

NPX™ Мар

Software User Manual

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Part 1: Introduction

Olink high-multiplex immunoassay panels provide an efficient and innovative tool for targeted human protein biomarker discovery, development and validation.

1. About this manual

This user manual provides you with the instructions needed for data processing when running Olink[®] Reveal, Olink[®] Explore HT, or Olink[®] Explore 3072/384 panels with a locally installed set up of software.



NOTE: The information in the NPXTM Map Software User Manual is believed to be accurate. However, the displayed screenshots may differ from actual user interface and should be considered as examples.

1.1 Intended use

NPX[™] Map is a data analysis software that is designed for Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 platforms. It allows for importing data, validating data quality, and normalizing Olink data for subsequent statistical analysis.

NPX Map is intended for Research Use Only. Not for use in diagnostic procedures.

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1.2 Intended target group

NPX Map is intended to be used by staff certified to run the Olink Reveal, Olink Explore HT or Olink Explore 3072/384 platform. Quality control should be performed by trained users that determine whether data from a run can be approved for further analysis.

2.List of abbreviations

- %CV Coefficient of Variance
- NGS Next Generation Sequencing
- PCR Polymerase Chain Reaction
- PEA Proximity Extension Assay
- QC Quality Control
- SD Standard Deviation

3.Associated documentation

3.1 Olink documentation

- Olink® Map Preprocessing Technical information, vx.x or later
- NPX[™] Map CLI Technical information, vx.x or later

Olink Reveal manuals

- Olink[®] Reveal Overview User Manual
- Olink[®] Reveal Laboratory Instructions

Olink® Explore HT manuals

- Olink[®] Explore HT Overview User Manual
- Olink[®] Explore HT F.A.S.T. lab instructions
- Olink[®] Explore HT Mosquito lab instructions
- Olink® Explore HT Sequencing using NovaSeq 6000 S4 User Manual
- Olink[®] Explore HT Sequencing using NovaSeq X User Manual

Olink® Explore 384/3072 manuals

- Olink[®] Explore Overview User Manual
- Olink[®] Explore 384 User Manual
- Olink[®] Explore 4 x 384 User Manual
- Olink[®] Explore 3072 User Manual
- Olink[®] Explore Sequencing using NextSeq 550 User Manual
- Olink[®] Explore Sequencing using NextSeq 2000 User Manual
- Olink[®] Explore Sequencing using NovaSeq 6000 User Manual
- Olink® Explore 384/3072 Sequencing using NovaSeq X Plus User Manual

All relevant Olink documentation is available from the Olink website www.olink.com/downloads.

3.2 Other documentation

• Article & lot configuration sheet - delivered together with the analysis kit.

4. Technical support

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

5.Process

5.1 Hardware and software requirements

5.1.1 System requirements for NPX[™] Map

Components	Minimum	Recommended
Operating System	Windows [®] 10 or Windows [®] 11	
Processor	Intel® Core™ i5	Intel [®] Core™ i7 or higher
Memory	16 GB RAM	32 GB RAM or more
Disk Space	500 GB	500 GB
Display scale of computer screen		Scale 100 % * 14" screen

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

5.1.2 System requirements for Preprocessing

Components	Minimum	Recommended
Operating System	Linux Ubuntu 20.04 LTS or Linux Ubuntu 22.04 LTS	
Other	Linux administrative knowledge	

5.1.3 Requirements for analysis

Files and information needed for analysis:

- Count files in CSV format and run metadata in JSON format (NGS raw data preprocessed using ngs2counts)
- Plate layout with sample names
- Data analysis reference ID number of reagents used (provided on the Lot configuration insert delivered with the kit)

5.2 Preprocessing runs

Preprocessing is a necessary step in NPX Map analysis. The Next Generation Sequencing (NGS) output from the sequencing instrument is converted to counts files containing the number of reads for each Olink sequence and a run metadata file containing additional information which is necessary for importing the counts files into the analysis software NPX Map or NPX[™] Map CLI.

The preprocessing can be performed in two different ways:

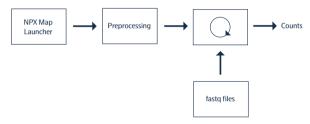
Alternative A

In this alternative, the preprocessing is done in the preprocessing software.



Alternative B

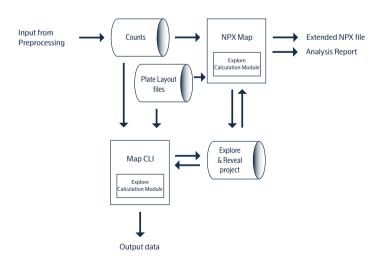
In this alternative, the fastq files are imported directly to the NPX Map.



