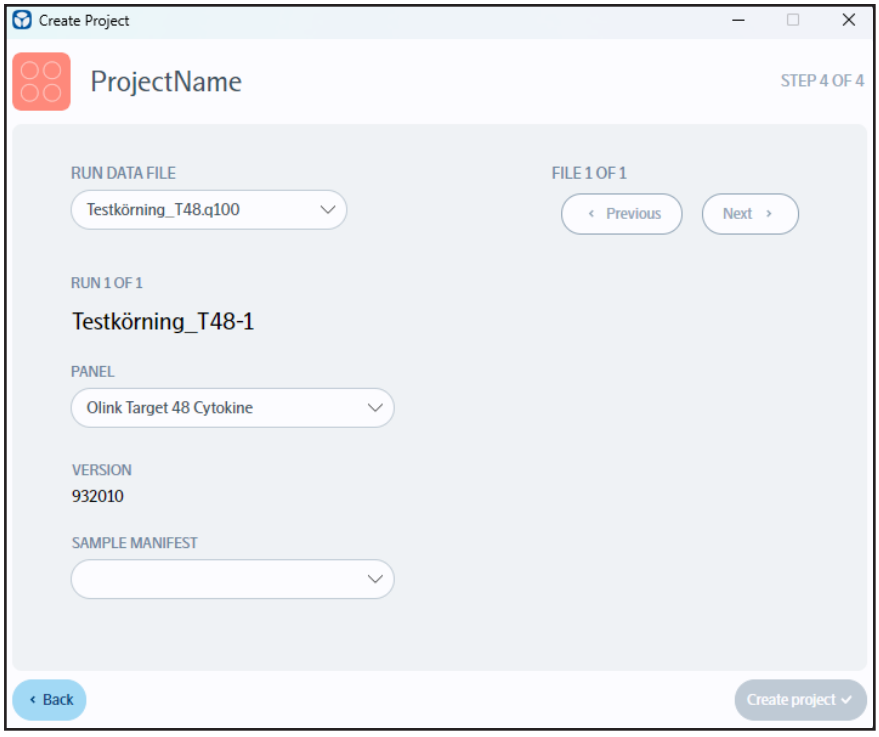
   The Sample Manifest is required to define the Sample Types. A template can be downloaded in this step clicking **Download template**. The template can then be uploaded to the software as a Sample Manifest.

NOTE: *Files must be closed when uploaded to the NPX Signature Software.*

5. Uploaded files will appear under each title/box and a confirmation of successful import will show.
   Click **Annotate Run Data**.



6. Choose for each Run Data file, the corresponding **Panel** and **Sample Manifest** for all Plates.
   A Sample Manifest cannot be used for multiple plates within the same panel.
   A Sample Manifest can be used for plates in different panels
   Click **Create Project**.



7. Browse for folder to save the project folder.*

* In order to reduce the risk of file corruption due to potential limitations in network connectivity while using a VPN, it is recommended to save projects locally. By working directly from local storage, interruptions can be minimized and it can be ensured that the progress is consistently saved. Once the project is complete, it can be uploaded or synchronized to a network location after disconnecting from the VPN or when network stability improves.

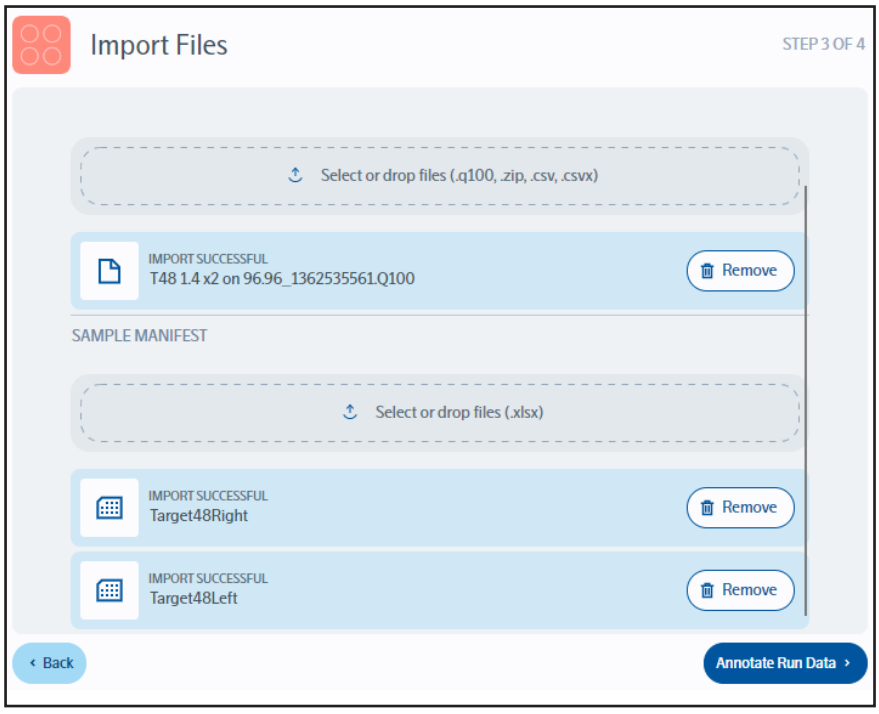
The project will be stored as a folder including a project file with the extension .osp (Olink Signature Project). It is possible to open the project by double-clicking on the .osp file.

For how to import a project created in NPX™ Signature Software v1.x, refer to *11.5 Migration of NPX™ Signature Software v1.x projects.*

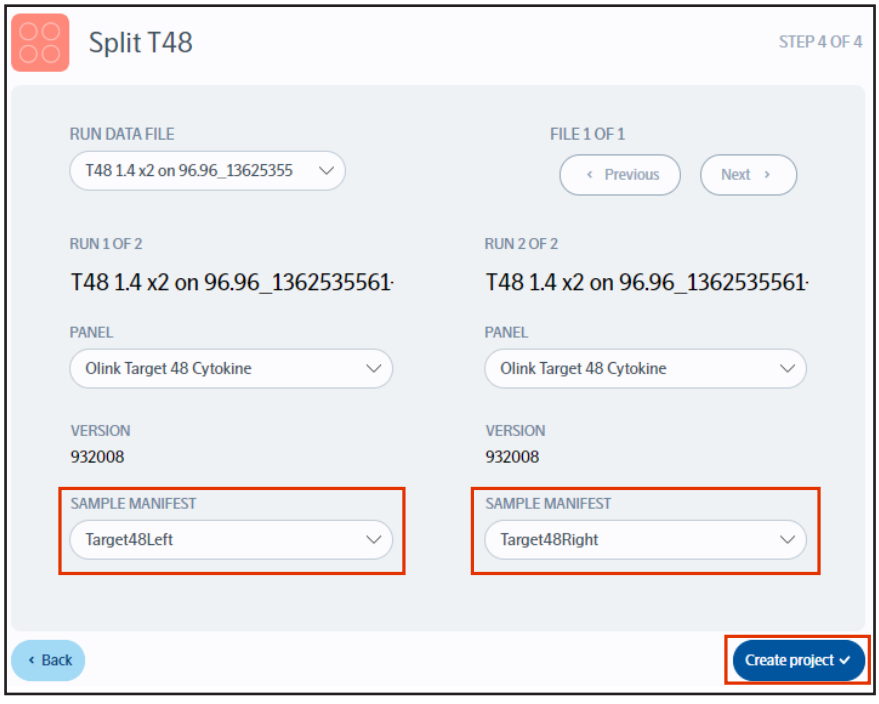
11.4 Import a split Olink® Target 48 in version 2.x

When downloading the templates for Olink Target 48, two files are provided. These templates are intended for use during project creation when a 96.96 run is being imported.

1. Under Product Type, select your product and fill in the project details.
2. Select run(s) and Sample Manifest.



3. Select Sample Manifests for the split run and click **Create project**.



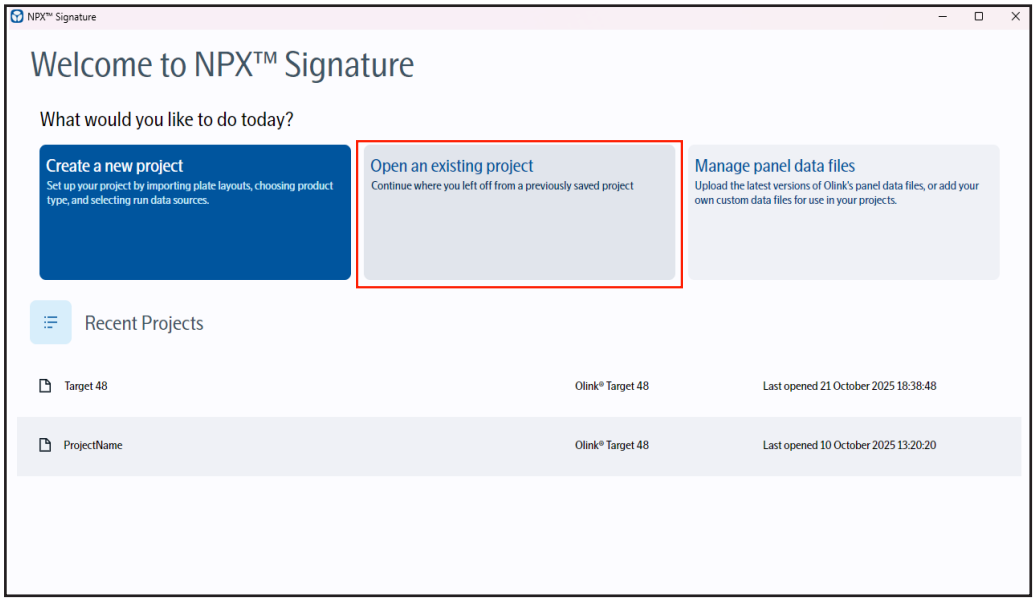
11.5 Migration of NPX™ Signature Software v1.x projects

Projects created in the old version of the NPX Signature Software (v 1.x) can be migrated into the new software (v 2.x). A migrated project will be saved as a new project (.osp). It will not replace the old project (.npx).

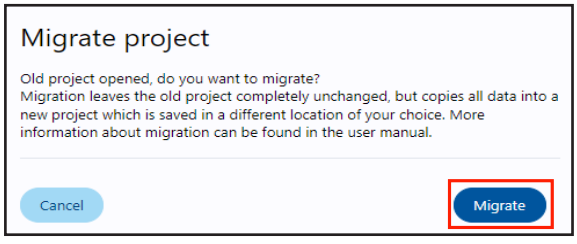
The old Panel Data File in the old NPX Signature Software v1 format will not be migrated in NPX Signature Software v2. A Panel Data File or an Panel Data Archive with the new format (NPXSv2) must be imported into the software before migrating the project. For more information about the Panel Data File, refer to *10. Files and information needed for analysis.*

11.6 Migrate a project

1. Start the NPX Signature Software and import Panel Data File, according to *11.2 Import Panel Data Files or Panel Data Archive.*
2. On the start page, click **Open an existing project.**



3. Browse for a project to migrate. Click **Open**.
4. A popup window will be prompted. Click **Migrate**.



5. Browse for a folder to save the migrated project in. Click **Select folder**.
   The project is migrated and the Project view will be shown.
6. The project is created and all files needed for quality control are imported.

11.7 Migration information

The following information must be considered when migrating a project.

- If all data points in an assay were manually failed individually in NPX Signature Software v1.x, NPX Signature Software v2.x will treat the assay as manually failed.
- A manual fail of data points will be migrated, with the exception of the above.
- A manual sample fail will be migrated.
- Normalization and QC will be re-calculated based on the migrated data. For more information, refer to *Part 2: Technology description.*
- It is not possible to have different normalization types within a project. Normalization types will be set to IPC normalization for Target 96 projects with mixed settings of normalization methods. Projects with consistent normalization types will retain the original setting.
- Sample Manifests not connected to any plates will not be migrated.
- SampleIDs with non-alphanumerical characters will be trimmed to only include alphanumerical characters and the following special characters ".", "-", "_", <space>, and "#". If the SampleID only contains non-alphanumerical characters, the SampleID will get a name based on the PlateID and WellID.
- PlateName will be changed to PlateID.
- PlateIDs with non-alphanumerical characters will be trimmed to only include alphanumerical characters and the following special characters ".", "-", "_", <space>, and "#".
- PlateID must be unique in NPX Signature Software v2.x. For duplicates, a suffix starting on 1, will be added to the PlateID name.
- SampleID of sample type Sample within a panel must be unique in NPX Signature Software v2.x. For duplicates, a suffix starting on 2 will be added to the SampleID name.

11.7.1 Changes in project name

During the migration, a copy of the project will be created, and the old project file will be kept as it is.

When saving the migrated project, a folder is created. NPX Signature Software creates a sub folder, with the same name as the NPX file. Both the name of the NPX file, and the project name, may change name, according to the following rules:

- Minimum name length: 1 characters
  If the length of the project name is shorter than minimum length, the new project name will be: "MyProject".
- Maximum name length: 150 characters
  If the length of the project name is longer than maximum length, all characters after the first 150 characters will be stripped.
- Allowed characters: [a-z][A-Z][0-9]_-.#(whitespace)
  If the project name contains other characters than above, these characters will be replaced with "-" (dash).
- Project name can't end with the character "." (dot)
  If the project name ends with ".", ALL "." in project name will be replaced with a "-" (dash).

Examples

BEFORE migration:	AFTER migration:
abs?!@#$%abs	abs---#--abs
(empty)	MyProject
abs åäö	abs ---
abs.123.	abs-123-
abs123_-.#	abs123_-.#

11.8 Perform quality control

Refer to section *14. Views* for guidance on how to use the different views to perform quality control. The following steps are suggested best practices; however, they do not need to be followed to successfully perform quality control.