For more information about the different preprocessing versions, refer to the NPX[™] Map Preprocessing Technical information.

5.3 CLI

QC and normalization of Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 data can be performed either using the NPX Map CLI or the NPX Map Software, or using a combination of the two.



NPX Map is a command-line interface (cli). The application is capable of performing normalization, Quality Control (QC) and CV computations on NGS data, and exporting the results on several supported formats.

For more information, refer to the NPX[™] Map CLI Technical Information.

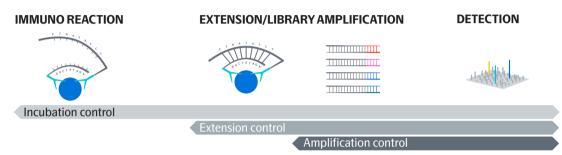
Part 2: Technology description

6.Overview

Olink Reveal, Olink Explore HT, and Olink Explore contains a built-in quality control system using internal and external controls, which enables full control over the technical performance of assays and samples.

6.1 Internal controls

Three internal controls are spiked into every sample for each panel and dilution. The internal controls are designed to monitor the quality of the assay's performance, as well as the quality of individual samples:



Incubation Control (Immuno Control)

The Immuno Control is a non-human antigen measured with PEA. This control is included in the immuno reaction and monitors potential technical variation in all three steps of the reaction.

Extension Control

The Extension Control is an antibody coupled to a unique pair of DNA-tags. These DNA-tags are always in proximity so this control is expected to give a constant signal independent of the immuno reaction. This control monitors variation in the extension and amplification step.

Amplification Control

The Amplification Control is a complete double-stranded DNA amplicon that does not require any proximity binding or extension step to generate a signal. This control monitors the amplification/sample indexing step.

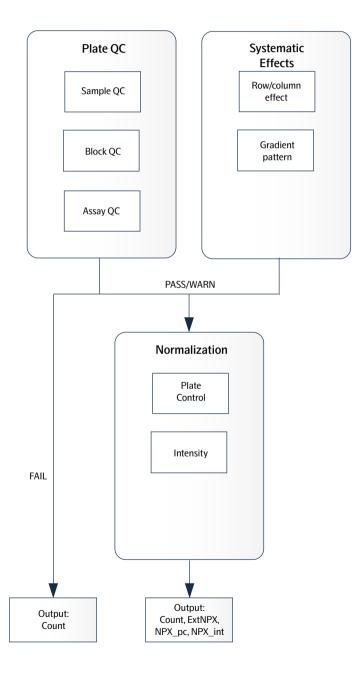
The Extension Control is used to calculate the NPX, refer to 7.4 Normalization, and the other two are used in the Quality Control.

7.The QC workflow

Three internal controls are added to each sample to monitor the quality of assay performance, as well as the quality of individual samples:

- Incubation Control •
- **Extension Control** .
- **Amplification Control** •

The Extension Control is used to calculate the NPX, refer to 7.4 Normalization, and the other two are used in Quality Control (described below).



7.1 Plate QC

The QC is performed directly on Counts, on one block per 96 samples. The Plate QC are divided into three parts: Sample QC, Block QC, and Assay QC. For acceptance criteria, refer to *8. QC criteria*.

7.1.1 Sample QC

Samples and external controls that fail Sample QC will not be considered for additional QC steps and not normalized. Only counts will be reported for those.

1. Low total number of counts per sample per block

Too low total counts for a sample might indicate that the sample is missing or the index was not added to the sample. Each sample and external control should have a minimum number of counts in a block, otherwise they fail.

2. Low number of counts of internal controls per block

Low counts in any of the internal controls might indicate a technical error in the workflow for the corresponding sample, for example a missing internal control.

- Each external control should have a minimum number of counts for each of the internal controls, otherwise they fail.
- Each sample should have a minimum number of counts for each of the internal controls, otherwise they fail or get a warning.
- 3. Deviation of counts in Plate Controls

Deviation of internal control's counts from the expected ranges in Plate Controls might indicate different technical errors.

- A Plate Control fails if the fraction of counts of all internal controls to total counts in logarithmic scale, deviates positively or negatively from the reference values. The reference ranges are block specific and kit lot related.
- 4. Unexpected signal in Negative Controls

Detection of high number of counts for many assays, relative to the counts of internal controls, in any Negative Controls might indicate that signals are from other sample types or not from pure buffer.

- A Negative Control fails if many assays get higher number of counts relative to the counts of internal controls.
- 5. Deviation of incubation control ratio per sample per block

The relative count levels of incubation control to other internal controls (ratio in logarithmic scale) should be consistent across the plate. Any sample where these ratios deviate from the expectation by an amount larger than a reference value indicates a technical error in the workflow.

- The expected incubation control ratios are given by the median of the plate controls and sample controls per plate per block.
- An external control fails if the incubation control ratio is much lower than the expectation.
- A sample gets a warning if the incubation control ratio is much lower than the expectation.

7.1.2 Block QC

Block QC is to quality control whether a block-plate is affected by technical errors. This step is based on the number of Plate and Negative Controls that pass Sample QC. A failed block is recommended to be rerun.

- 1. Based on Plate Controls:
 - A block-plate fails if <50% of Plate Controls pass the Sample QC (a minimum of 3 Plate Controls is required to pass the block-plate).
- 2. Based on Negative Controls:
 - A block-plate fails if none of the Negative Control pass the Sample QC.

7.1.3 Assay QC

Detection of high number of counts for any assay, relative to the internal controls, in any of the Negative Controls is considered as unexpected signal. This step is performed on Negative Controls that pass Sample QC.

• An assay gets a QC warning if it gets high number of counts relative to the counts in internal controls in all Negative Controls.

7.2 Systematic effect detection

Systematic effects are defined as a systematic pattern on a plate caused by a deviation in the workflow such as instrument failure or human error. A pattern corresponds to a particular type of failure. Detection of systematic effects is complementary to the Plate QC and is performed on NPX. All samples and data-points (passed, failed, and warned) are included in the computation.

Systematic effects are categorized into patterns that impact the full plate or impacting a row or/and column. To detect the patterns, three main criteria are considered:

- The frequency of assays representing the effect
- The intensity of the effect on NPX deviation
- Number of samples showing the effect

A pattern is detected per assay/block/plate unit. If enough assays (internal controls are not included) are affected, the block will get a systematic effects warning corresponding to that effect in NPX Map (Quality Control Summary view). Systematic effects may be due to either non-randomized plate design or technical errors and should be investigated further when warned.

7.2.1 Types of systematic effects

- Row
- Column
- Four_column
- Alternating_column
- Row_gradient
- Column_Gradient
- Diagonal_Gradient

7.3 Summary notes for exported data

- Data related to all assays, including the internal controls, will be presented in the output NPX file.
- Data related to all samples, including the external controls, will be presented in the output NPX file.
- Data related to any failed sample or block that do not pass QC criteria will not be normalized and therefore no NPX will be computed. Counts are reported for the failed data-points in the NPX file.
- Any data-points with a QC warning should be used with caution.

For any more information about Plate QC and systematic effects, please contact support@olink.com.

7.4 Normalization

The two between-plate-normalization methods are called Plate Control (PC) and Intensity normalization. They both adjust each assay per plate to a median, but they differ in how these medians are calculated. An important concept when selecting normalization procedure is randomization, which in this context applies to the sample placement across the plates. For details, refer to <u>Randomization FAQ</u> on the Olink website.

Plate Control normalization is performed by default. Intensity normalization should be selected for all Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 projects where samples are randomized. Contact support for study design assistance and choosing the appropriate normalization type.

7.4.1 Converting counts to NPX

The Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 system's raw data output is counts, where each combination of assay and sample is given an integer value based on number of copies detected. These raw data counts are converted into NPX values for use in the continued analysis.

NPX generation

The NPX values are calculated in two main steps. First, the assay counts of a sample are divided with the Extension Control for that sample and block, and a log2 transformation is applied (1). The resulting scale has increasing values with increasing concentration for each assay.

The second step is either Plate Control normalization (2a) or Intensity normalization (2b). Plate Control normalization subtracs the median of the Plate Controls. Intensity normalization subtracs the median of all samples (excluding the control strip).

Steps in the NPX generation described in equation form, where *i* refers to a specific assay, *j* refers to a sample, and ExtNPX defines an extension normalized NPX value.

- 1. ExtNPX_i,j = log2(counts(sample_j,Assay_i)/counts(ExtCtrl_j))
 - Relate counts to known standard (Extension Control).
 - For all assays and all samples, including Negative Controls, Plate Controls, and Sample Controls.
 - Log2 transformation gives more normally distributed data.

2a. NPX_i,j = ExtNPX_i,j - median(ExtNPX(Plate Controls_i))

- Normalize by median of Plate Controls.
- For each assay, per plate.
- 2b. NPX_i,j = ExtNPX_i,j median(ExtNPX(Samples_i))
 - Normalize by median of samples (excluding control strip).
 - For each assay, per plate.

7.4.2 CV calculation

To evaluate the reproducibility and repeatability of data related to each plate in the project, and to assess the within- and between-plate variation in the project, two types of CV are computed for each of the preselected assays: 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.