1. Go to **Plate Details.**
   - ☐ Ensure that it is the correct Sample Manifest.
   - ☐ Ensure that it is the correct number of samples and Sample ID .
   - ☐ Ensure that it is the correct file path and correct combination of Sample Manifest and file path.
2. Go to **QC Summary.**
   - ☐ Check the overview of number of earned and failed samples and assays. Check if something sticks out, if there's a plate issue.
3. Go to **Sample QC.**
   - ☐ Check the warned assays. If there are many warned assays, go to **Run QC** to identify which assays stand out.
   - ☐ Check the rest of the values.

   3.1. If there are many errors, go to **Heat Map**.
   - ☐ Check if any of the warned or failed assays contain outliers.

   3.2. If an outlier is detected, go to **Workspace**.
   - ☐ Set the sample as failed.

   3.3. Go back to **Sample QC**.

   3.4. Check that the results are satisfying. If not, repeat step 4.1–4.3.
4. Go to **Plate Data**.
   - ☐ Check internal controls in Ct Value. Check differences and that the values are within the interval.
   - ☐ Check the NPX Value and scroll and see if there's a pattern. If so, compare with Ct Value.
5. Go to **Heat Map**.
   - ☐ Check all samples in NPX Value. Ensure that no values appear unusual or indicate potential outliers.
   - ☐ Check row and column, plate by plate, to identify any systematic errors.
   - ☐ Check sample type one by one.
6. Go to **Detectability**.
   - ☐ Ensure that the detectability is satisfying.
   - ☐ Ensure that no LOD values in the table are missing out.
7. Go to **Plate Variation**.
   - ☐ Check each assay individually, first in Ct Values and then in NPX Values, to ensure that the randomization appears correct. All boxes should be on the same level, which is especially important when working with more than one plate.
8. Go to **Metrics**.
   - ☐ For all plates, ensure that the Intra CV is not too high.
9. Go to **Amplification Curves**.
   - ☐ Check for spikes in the curves.

11.8.1 Bimodal assays (Olink® Target 96)

Some assays can show a bimodal profile. This can be dependent on the sample matrix. The plate view pattern and a high assay CV can indicate if an assay is bimodal. In that case it is recommended to use IPC Normalized NPX values for these assays.

For bimodal assays the recommendations are to:

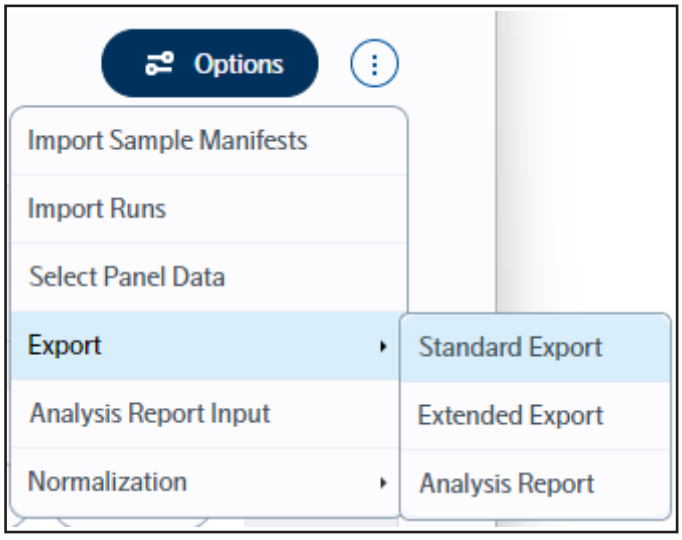
- Select Intensity Normalization.
- Export Extended Report for the whole project.
  The extended report will include both Intensity Normalized and IPC Normalized NPX values.

No assay is set per default as bimodal in NPX Signature Software v2.x. High assay CV and distribution in the Plate Variation view are a hint to detect bimodal assays.

For more information about bimodal assays, please refer to *https://olink.com/knowledge/publications.*

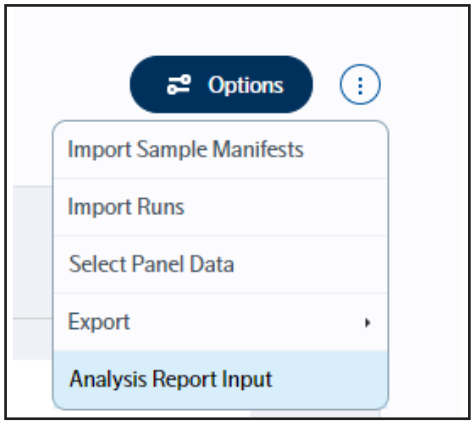
11.9 Export result files

Three different files can be exported from the NPX Signature Software: Standard Export, Extended Export, and Analysis Report. For more information about the files and file formats, refer to *12. Result files.* To export the file, go to **Options → Export**, and select the preferred file type to export.

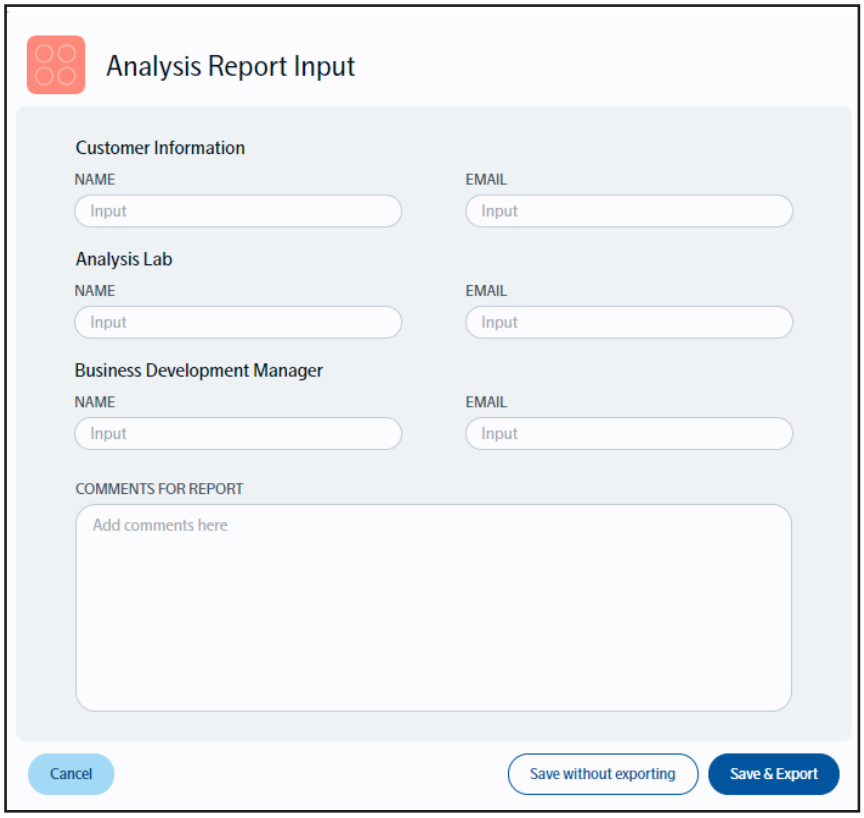


11.9.1 Preparing to Export the Analysis Report

To ensure that all required report details and metadata are included in the analysis report, the input form must be completed prior to exporting the file. Navigate to **Options → Analysis Report Input**.



Fill in the required fields, then choose either **Save without exporting** or **Save & Export**.



12. Result files

Results can be exported in 3 formats. Standard (.csv) or extended (.csv) and as Analysis Report (.pdf).

12.1 CSV files

CSV export files can be exported based on either relative quantification or absolute quantification, depending of the project type. Additionally, they are available in two formats: standard and extended. Below is an overview of the different file types and what each version includes.

In some regional configurations, Excel uses a comma (,) as the decimal separator instead of a period (.). The NPX file uses periods, and if Excel expects commas, values may be misinterpreted as thousands.

To verify Excel's decimal separator setting:

1. Open Excel.
2. Navigate to **File → Options → Advanced → Editing Options**.
3. Confirm that the decimal separator matches the format used in the exported NPX file, which is the period (.).

12.1.1 CSV files for RELQ

CSV files for Relative Quantification (RELQ) are for Olink Target 96 and Olink Focus panels with relative quantification.

Column	Description	Type/example	Standard	Extended
Product	Type of product.	String TARGET96, FOCUS	√	√
Panel	Panel name.	For example "Olink Target 96 Cardiovascular II"	√	√
PanelVersion	Version of Panel Data File used.	For example 902001	√	√
PlateID	Name of the plate the sample was run on.		√	√
WellID	Well on 96-well plate where sample was run.		√	√
SampleID	The annotated Sample ID.		√	√
SampleType	The annotated Sample Type, listed as included in the product and data export file type.		√	√
Assay	Short name for assay, taken from Panel Data File.		√	√
UniProt	UniProt ID for assay.		√	√
OlinkID	OlinkID for assay.		√	√
Ct	Cycle threshold reading from qPCR instrument.	Decimal number	√	√
NPX	NPX for chosen normalization.	Decimal number or NA	√	√
LODNPX	Limit of detection on NPX scale.	Decimal number or NA	√	√
BelowLOD	Indicates if value is strictly below LOD.	TRUE, FALSE, or NA	√	√
SampleQC	Shows the QC status of the sample.	PASS, WARN, FAIL, or MANUAL_FAIL	√	√
AssayQC	Shows the QC status of the assay.	PASS, WARN, FAIL, or MANUAL_FAIL	√	√

MissingFreq.	Frequency of missing data (below LOD or NaN).	Decimal number	√	√
Normalization	Type of normalization used in project.	IPC Normalized or Intensity Normalized (v.3)	√	√
SoftwareVersion	Software version of the NPX Signature Software used for panel calculations and normalization.		√	√
SoftwareName	Name of software.	NPX Signature	√	
AssayType	Type of assay.			√
IPCNormalizedNPX	IPC normalized NPX values.	Decimal number or NA for Target 96 NA for Focus		√
LODIPCNormalizedNPX	LOD for IPC normalized NPX.	Decimal number or NA		√
QCDeviationDetCtrl	QC deviation for Incubation Control	Decimal number		√
QCDeviationIncCtrl	QC deviation for Detection Control.	Decimal number		√

12.1.2 CSV files for ABSQ

CSV files for ABSQ, Absolute Quantification, for ABSQ are for Olink Target 48, Olink Flex and Olink Focus panels with absolute quantification.

Column	Description	Type	Standard	Extended
Product	Type of product	TARGET48, FLEX, FOCUS	√	√
Panel	Panel name	For example "Olink Target 48 Cardiovascular II"	√	√
PanelVersion	Version of Panel Data File used.	For example 902001	√	√
PlateID	Name of the plate the sample was run on		√	√
WellID	Well on 96-well plate where sample was run.		√	√
SampleID	The annotated Sample ID		√	√
SampleType	The annotated Sample Type, listed as included in the product and data export file type.		√	√
Assay	Short name for assay, taken from Panel Data File.		√	√
UniProt	UniProt ID for assay.		√	√
OlinkID	OlinkID for assay.		√	√
Ct	Cycle threshold reading from qPCR instrument.	Decimal number	√	√
NPX	NPX value.	Decimal number or NA	√	√
LODNPX	Limit of detection on NPX scale.	Decimal number or NA	√	√
BelowLOD	Indicates if value is strictly below LOD.	TRUE, FALSE, or NA	√	√
QuantifiedValue	Quantified value of the sample.	Decimal number	√	√
Unit	Unit for assay from Panel Data File.	TRUE, FALSE, or NA	√	√
LODQuant	Limit of detection on quantified scale based on the Negative Controls.	Decimal number	√	√
LLOQ	The lower limit of quantification as set in the Panel Data File.	Decimal number	√	√

LQL	The higher value of either the LLOQ from the Panel Data File or the LODQuant value computed based on the Negative Controls.	Decimal number	√	√
BelowLQL	Indicates if QuantifiedValue is strictly below LQL.	TRUE, FALSE, or NA	√	√
ULOQ	The upper limit of quantification as set in the Panel Data File.	Decimal number	√	√
AboveULOQ	Indicates whether Quantified value is strictly above ULOQ.	TRUE, FALSE, or NA	√	√
SampleQC	Shows the status of the sample.	PASS, WARN, FAIL, or MANUAL_FAIL	√	√
AssayQC	Shows the status of the assay.	PASS, WARN, FAIL, or MANUAL_FAIL	√	√
MissingFreq	Frequency of missing data (below LOD or NaN).	Decimal number	√	√
Normalization	Type of normalization used in project.	For example Calibrator Normalized	√	√
SoftwareVersion	Software version of the NPX Signature Software used for panel calculations and normalization.		√	√
SoftwareName	Name of software.	NPX Signature	√	√
AssayType	Type of assay.			√
QCDeviationDetCtrl	QC deviation for Incubation Control	Decimal number		√
QCDeviationIncCtrl	QC deviation for Detection Control.	Decimal number		√

12.2 Analysis Report

The analysis report is a PDF file that can be generated from the NPX Signature Software. The analysis report summarizes the quality analysis of the project. It contains basic information and descriptions of the project.

Go to **Options** → **Export** → **Analysis Report**. Browse for preferred folder to save the Analysis Report and click **Save**.

To ensure that all required report details and metadata are included in the analysis report, the input form must be completed prior to exporting the file. To edit the Analysis Report Input, go to **Options → Analysis Report Input**. Fill in the required fields, then click **Save & Export**.

The Analysis Report contains the following:

- Project information
  - Sample matrix
  - Project specific comments
- Quality Control
  - Summary of Quality Control of samples
  - Summary of Quality Control of assays
  - Intra- and Inter-Assay Coefficient of Variance (%CV)
    - Intra-Assay %CV
    - Inter-Assay %CV
- Protein detection and quantification results
  - Number of proteins with NPX above LOD in >75% of the samples
    - No of detected proteins / Total numbers of proteins
    - Detected proteins (%)
  - Number of proteins quantified within LOQ in >50% of the samples (only for AbsQ products)
  - Data output
- Samples that did not pass QC
- Observed deviations
- Downstream analysis
- Software version information and copyright information

12.2.1 Customize the Analysis Report

It is possible to add a custom logo image to the title page of the Analysis Report. Supported image formats are: bmp, png, jpeg, and gif.

4. Open a text file and fill in the following information:

```
{
"LogoFilePath": "<path to file>",
"LabName": "<Service Lab Name>"
}
```

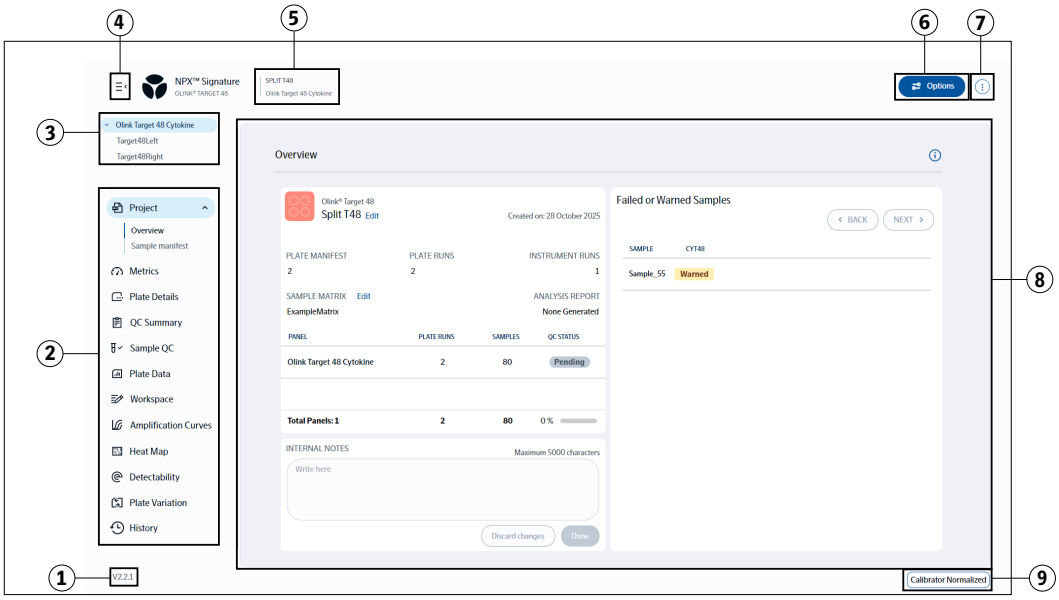
   Where <path to file> is the complete path to image file, including the file name of the image and <Service Lab Name> the name of laboratory or company or similar. For example:

```
{
"LogoFilePath": "C:/pictures/IMG_01234.JPG",
"LabName": "<SuperLab>"
}
```

5. Name the file `certificate.json`. Remove the file format .txt, the file type must be .json.
6. Place the file in the folder `%LocalAppData%/Olink/NPX-Signature/Settings`.

Part 4: User Interface

13.General



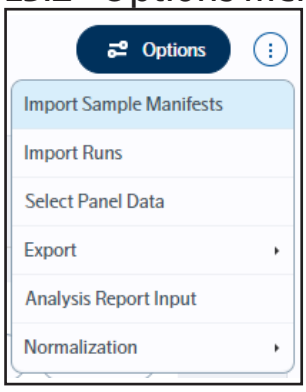
No	Part	Description
1	Version number	Specifies the version number of the software.
2	View menu	For more information about the different views, refer to *14. Views.*
3	Plate Selection	Select plate and panel to display.
4	Minimize	Minimize or maximize the left menu.
5	Header section	Displays the name of the active project, along with the selected panel and plate listed below.
6	Options	Menu for modifying the project data and for exporting results. Refer to *13.2 Options menu.*
7	Project Actions menu	Menu for handling the project and software settings. Refer to *13.3 Project Actions menu.*
8	Main view	Shows the selected view from the View menu.
9	Normalization	Shows the selected normalization method. To change normalization method, refer to *13.2 Options menu.*

13.1 Plate Selection

This menu shows the selected panel and plate. Click the small arrow to show or hide the list of plates. Select a specific plate by clicking its name.
The navigation allows the use of shortcuts to move between panels and plates. If there is no next or previous plate or panel available, the shortcut will have no effect.

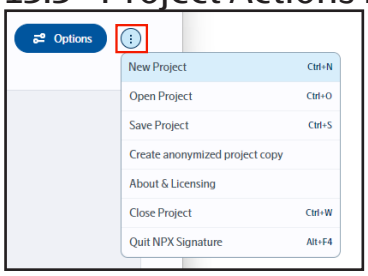
- Press the **Ctrl** key and the **.** (period) key at the same time to move to the next plate.
- Press the **Ctrl** key and the **,** (comma) key at the same time to move to the previous plate.
- Press and hold **Ctrl** and **Shift**, then press the **.** (period) key to move to the next panel.
- Press and hold **Ctrl** and **Shift**, then press the **,** (comma) key to move to the previous panel.

13.2 Options menu



Menu	Expansion	Description
Import Sample Manifests	–	Import Sample Manifest. Refer to *11.3 Create a new project*
Import Runs	–	Import run files. Refer to *11.3 Create a new project.*
Select Panel Data	–	Change Panel Data File versions for the imported panels.
Export	Standard Export Extended Export Analysis Report	Export the result files. Refer to *12. Result files.*
Analysis Report Input	–	Use this option to enter the required report details and metadata for the Analysis Report. The information provided in this section will be included in the final exported report.
Normalization	IPC Normalized Intensity Normalized (v.3)	Change the normalization method. This applies only to Target 96.

13.3 Project Actions menu



Menu	Description
New Project	Create a new project. Refer to *11.3 Create a new project.*
Open Project	Open a saved project.
Save Project	Save the current project.
Create anonymized project copy	Create a copy of the current project, that is anonymized.
About & Licensing	View software version, copyright, and license information
Close Project	Close the current project.
Quit NPX Signature	Close down the software.

14. Views

Project
Provides a summary of the project, including the number of runs, panels, sample manifests, and any samples with warnings or failures.

Metrics
Shows evaluation of systematic bias. Gives an indication of the overall data quality of the selected panel and includes results of the selected panel.

Plate Details
Shows the plate layout, run details, panel information and plate status. Sample Manifest can be applied and plates can be excluded or deleted.

QC Summary
The Quality Control Summary view displays a summary of the plate info and QC details per plate for the entire project

Sample QC
Shows a diagram of the deviation from Median / of the incubation and detection controls in NPX for all samples and external controls of the selected plate, together with a table of Assay QC and Run QC.

Plate Data
Shows the values of NPX, Ct, and Quantified values (if applicable) for internal controls and assays for each sample per plate. Assess if there are patterns in the plate (row or column) representing a possible technical error.

Workspace
Displays data of a plate or panel in a grid view, depending on the based on the option chosen in the upper left corner. When either NPX or Quantified Values is selected, the LOD (Limit of Detection) is shown at the bottom of the view. This view also allows for manual QC (manually failing data), and reviewing both the values and status for all samples and external controls.

Amplification Curves
Shows the amplification curves for all samples and plates in Ct.

Heat Map
Displays the values of datapoints in the plate or panel in a red-and-blue color scheme. Raw intensity levels (1 and 2) are shown in grayscale, providing a visual representation of the sample results.

Detectability
Shows a bar chart of the frequency of missing data per assay, indicating the percentage of data above LOD for all plates in the selected panel. Table Data shows LOD values for each assay and plate within the selected panel.

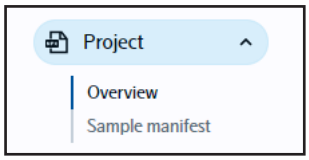
Plate Variation
Shows box plots representing the deviation from the plate median of each sample, both across plates and within the selected panel.

History
Shows a log of all actions performed in the project.

Help Panel
A help panel can be accessed using the Information icon in the upper-right corner of each view. The panel provides information about the features and functions specific to that view.

14.1 Project

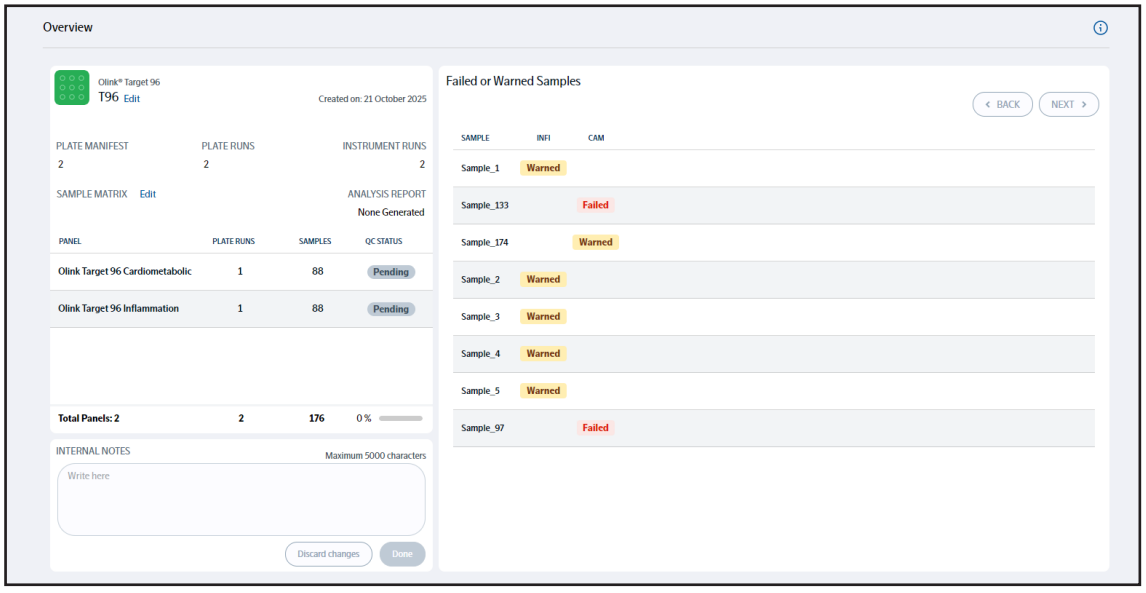
The Project view contains two different pages: Overview and Sample Manifest. Expand the Project menu to reach the different views.



14.1.1 Overview

General:

The Overview provides a summary of the project, displaying the number of Sample Manifests, plate and instrument runs, panels and other key metrics. It also highlights any samples that have failed or triggered warnings.



Useful for:

- Showing information on the panels, one panel at the time.
- Monitoring the progress of the QC process, shown as a percentage at the bottom of the view.
- Checking whether an Analysis Report has been generated and viewing the date and time of the most recent report.

Functions:

- Failed and warned samples are shown to the right.
- Project Name and Sample Matrix can be edited by clicking the **Edit** link next to the respective fields.
- Internal notes can be added in the dedicated field.

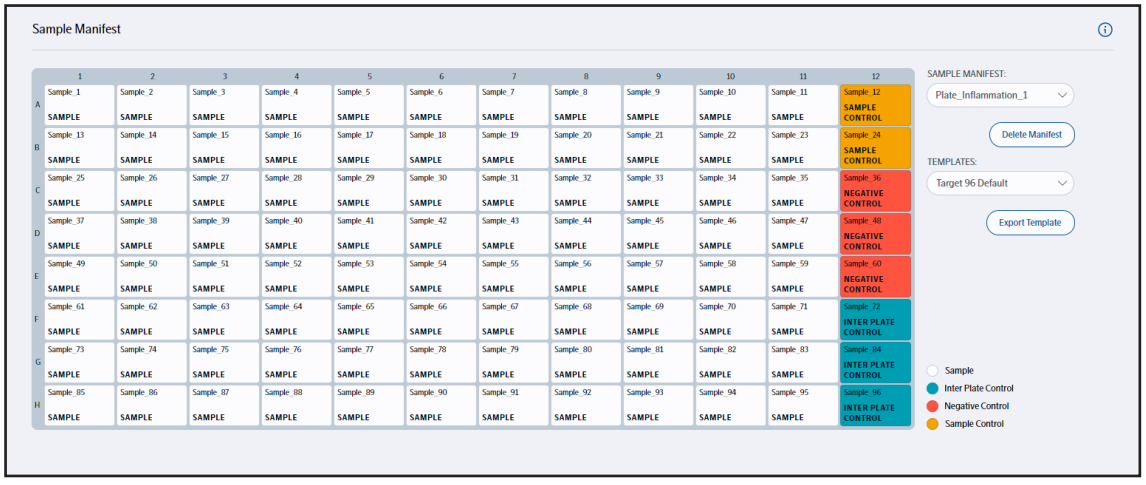
14.1.2 Sample Manifest

General:

The Sample Manifest view shows the Sample Manifest, use the dropdown list to select Sample Manifest to view. Viewing them does not change the Sample Manifest used for the selected plate.

The Sample Types are defined in the Sample Manifest. Changes to Sample Types in the sample positions must be done directly in the Sample Manifest and then be uploaded again. To upload the new Sample Manifest, go to the **Options** menu and select **Import Sample Manifests**.

The Sample Types are based on the color code that differs between the products. Definitions of the colors are shown to the right of the plate layout.



Useful for:

- Getting an overview of each Sample Manifest imported in the project.

Functions:

- Use the **Sample Manifest** dropdown list to Sample Manifest to view.
- Click **Delete Manifest** to delete the selected Sample Manifest. A Sample Manifest cannot be deleted if it is connected to a run data.
- Use the **Template** dropdown menu to select template to use.
- Click **Export Template** to export the selected template as a .xlsx (Excel file).

14.2 Metrics

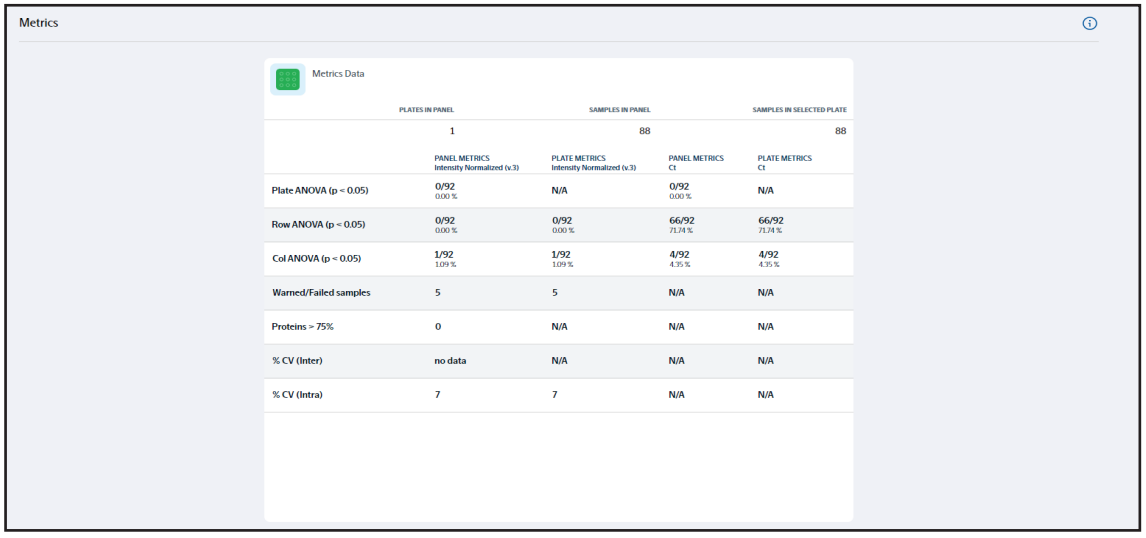
General:

The Metrics view displays parameters related to systematic bias that need to be evaluated for each plate and panel, along with their recommended values.

In addition to numeric values, the table may include the following indicators:

- **N/A (Not Applicable)**: This label is used for metrics that are undefined in the given context. For example, %CV (Inter) for Plate Metrics will always be marked as N/A, since %CV Inter for a single plate is not defined.
- "**no data**": Appears when there is insufficient data to calculate a metric. For example, %CV (Inter) for Panel Metrics will display "no data" if the panel contains only one plate. At least two plates are required to calculate this metric.

Note: The Metrics view may take some time to load due to the number of calculations.



Useful for:

- Checking the tendencies regarding systematic bias.
- Getting an overview of the different ANOVA's. They give the following indications:
  - Row ANOVA:
    May indicate pipetting errors or issues with sample randomization. High values suggest row-wise randomization or an issue with a pipette channel. If elevated, inspect the Plate Data and heat map for row-based patterns.
  - Column ANOVA:
    Similar to Row ANOVA, it can point to pipetting errors or poor randomization. High values suggest column-wise randomization or pipetting inconsistencies. If elevated, review the Plate Data and heat map for column-based patterns.
  - Plate ANOVA:
    Highlights variation between plates.
- Checking the quality of the laboratory performance, including protein detection consistency.
- Comparing different normalization methods (Only for Olink Target 96).

Functions:

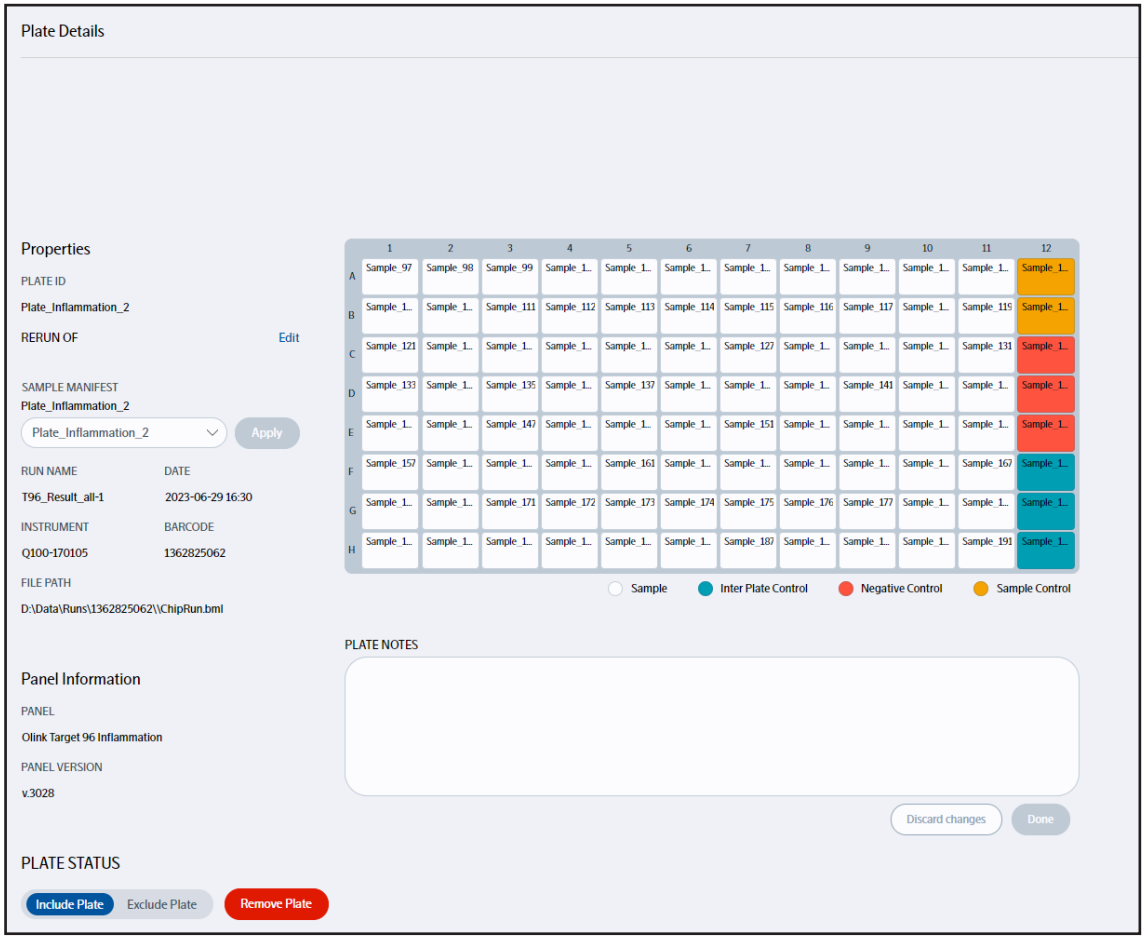
For each panel, the following information is displayed:

- Plate ANOVA
- Row ANOVA
- Col ANOVA
- Warned/Failed samples
- Proteins >75%
- %CV (Inter)
- %CV (Intra)

14.3 Plate Details

General:

Plate Details displays information about the selected plate, along with options to include, exclude, or delete plates. However, if a value is provided for 'Rerun Of', that value will be used for sorting instead of the Plate ID.



Useful for:

- Include, exclude, or delete the selected plate.

Functions:

- For each plate, the following information is displayed:
  - Properties

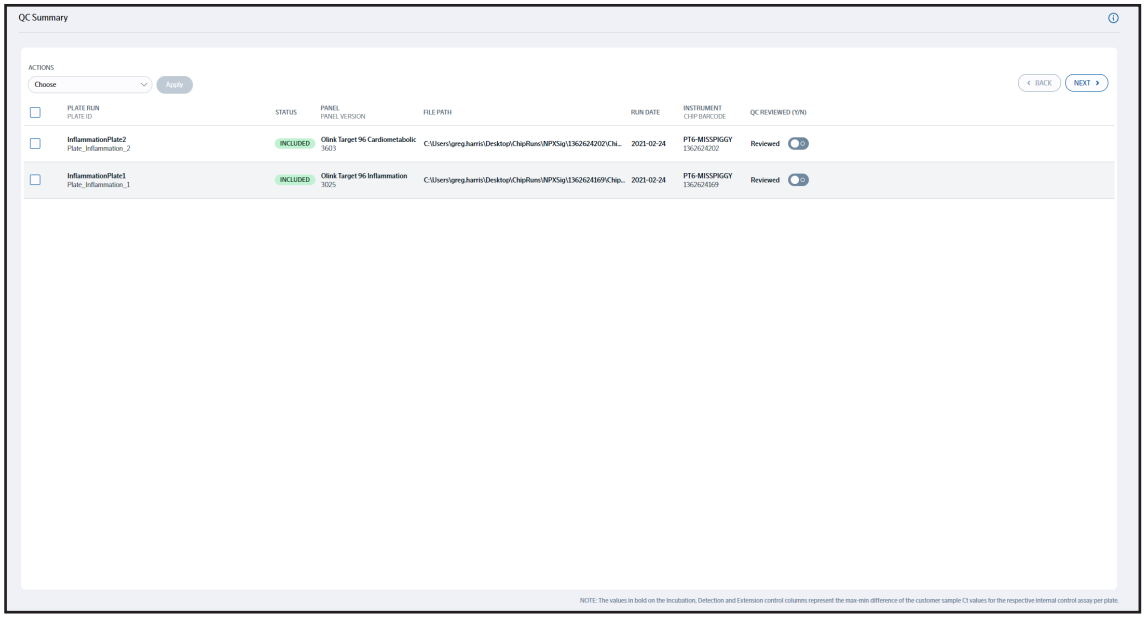
    - Plate ID
    - Entered Rerun
    - Selected Sample Manifest
    - Run name
    - Date
    - Instrument
    - Barcode
    - File path
  - Panel Information
    - Panel type
    - Panel version
- To annotate a plate as a rerun, use the **Edit** link located next to the **Rerun of** label. Click **Edit** and a text box will appear, allowing a note to be entered indicating that the plate is a rerun.
- Use the Sample Manifest dropdown menu to change Sample Manifest. Click **Apply**.
- Use the Plate Status buttons to Include or Exclude the plate, or to delete it.
  - An included plate is included in the QC calculations.
  - An excluded plate is not included in the QC calculations.
  - A deleted plate is deleted from the project. This action cannot be undone.
- Add comments in the **Plate Notes**. Comments will not be included in the Analysis Report.

14.4 QC Summary

General:

The QC Summary shows an overview of all plates in the project. Note that the list is too wide to be shown in one view. Use the **< Back** and **Next >** buttons or the scrollbar to see all information for each plate.

The values in bold on the Incubation, Detection and Extension control columns represent the max-min difference of the customer sample Ct values for the respective internal control assay per plate.



Useful for:

- Quickly assessing if there are plates with a lot of warned or failed samples and assays.
- Quickly assessing the Ct ranges of the internal controls for all plates.
- Quickly getting an overview of median absolute deviation of internal controls for all plates.
- Giving a summary of all plates in the project.
- Setting the QC status of a plate as reviewed.
- Checking that the file path and run names are correct, to verify that the correct Sample Manifest has been applied.

Functions:

- Select all plates by ticking the top box.
- Select plate(s). Use the Actions dropdown menu to Include or Exclude the selected plates or to remove the selections from the project.
- Set a plate QC as QC reviewed by sliding the toggle switch for QC Reviewed (Y/N) to the right. To set the status to not reviewed, slide the toggle switch to the left. This will change the QC status progress bar in the Project Overview.

14.5 Sample QC

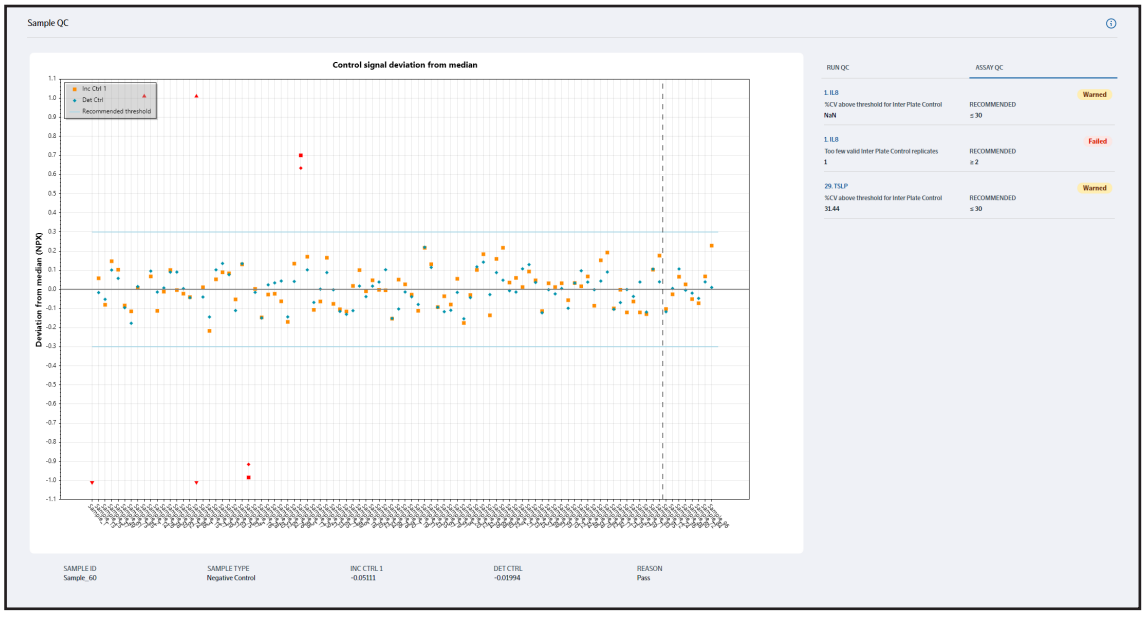
General:

The Sample QC view shows a scatter plot of deviation from median of the incubation and detection controls in NPX for all samples and external controls of the selected plate. To the right, information about Assay QC and Run QC are shown in different tabs, together with recommended values. Both warned and failed samples and assays are shown in the tables, but not manually failed ones.

The scatter plot uses the following symbols:

- **Light blue line:** The recommended threshold.
- **Orange square:** Incubation Control 1
- **Blue-green diamond:** Detection control
- **Red cross:** Indicates values that are "Not a Number," (a replacement for data values below the LOD).
- **Red data points (squares or diamonds):** Samples warned because Incubation Control 1 or Detection Control deviated by more than ±0.3 NPX or had missing values.
- **Outliers (red square or red diamond):** Samples with deviations beyond ±1 NPX. Displayed at 1.0 for visibility. A red square marks an outlier for Incubation Control 1, and a red diamond marks one for the Detection Control.
- **Vertical dashed line:** Separates sample data (left) from External Controls (right).

The X axis shows the samples and the Y axis the median NPX values.



Useful for:

- Get an overview of the samples and deviation from the median NPX of the incubation and detection controls.
- Find warned and failed samples and outliers.

Functions

- Hover over a sample to show the following information:
  - Sample ID
  - Sample Type
  - Inc Ctrl 1 (deviation from median)
  - Det Ctrl (deviation from median)
  - Reason (reason for sample warning or failed)

- The Run QC table shows the following information together with corresponding acceptance criteria:
  - Number of warned/failed samples
  - Number of warned/failed assays
  - Inc Ctrl 1 Samples (Median absolute deviation for the Incubation Control in samples)
  - Det Ctrl Samples (Median absolute deviation for the Detection Control in samples)
  - Inc Ctrl 1 External Controls (Median absolute deviation for the Incubation Control in control samples)
  - Det Ctrl External Controls (Median absolute deviation for the Detection Control in control samples)
- The Assay QC table lists all warned and failed assays, along with a description explaining the reason for each warning or failure, and the corresponding acceptance criteria.

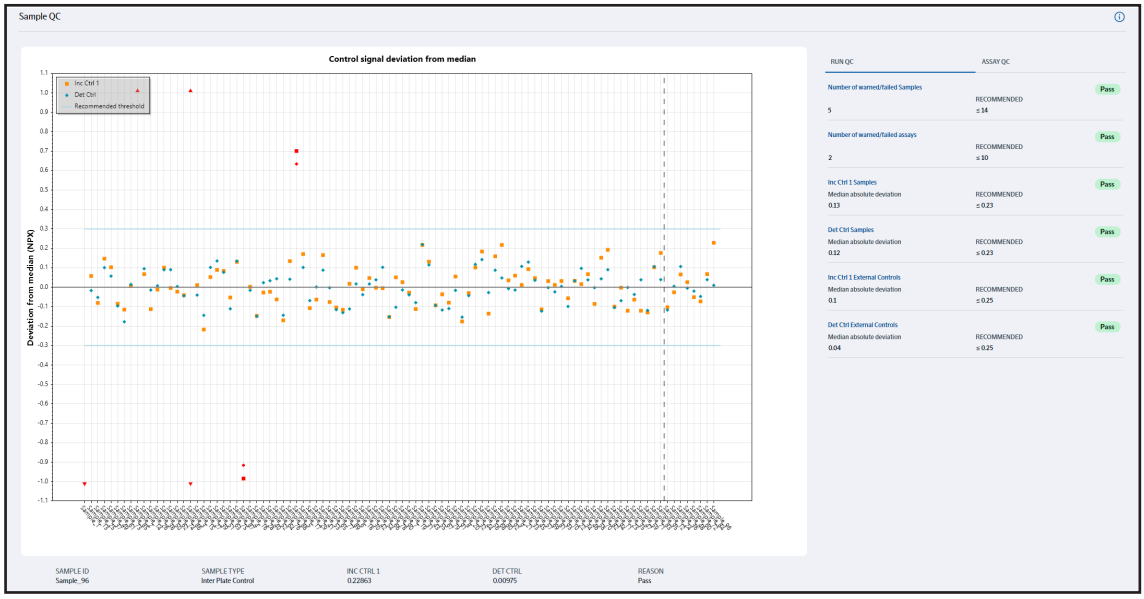
Value ranges

Values between ±0.3:
Samples with internal controls inside the range passes the QC criteria.

Values between 0.3 and 1 / -0.3 and -1:
Samples with internal controls in this range are automatically warned.

Values outside the range of ±1
Samples with internal controls outside this range are outliers. They are also warned.

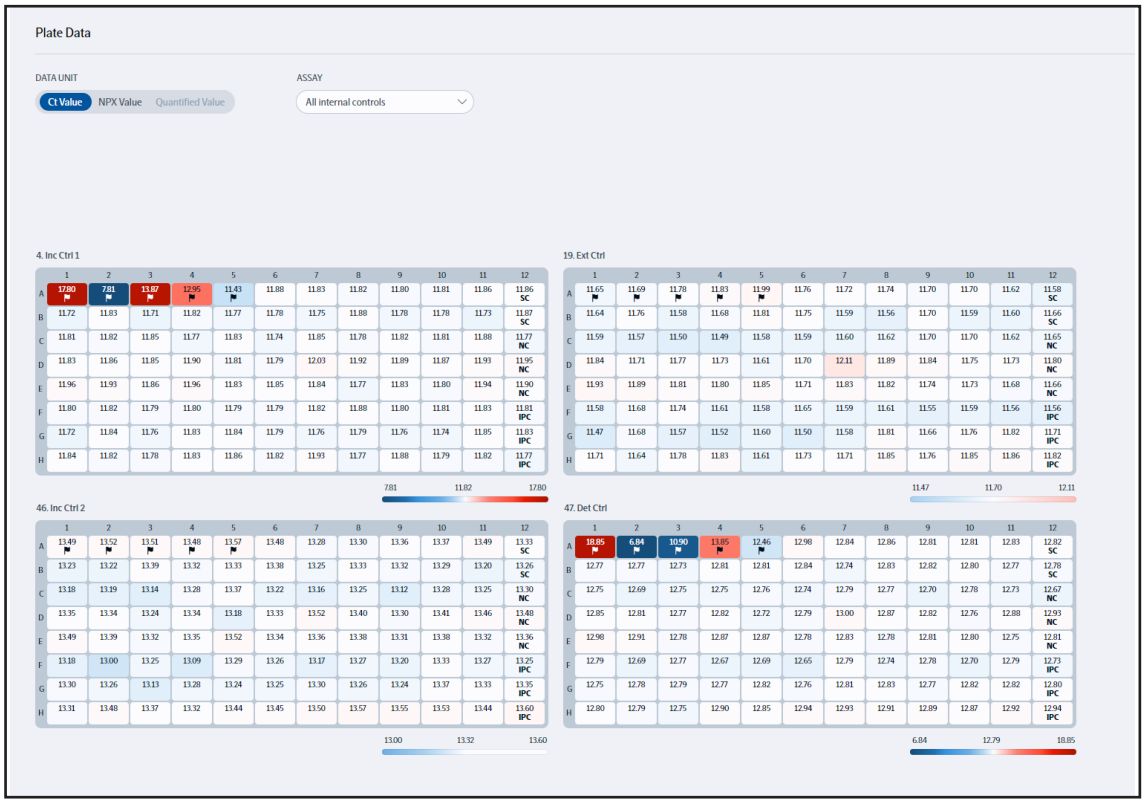
All outliers are automatically set to 1.0 in the graph. A red square marks Incubation Control and a red diamond Detection Control. To see the exact value, mark the outlier by clicking at it. The exact value is shown in the bottom of the screen. To visualize the outlier, hover over the plot while scrolling out using the mouse to change the scale of the plot.



14.6 Plate Data

General:

The Plate Data view displays NPX Value, Ct Value, and Quantified Value (if applicable) for both internal controls and assays for each sample on the plate. Review the data for any patterns across rows or columns that may indicate potential technical errors.



Useful for:

- Get an overview of warned or failed samples.
- Check that all internal controls are good. A Ct range for each internal control is within 1 Ct per plate indicates evenly pipetted samples and reagents.
- Scroll through all assays to find patterns. Patterns can indicate that there are laboratory technical errors.

Functions:

- Change data unit by selecting either Ct Value, NPX Value, or Quantified Value (if applicable).
- Use the Assay dropdown menu to change which assay to display, or use the arrow keys for mouse scrolling wheel for faster toggling.
- The bar below the plate shows the range of Ct Value, NPX Value, or Quantified Value (if applicable). The blue values are below median and red values are above median.

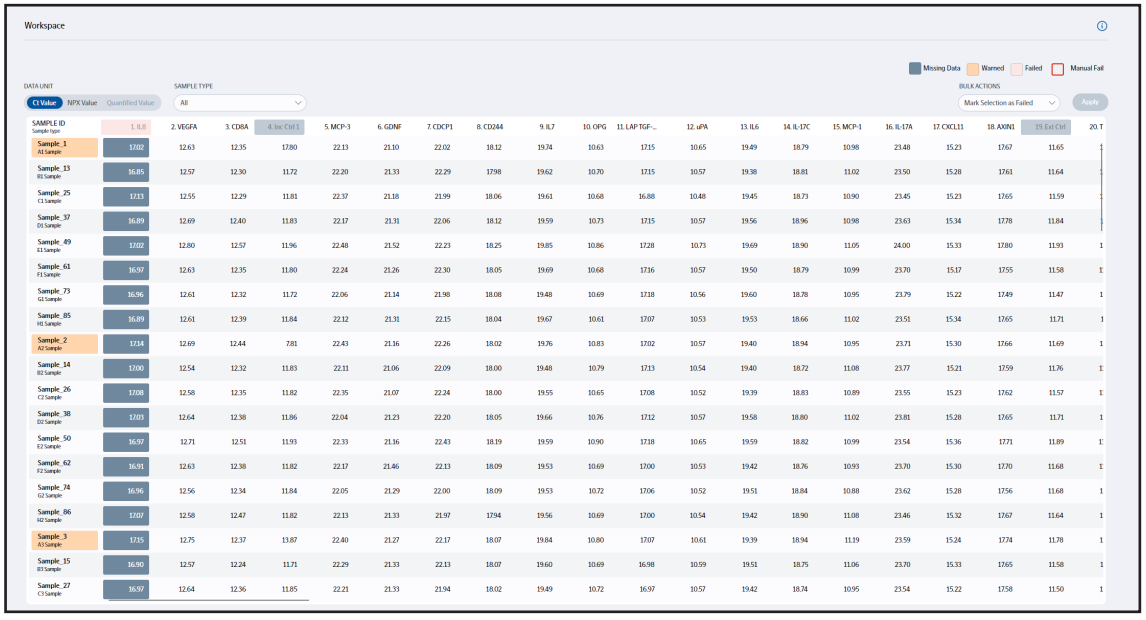
14.7 Workspace

General:

The Workspace view shows values and status for all samples and external controls. In the workspace view, samples and assays can be manually failed. Single datapoints can be falied without failing an entire sample or assays.

The table uses the following colors:

- Dark grey: Missing Data
- Orange: Warned
- Light red: Failed
- Red border: Manual Fail
- Light grey: <LOD / <>LOQ (Only for Quantified values)



Useful for:

- Create manual fails.

Functions:

- Use the Data unit to show Ct Value, NPX Value, or Quantified Value (if applicable).
- Use the Bulk actions dropdown menu to Mark Selection as Failed. Click **Apply**. To undo this action, select **Reset To Automatic QC** in the dropdown list.

QC

- Apply manual QC. It is possible to fail a single datapoint, a sample or an assay.
- Automatic QC will be recalculated and exclude manually failed data.
- After applying manual QC, double check Sample QC, Run QC, and Assay QC.

14.8 Amplification Curves

General:

The Amplification Curves view displays the curves for selected samples, assays, and data points from the current plate, regardless of inclusion status. To select multiple items, hold the **Ctrl** key while clicking.

The Amplification Curves only displays Ct Values. The Ct refers to the number of cycles required to cross the detection threshold of the PCR amplification curve. Based on Ct values, the arbitrary, relative quantification unit NPX is calculated.

The linear portion of each curve is in the exponential phase of PCR, where the amount of product, and therefore the signal, doubles after each cycle. The clustering of the amplification curves for each dilution should be tight.

The Y-axis shows on a log scale ΔRn, the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification and the X-axis the number of cycles.

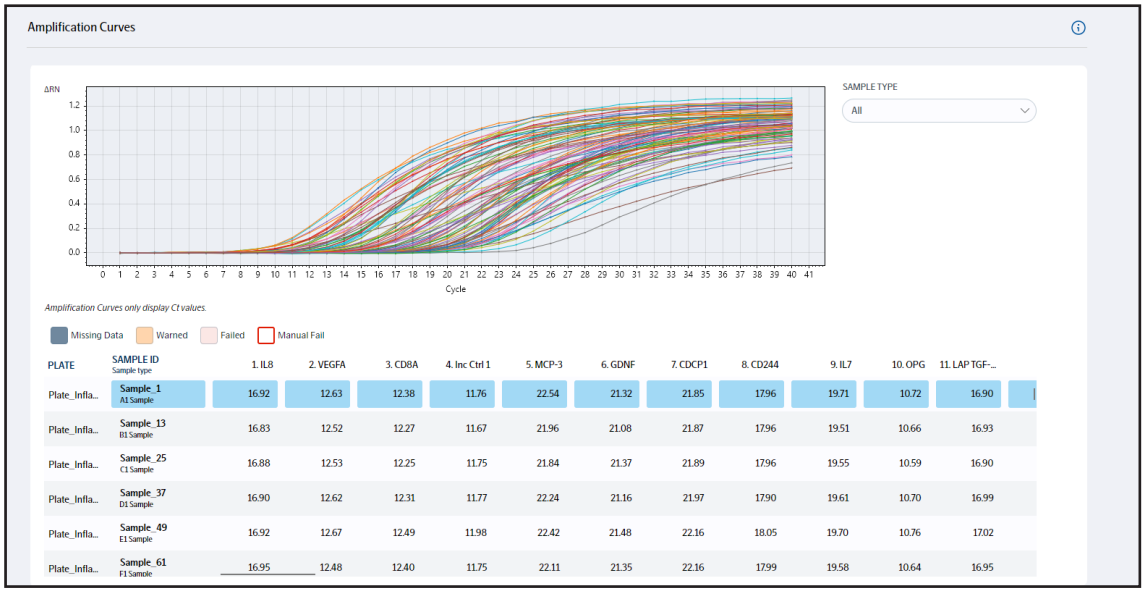


PLATE	SAMPLE ID (Sample type)	1. IL8	2. VEGFA	3. CD8A	4. Inc Ctrl 1	5. MCP-3	6. GDNF	7. CDCP1	8. CD244	9. IL7	10. OPG	11. LAP TGF-...
Plate_Infla...	Sample_1 (A1 Sample)	16.92	12.63	12.38	11.76	22.54	21.32	21.85	17.96	19.71	10.72	16.90
Plate_Infla...	Sample_13 (B1 Sample)	16.83	12.52	12.27	11.67	21.96	21.08	21.87	17.96	19.51	10.66	16.93
Plate_Infla...	Sample_25 (C1 Sample)	16.88	12.53	12.25	11.75	21.84	21.37	21.89	17.96	19.55	10.59	16.90
Plate_Infla...	Sample_37 (D1 Sample)	16.90	12.62	12.31	11.77	22.24	21.16	21.97	17.90	19.61	10.70	16.99
Plate_Infla...	Sample_49 (E1 Sample)	16.92	12.67	12.49	11.98	22.42	21.48	22.16	18.05	19.70	10.76	17.02
Plate_Infla...	Sample_61 (F1 Sample)	16.95	12.48	12.40	11.75	22.11	21.35	22.16	17.99	19.58	10.64	16.95

Useful for:

- Identifying and examining irregular amplification patterns.
- Viewing threshold and baseline values for the run.
- Detecting spikes in the curves , which may indicate sample preparation issues.

Functions:

- Select a sample to show the amplification curves.
- Select multiple samples, assays, or data points by holding the Ctrl key while clicking.
- Use the filter menu to select Sample Type.

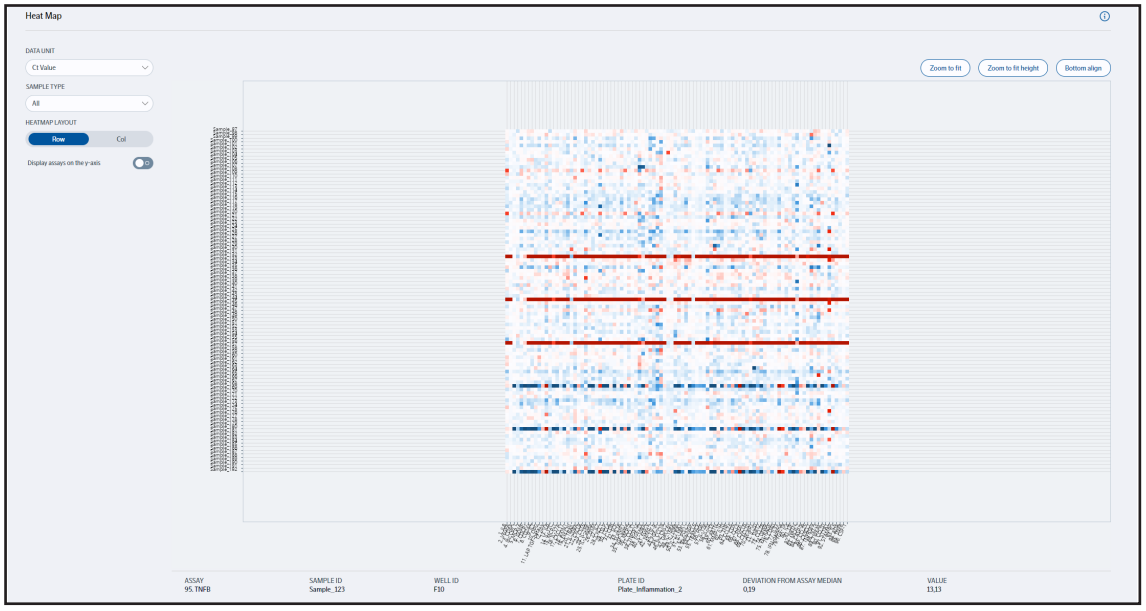
14.9 Heat Map

General:

The Heat Map view contains a color-coded view for all values of all assays for samples and external controls in the chosen plate or all plates. By default, assays are displayed horizontally and samples are displayed vertically. The orientation can be changed using the toggle switch. When the orientation is switched, samples are displayed horizontally and assays are displayed vertically.

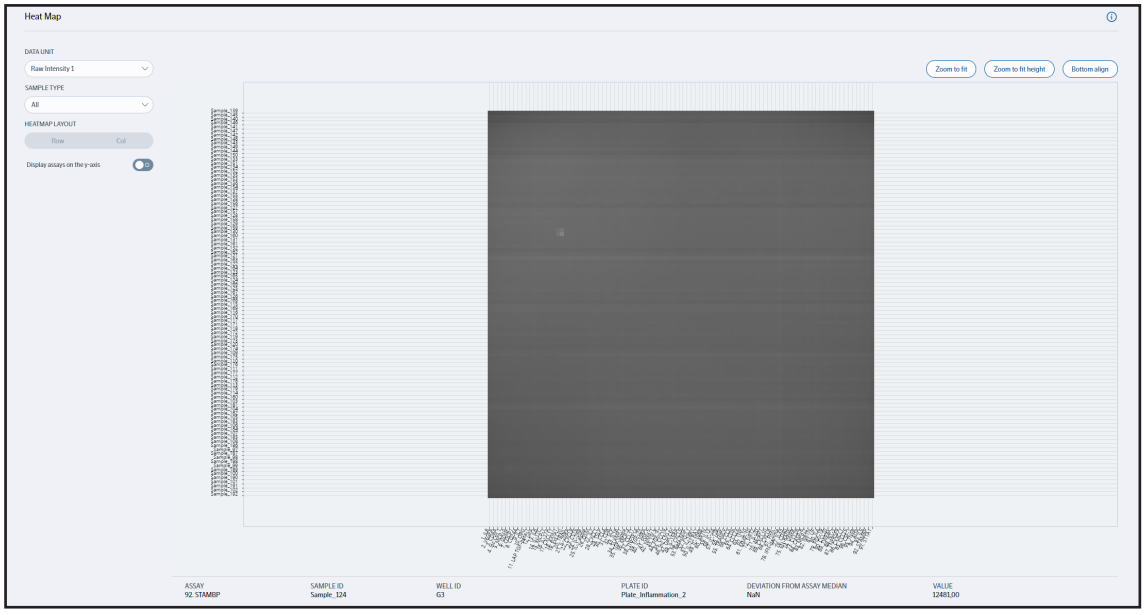
The heat map displays deviations from the plate median for the selected assay using the following colors:

- Blue: values below median
- Red: values above median



When raw data is available, the Heat Map can display Raw Intensity 1 (FAM-MGB fluorescence at qPCR cycle 5) and Raw Intensity 2 (FAM-MGB fluorescence at the final qPCR cycle). These raw intensity values are presented in grayscale and are always displayed using the Chip layout.

Note: The Deviation from assay median will not be displayed when either Raw Intensity 1 or Raw Intensity 2 is selected as the data unit.



Useful for:

- Finding systematic patterns within a plate and across multiple plates in a panel. It also facilitates comparison of external controls to detect outliers. Such outliers may indicate contamination and might need to be failed manually in the Workspace view, or they may suggest that an analyzed sample differs from the rest of the cohort.
- Get an overview of the alignment of the samples.

Functions:

- Change data unit by using the Data Unit dropdown menu. Select one of the following options: Ct Value, NPX Value, Quantified Value (if applicable), Raw Intensity 1, or Raw Intensity 2.
- Change heat map layout to sort the samples row-wise or column-wise based on the plate layout.
- Use the Sample Type dropdown menu to select display samples or external controls.
- Use the toggle switch to change the orientation of assay and sample display.
- Hover over any datapoint in the heat map to view detailed information, including:
  - Assay
  - Sample ID
  - Well ID
  - Plate ID
  - Deviation from assay median (not available when Raw Intensity 1 or Raw Intensity 2 is selected)
  - Value
- Zoom functions:
  - Zoom in or out using the mouse scroll wheel or the arrow keys on the keyboard.
  - **Zoom to fit:** will adjust the heat map to fit in the view.
  - **Zoom to fit width:** willl adjust the heat map to fit vertically.
  - **Left align:** will adjust the heaat map to align on the left of the view.
- To navigate the map:
  - Click and drag with the left mouse button, or use the arrow keys (Up, Down, Left, Right) to move the view.
    - Hold Shift and drag up or down to move vertically.
    - Hold Ctrl and drag left or right to move horizontally.

14.10 Detectability

General:

The Detectability view provides a visual summary of protein detectability across the entire panel. The bar chart shows the selected panel, and the list shows LOD values for all plates in the panel. The bar chart uses the following color code:

- Blue: Valid data. Values above LOD, within the Limit of Quantification (LOQ) if applicable, and not manually failed or failed by automatic QC.
- Red: >LOD (for Olink Target 96 and Olink Focus with RelQ).
  <>LOQ (for Olink Target 48, Olink Flex, and Olink Focus with AbsQ).
- Grey: Missing data. Data not manually failed or failed by automatic QC.

Useful for:

- Check all detectability within the panel to get an indication of the condition of the samples.
- Check for data below LOD / outside LOQ.
- Check every assay at the same time.
- To verify consistency across plates, use the 'Limit of Detection' table to compare LOD values for each assay. LOD outliers can indicate contamination of negative controls.

> **NOTE:** *For the Olink Target 48 panels, some assays can be expected to have LOD values above 2.5 NPX. This should not affect the number of samples that can be quantified above the plate LOD. For the Olink Target 96, the optimal range for LOD is ±2.5 NPX.*

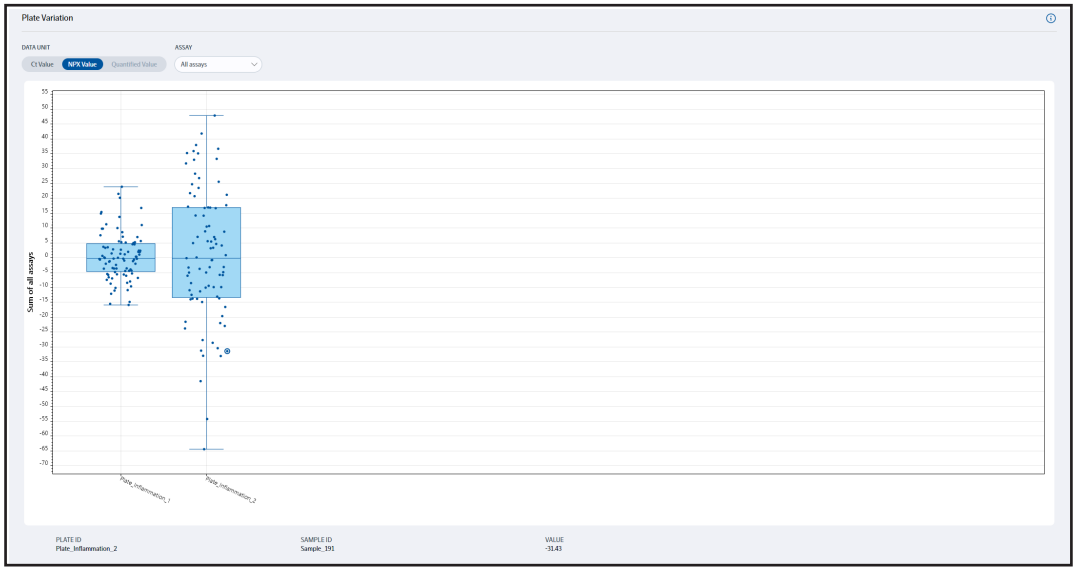
Functions:

- Mark an assay in the diagram to highlight it in the table, and mark an entry in the table to highlight it in the diagram.

14.11 Plate Variation

General:

The Plate Variation view shows the distribution of sample values and their deviation from the plate median for each assay. It uses box plots to visualize Ct, NPX, or Quantified values (if applicable) for samples that have passed Sample QC.



Useful for:

- Comparing intensity distributions across plates.
- Identifying systematic variance in total intensity between plates.
- Detecting potential contamination by checking if box plots are aligned. Misaligned boxes may indicate contamination.

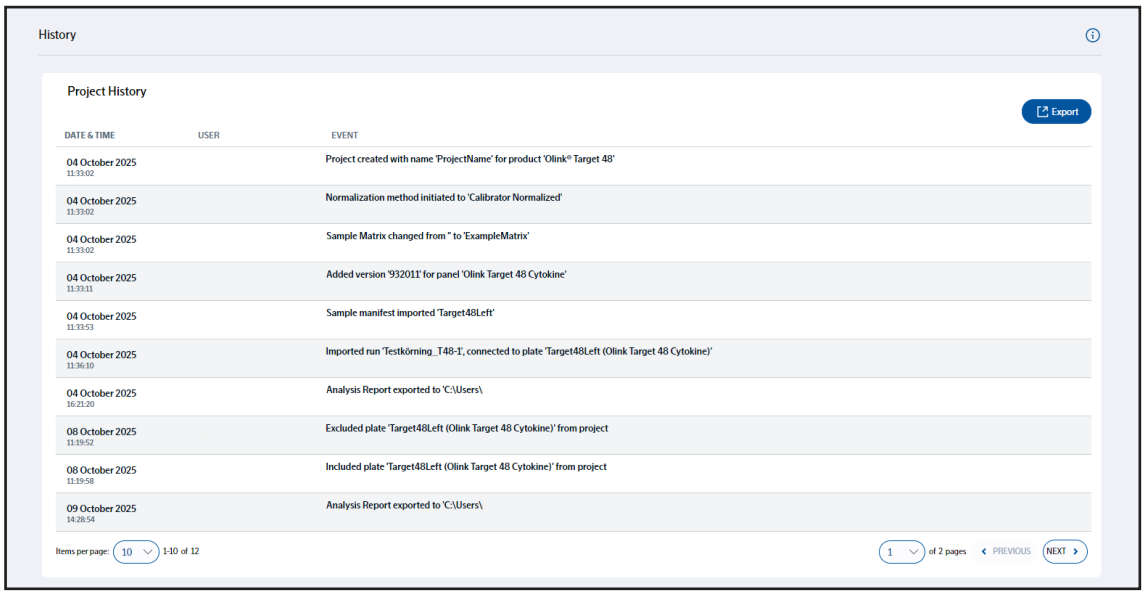
Functions:

- Switch data units by selecting between Ct, NPX, or Quantified values (if available).
- Use the Assay dropdown menu to select specific assays, or quickly switch between assays with the up/down arrow keys or scroll wheel while hovering over the dropdown.
- Hover over the chart and scroll to zoom in or out to view different parts of the box plots.
- Click, hold and drag the plot to move it to view different parts of it.

14.12 History

General:

The History view shows all events for the current project. Everything that is entered into the project are listed in the Project History list.



Useful for:

- Seeing what actions have been taken on the project.

Functions:

- Click on the Export button to export the project history as a CSV file.

15.Keyboard shortcuts

The application supports several keyboard shortcuts that help perform common actions more efficiently.

Action	Shortcut	Description
Plate Selection		
Move to the next plate	Ctrl + .	Press the **Ctrl** key and the **.** (period) key at the same time.
Move to the previous plate	Ctrl + ,	Press the **Ctrl** key and the **,** (comma) key at the same time.
Move to the next panel	Ctrl + Shift + .	Press and hold **Ctrl** and **Shift**, then press the **.** (period) key.
Move to the previous panel	Ctrl + Shift + ,	Press and hold **Ctrl** and **Shift**, then press the **,** (comma) key.
Project Actions menu		
Create a new project.	Ctrl + N	Press the **Ctrl** key and the **N** key at the same time.
Open an existing project.	Ctrl + O	Press the **Ctrl** key and the **O** key at the same time.
Save the current project.	Ctrl + S	Press the **Ctrl** key and the **S** key at the same time.
Close the current project.	Ctrl + W	Press the **Ctrl** key and the **W** key at the same time.
Exit the application.	Alt + F4	Press the **Alt** key and the **F4** key at the same time.
Heat Map		
Move the view up, down, left, or right.	Arrow keys (Up/Down/Left/Right)	Move the view up, down, left, or right by press the **Arrow** keys.
Zoom in or out of the heat map	Ctrl + Arrow keys (Up/Down/Left/Right)	Press the **Ctrl** key and the **Arrow** keys at the same time.

Part 5: Troubleshooting

16.Introduction

This chapter describes issues that may arise during use of NPX Signature Software, or data issues during analysis of the studies, and how to solve these issues.

NOTE: *The figures in the Troubleshooting sections are schematic only and do not reflect the current interface of NPX Signature Software.*

16.1 Warning messages

The list of warning messages is not complete. Other messages may appear. Please contact support@olink.com if further guidance is needed.

In version 2.2 of NPX Signature, the term 'study' has been replaced with 'project'.

The following warnings may be displayed:

- Creating a New Project from the Welcome Screen
  - Creating a New Project and then clicking the Back button will discard all changes and prevent the creation of a project.
    - Select **Cancel** to continue working on the current project.
    - Select **Continue** to discard progress and return to the start screen.
- Opening and closing the Select Panel Data view without selection
  - Opening the Select Panel Data view and closing it without selecting a version will result in loss of all progress and no project will be created.
    - Select **Cancel** to return and continue with the project.
    - Select **Continue** to close the application and discard the project.
- Closing the Panel Data view after selecting a Panel Data version
  - After selecting a Panel Data version, closing the view will trigger a warning. Three options are available:
    - **Cancel**: Returns to the Panel Data view to continue editing.
    - **Don't save**: Closes the application and discards all unsaved changes.
    - **Save without run data**: Saves the project without including run data.
- Changing the Product Type
  - Changing the Product Type after selecting it, along with the Project Name and Panel Data version, will reset all configuration settings.
    - Select **Cancel** to keep the current Product Type and continue.
    - Select **Continue** to change the Product Type and lose the existing configuration.
- Navigating back after selecting a Run Data Source and a Sample Manifest
  - When navigating back in the annotation process (step 4 of 4), after both Run Data Source and Sample Manifest have been selected, a warning message will appear, indicating that all annotations made in step 4 will be lost.
    - Select **Cancel** to continue with the current selections and annotations.
    - Select **Continue** to remove the selected files and discard all annotations.

- Creating a New Project while another Is open
  - If a project is open and New Project is selected from the Project Actions menu, any unsaved changes in the current project will be lost. .
    - To save changes in the current project, click **Save**.
- Opening a project while another Is already open
  - If a project is open and Open Project is selected from the Project Actions menu, any unsaved changes in the current project will be lost.
    - To retain the current project, click **Save & close**.
- Closing a project or the application
  - If a project is open and Close Project is selected from the Project Actions menu, or if the application is closed, any unsaved changes will be lost.
    - To save the project, click **Save & close**.

17.Deviating controls

If the normalized value for internal controls for a specific sample deviates from the rest of the sample set, the sample does not pass Sample QC and is warned in the NPX Signature Software.

Issue	Explanation	Reason	Action
A sample is warned.	A sample is warned when one or several internal controls deviate from the plate median for that specific sample.	All warned samples in a project are shown in the *14.1.1 Overview* in the right window called *Failed or Warned samples.*	The behavior of the internal controls makes it possible to understand why the sample is warned. See the rest of this section for more information. For more detailed information about warned samples, go to the *14.5 Sample QC* view (*Figure A*) and the *14.6 Plate Data* view (*Figure B*)

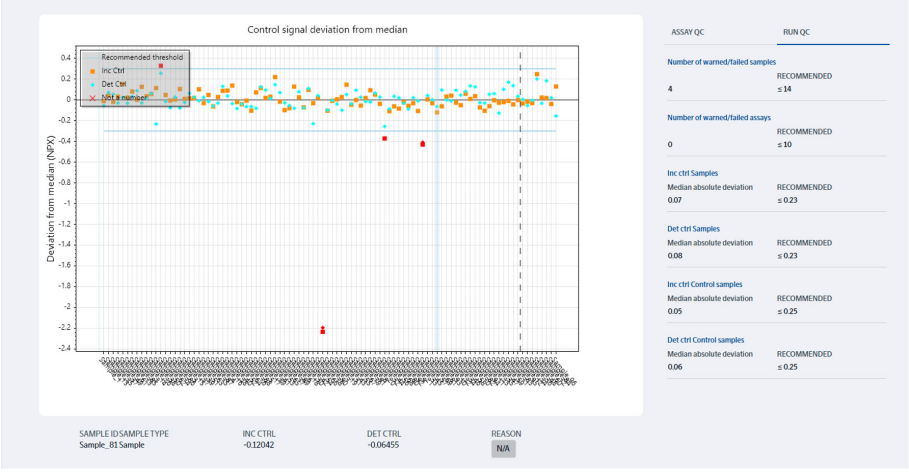


Figure A. The Sample QC view shows the warned samples in red, both by Incubation Control and/or Detection Control. These data-points are outside the recommended threshold of ±0.3 NPX limit which is visualized with the light blue lines. In the Sample QC view, the Run QC view indicates the number of warned/failed samples in the plate (4))

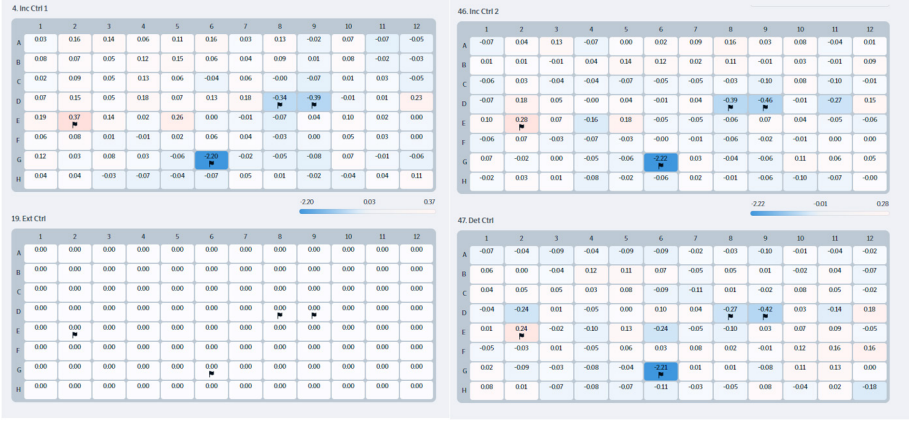


Figure B. Plate Data view corresponding to samples shown in Figure A . The 4 warning samples are shown with a flag symbol, some with lower or higher NPX values for different internal controls.

17.1 Sample warned by Incubation Control

Issue	Explanation	Reason	Action
A sample is warned due to the Incubation Control.	The NPX Value of the Incubation Control for this specific sample deviates more than 0.3 NPX from the plate median.	If the Incubation Control(s) deviate, but not the Extension and Detection Controls, this is an indication that something (most likely in the sample) is affecting only the Incubation Controls.	Refer to *17.1.1 Common reasons for deviating Incubation Control* for examples.

17.1.1 Common reasons for deviating Incubation Control

Possible cause	Explanation	Solution
Sample matrix	If the sample matrix for the warned sample is different from the others on the same plate, the incubation environment can be different (*Figure C*). This can make the reaction slightly more or less efficient.	Perform the QC evaluation for one sample matrix at a time.
Sample volume or concentration	The sample volume is too high or too low or Incubation mix is to high or to low.	Set the affected samples as failed in the *14.7 Workspace* view. If applicable, rerun from the Incubation step and make sure the correct volume is used.
Sample quality	If the samples have pre-analytical variations this can introduce deviation within internal controls.	For all sample quality considerations please refer to the "Pre-analytical variation in protein biomarker research" White Paper, refer to section *3. Associated documentation.*
Sample Type	Olink assays are validated for customer's matrix. Other sample matrices can contain factors that interfere with the immuno reaction step.	Standardize samples as much as possible (for example: for lysed cells and tissue, use similar concentration of protein in each sample).

Figure C. The Plate Data view shows Ct Value deviation for internal controls due to different sample matrices being present in the same plate (Rows A-D one sample type, Rows E–H a different sample type), and thus affecting the incubation environment. This leads to warning samples.

18.Sample warned by Detection Control

Issue	Explanation	Reason	Action
A Sample Control is warned by the Detection Control.	The NPX Value of the Detection Control for this specific sample deviates more than 0.3 NPX from the plate median.	One example can be seen in *Figure A*. Data is normalized using the Extension Control. Since both Incubation Control and the Extension Control display decreased Ct-values, the normalization step will adjust the data. The Detection Control that did not show decreased Ct-values will be overcompensated by normalization against the Extension Control and thereby deviate from the rest of the sample set after normalization.	This type of flag is generally caused by the same reasons as warned Incubation Control. It is not uncommon that both the Incubation Control and Detection Control flag simultaneously, but with opposite "direction" in the *14.5 Sample QC*. This is seen when the warned sample affects both the extension and immunoassay step, but to different extent. See examples in *section 17.1.1*.

18.1 Deviating external controls

Issue	Explanation	Action
High Intra-CV value	Intra-CV value > 15% for Sample Controls within the same plate. The Sample Control replicates differ within the sample plate.	Check sample annotation of the Sample Controls. Check the row pattern for rows containing Sample Controls to make sure that none of the rows is an outlier (for example issue with a channel on one of the multichannel pipettes). If one Sample Control replicate deviates from the other(s) and this pattern can be confirmed for several assays, this could indicate a manual issue with this replicate. In such case, that replicate can be failed and the remaining replicates used for CV calculation. Please contact *support@olink.com* for further guidance.
High Inter-CV value	Inter-CV value > 25% for Sample Control within the same plate. The Sample Control replicates differ between sample plates.	• Check sample annotation of the control samples and that they pass QC. • If one Sample Control replicate deviates from the other(s) two and this pattern can be confirmed for several assays, this could indicate a manual issue with this replicate. In such case that replicate can be failed and the remaining used for CV calculation. • If applying intensity normalization, check for proper randomization between the plates within the project. (Only for Olink Target 96) Please *support@olink.com* for further guidance.

Issue	Explanation	Reason	Action
High LODs that affect the number of samples that can be quantified above LOD.	Calculated LODs are out of expected range.	• Wrong Panel Data File. • Wrong panel. • Contamination of Negative Controls. • Wrong annotation of Negative Controls, Sample Controls or Calibrators.	• Confirm annotation of Negative Controls, Sample Controls and Calibrators. • Confirm datafile; reanalyze data. • If applying intensity normalization, check for proper randomization between the plates within the project. Please contact *support@olink.com* for further guidance.
Assay warning for Sample Control precision in Olink Target 48 Olink Flex Olink Focus.	Sample Control intra CV > 30% for the given assay.	• Sample Control replicates differ within the same plate.	• Check sample annotation of the Sample Controls. • Check the row pattern for rows with Sample Controls to make sure that none of the rows is an outlier (for example issue with a channel on one of the multichannel pipettes). • If one Sample Control replicate deviates from the other two and this pattern can be confirmed for several assays, this could indicate a manual issue with this replicate. In such case that replicate can be failed and the remaining used for CV and accuracy calculation. Please contact *support@olink.com* for further guidance.

Assay warning for Sample Control accuracy in Olink Target 48 Olink Flex Olink Focus.	Sample Control accuracy is > ±30% from expected value.	• Wrong Panel Data File version selected.	• Check that the correct Panel Data File version is applied. • Check sample annotation of the Sample Controls and that they pass QC. • Check the row pattern for rows containing Sample Controls to make sure that none of the rows is an outlier (for example issue with a channel on one of the multichannel pipettes). • If one Sample Control replicate deviates from the other two and this pattern can be confirmed for several assays, this could indicate a manual issue with this replicate. In such case that replicate can be failed and the remaining used for CV and accuracy calculation. Please contact *support@olink.com* for further guidance.
Assay warning for Calibrator precision in Olink Target 48 Olink Flex Olink Focus	Calibrator CV > 30% for the given assays.	Calibrator replicates differ within the same plate.	• Check sample annotation of the Calibrators. • Check the row pattern for rows with calibrators to make sure that none of the rows is an outlier (for example issue with a channel on one of the multichannel pipettes). If one Calibrator replicate deviates from the other two and this pattern can be confirmed for several assays, this could indicate a manual issue with this replicate. In such case that replicate can be failed and the remaining used for CV calculation. Failing a replicate will result in renormalization, and NPX and pg/mL Vaules will change. Please contact *support@olink.com* for further guidance.

19.Inconsistent results detected

19.1 Variation between plates - common reasons

Issue	Explanation	Reason	Action
The “Plate ANOVA” value in the *14.2 Metrics* view (*Figure D*) shows a high value. The variation is also shown on the *14.11 Plate Variation* view. (*Figure E* Plate variation should be solved by intensity normalization.)	The total NPX varies between plates.	• May be caused by insufficient randomization, meaning that the distribution of samples from different groups is not the same for all plates in the project. • Other option is different sample groups and/or matrices are part of the plates.	If the samples are completely randomized, make sure that intensity normalization has been used. In case of different sample groups, create individual projects. The results will then look like the example in *Figure F* the same results as in *Figure E* after intensity normalization.
High Inter-CV value	Inter-CV value > 25%.	• The Sample Control differ between sample plates. • May be caused by insufficient randomization when using intensity normalization. The distribution of samples from different groups is not the same for all plates in the project. Using intensity normalization will than results in higher Inter-CV values.	• Check sample annotation of the Sample Controls and that they pass QC. If intensity normalization has been used, ensure that randomization assumption is valid by comparing with IPC normalization • Check if all samples are from the same project.

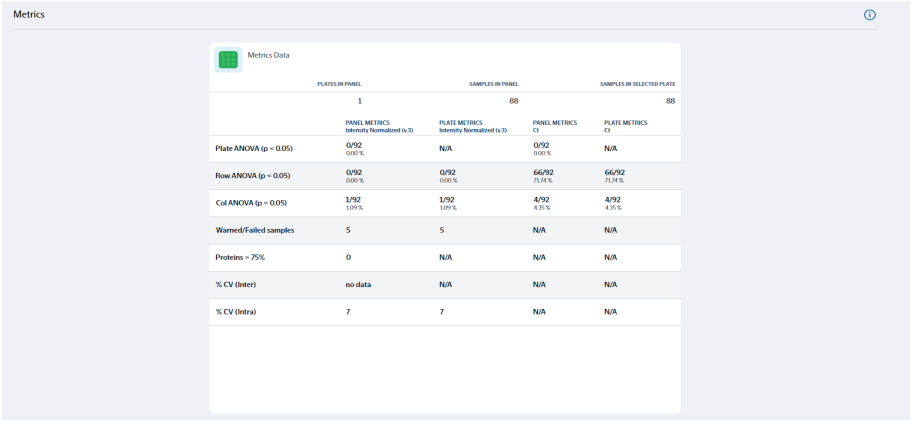


Figure D. The Metrics view with a Plate ANOVA value that indicates variation between plates.

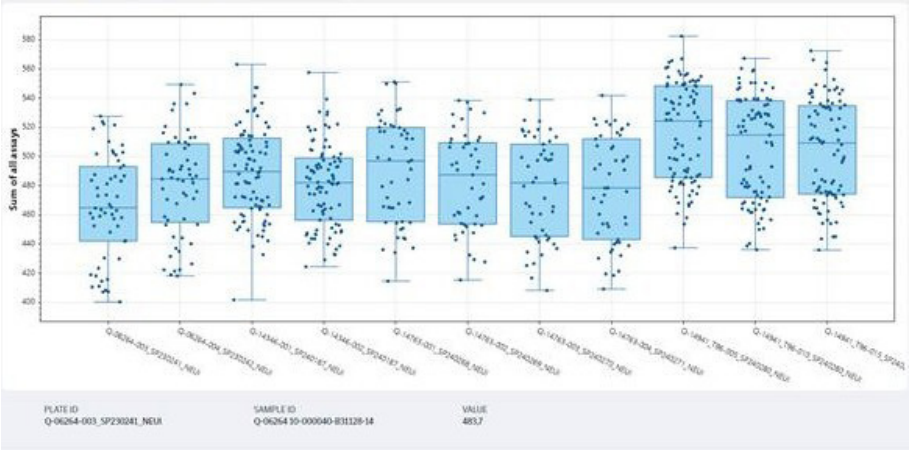


Figure E. Plate variation indication technical deviation.

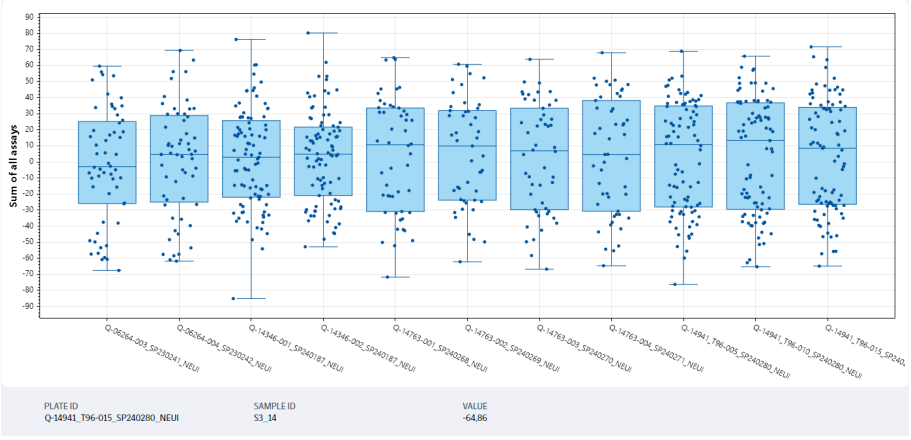


Figure F. The same results as in Figure E, after intensity normalization.

19.2 Variation within a plate - common reasons

Issue	Explanation	Reason	Action
The columns or row ANOVA in the *14.2 Metrics* view show high values. The effects are visualized in the *14.6 Plate Data* tab.	Areas of a plate (columns and/ or rows) have systematically higher or lower values (Ct and/or NPX) than the rest of the plate.	• The variation is caused by the sample position on the plate. Within-plate effects are often caused by laboratory mistakes. • The variation is caused by suboptimal randomization and/ or different matrices on the same plate.	See examples in the next table. **NOTE:** *Make sure that the deviation cannot be explained with biological variation, i.e. that a unique sample group is placed on one column/row.*
Position effects/patterns: visible only for Internal Controls			
All Internal Controls are affected on Ct-level in a similar manner (all increased or decreased.)	One sample column or row consistently show deviating values.	• Pipetting error, for example pipette not pre- conditioned. • Different matrices on the plate.	• Exclude data from analysis and/or rerun. • Create individual projects per matrix.
		Mistake during pipetting of the incubation mix, extension mix, detection mix, or samples.	
		Evaporation due to incomplete sealing of plate during incubation or PCR steps.	Exclude data from analysis and/or rerun.
		Different sample matrices.	QC matrices separately.
		Forgotten to pause the PCR machine resulting in only a few minutes 50 °C instead of 20 minutes	Rerun from Incubation needed.
Incubation Controls and Extension Control differ from Detection Control on Ct-level.	Incubation and Extension Controls show the same pattern, Detection Control shows another pattern.	• PCR instrument not good at keeping 50 °C, Detection Control not affected by extension step. • Enzyme not added resulting is suboptimal extension step.	Rerun from Incubation needed.
		May be pre-analytical (interfering factors may affect all controls but the Detection Control).	Contact *support@olink.com* for further guidance.
Incubation Control differ from Extension and Detection Controls.	Incubation Control shows deviating pattern.	• Pre-analytical factors in samples that interfere with the immuno reaction. • Contamination in samples, for instance GFP present.	Few deviating samples: • Exclude samples from subsequent statistical data analysis. • > 1/6th of samples deviating: Contact *support@olink.com* for further guidance.
Position effects/patterns: visible for samples and Internal controls			
Columns/rows/parts of sample plate.	Patterning that follows sample naming/sample matrices.	• Different sample matrices. • Suboptimal randomization.	QC matrices separately.

Issue	Explanation	Reason	Action
Gradient over the sample plate.	Upper or lower part or left and right part of the plate affected with clearly deviating values.	• IFC issue. • Poor vortexing in extension step. • Poor mixing of detection mix. • Uneven temperature in PCR block.	Rerun. Contact support@olink.com for further guidance.

19.2.1 Common problems and reasons for within-plate effects.

Problem	Possible cause	Figure reference
First column different than the rest of the plate (observed for Internal Controls).	The pipette was not preconditioned.	
A specific column or columns is/are different than for the rest of the plate (observed for internal Controls).	A mistake when the incubation solution, extension mix, detection mix, or samples were pipetted.	*Figure G*
One row consistently different from the rest of the plate (observed for Internal Controls and/or samples).	The pipette tip in that specific position was not tight enough.	
Deviating Plate Variation distribution for single assays on one plate.	Primer contamination.	*Figure H*
A gradient from row A to row H.	Pipetting was done at an angle. The plates were not sufficiently vortexed.	*Figure I*

Figure G. Ct Values that show results of laboratory mistakes. Refer to table below for a description of errors performed on this run. The figure shows a Olink Target 96 project, but the behaviour is similar for other products.

Figure H. Ct Values that show primer contamination of Incubation Control 2.

	Column 4 (correct)	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Incubation mix (µL)	3	3	3	3	3	**6**	3
Sample volume (µL)	1	1	1	**0**	**2**	1	1
Extension mix (µL)	96	96	**192**	96	96	96	96
Detection mix (µL)	7.2	**14.4**	7.2	7.2	7.2	7.2	7.2
PCR product (µL)	2.8	2.8	2.8	2.8	2.8	2.8	**5.6**

Figure I. Within plate effects due to incorrect or insufficient vortexing of the sample plate during sample dilution.

19.3 Extreme outliers - common reasons

Issue	Explanation	Reason	Action
Extreme blue or red lines in heat map (when only selecting “samples” in the dropdown menu) or outlier dots in Plate Variation view. **NOTE:** *Extreme outliers can be discovered in different views (Plate Variation, Assay QC, Heat map).*	Sample with extremely low or high protein quantification.	• Incorrect dilution of samples. • No sample added. • Same columns two times sample added.	Fail sample.
		Sample contains only a buffer with no protein content.	Investigate if a sample ressembles a Negative Control. If so, do not fail the sample.

19.4 High NPX signals - common reasons

Issue	Explanation	Reason	Action
Unexpectedly high NPX signals in diluted panel for the majority of samples.	• Low variation between samples. • Saturated assays (may be visualized in *14.11 Plate Variation* view).	Samples were not diluted.	Rerun from sample dilution step.

20.Revision history

Version	Software version	Date	Description
v2.2	2.2	2025-11-07	*6.2 Install software:* Updated the installation process. *8.4 Intensity normalization (Olink® Target 96):* Added new information. *8.5 CV calculation:* Added new section. *8.8 Run QC:* Added new information. *8.9 QC criteria:* Added new section. *10.3 Sample Manifest file:* Added new information about the Sample Manifest file. *11.2 Import Panel Data Files or Panel Data Archive:* Updated content and images. *11.3 Create a new project:* Updated content and images. *11.4 Import a split Olink® Target 48 in version 2.x:* Added new section. *11.6 Migrate a project:* Updated content and images. *11.8 Perform quality control:* Updated content and images. *11.9 Export result files:* Updated content and images. *11.9.1 Preparing to Export the Analysis Report:* Added new section. *12.1 CSV files:* Added new information. *12.2 Analysis Report:* Updated and expanded content. *13. General:* Updated content and images. *14. Views:* Updated content and images. *15. Keyboard shortcuts:* Added new section. *16.1 Warning messages:* Updated and added new information.
v2.0	2.0	2025-02-12	NEW

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1606, v2.2, 2025-11-07