In each block, pre-selected assays with good performance in Olink Sample Controls are used so that any deviating CV actually indicates a potential technical issue within the workflow. The CV is calculated for NPX of those selected assays (i), using the assumption of a log-normal distribution, in Sample Controls using the formula below. The CV is part of the UI assessing run quality, and is presented in a CV Table showing average CV:s over the blocks

 $CV_i = 100 \sqrt{(e^{Slni^2} - 1)}$, where $Sln_i = ln(2) \times SDisk$

8.QC criteria

Criteria	FAIL NPX is not calculated, exclude from statistical analysis	WARN <i>NPX is calculated, assess further, use</i> data with caution
Sample QC		
Total counts per sample	< 10 000	N/A
Incubation control counts per sample	< 150	< 500
Extension control counts per sample	< 150	< 1000
Amplification control counts per sample	< 150	< 500
Internal control count fractions per sample	N/A	Log2 of incubation-to-amplification control count ration < -3.5 and,
	N/A	Log2 of incubation-to-extension control count ratio < -3.5 and,
	N/A	Absolute value of log2 of extension-to- amplification cotrol count ratio >3.5
External Control QC		
Total counts per sample	< 10 000	N/A
Incubation control counts per sample	< 500	N/A
Extension control counts per sample	< 1000	N/A
Amplification control counts per sample	< 500	N/A
Plate control internal control counts relative to assay counts	< or > internal control reference range to assay counts	N/A
Negative Control internal control counts relative to assay counts	Negative control fails	N/A
Internal control count fractions per sample	Log2 of incubation-to-amplification control count ratio < -3.5 and,	N/A
	Log2 of incubation-to-extension control count ratio < -3.5 and,	N/A
	Absolute value of log2 of extension-to- amplification control count ratio >3.5	N/A
Block QC		
Plate Controls passing external sample QC	For Olink Explore HT: < 50% (minimum 3 Plate Controls must pass QC)	N/A
	For Olink Explore 3072/384:: All 3 Plate Controls must pass QC.	N/A
Negative Controls passing external sample QC	<1	N/A
Systematic effect (NPX)	N/A	>10% of assays Systematic effect identified
Assay QC	· · · · · · · · · · · · · · · · · · ·	·
Assay count relative to internal control count in negative control	N/A	Assay count > any internal control count

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

IРХ™ Мар		- 0
Welcome to NPX [™] Map		
What would you like to do today?		
Create a new project Set up your project by importing plate layouts, select reference IDs, and import an NGS run folder	Open an existing project Continue where you left off from a previously saved project	Panel Data Archive Upload a new Panel Data Archive for use in your projects
		Process FASTQ data Convert new sequencing FASTQ output to an NGS run folder with counts that can be imported into a project
Reveal_training		Last opened: 2025-01-19 at 15:51
New_Project		Last opened: 2025-01-08 at 10:53
Explore_HT		Last opened: 2024-12-18 at 10:42
0 test		Last opened: 2024-12-17 at 15:55
New_Project		Last opened: 2024-12-16 at 09:37
D Reveal_manual		Last opened: 2024-12-16 at 08:52
Manual test		l act opened: 2024-12-12 at 11-38

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

- 1. Process fastq data
- 2. Upload Panel Data Archive
- 3. Create a new project or open an existing project
- 4. Import NGS run data
- 5. Perform quality control
- 6. Export data files and Analysis Report
- 7. Finalize the project

10.Operating workflow

10.1 Process FASTQ data

It is possible to add one or more FASTQ runs in NPX Map. The FASTQ file is a text file that contains biological sequence and its corresponding quality scores.

1. Click on **Process FASTQ data**.

NPX™ Map		- 0
Welcome to NPX™ Map		
What would you like to do today?		
Create a new project Set up your project by importing plate layouts, select reference IDs, and import an NGS run folder	Open an existing project Continue where you left off from a previously saved project	Panel Data Archive Upload a new Panel Data Archive for use in your projects
		Process FASTQ data Convert new sequencing FASTQ output to an NGS run folder with counts that can be imported into a project
Reveal_training		Last opened: 2025-01-19 at 15:51
New_Project		Last opened: 2025-01-08 at 10:53
Explore_HT		Last opened: 2024-12-18 at 10:42
test		Last opened: 2024-12-17 at 15:55
New_Project		Last opened: 2024-12-16 at 09:37
Reveal_manual		Last opened: 2024-12-16 at 08:52

2. Click on the Settings menu in the upper right corner to configure a new sequencing instrument

NPX™ Map	-	
Process FASTQ data		
+ Newran	🗈 Open output folder	a
	No Data to display Add instruments in setting to be able to start a new run	

3. Select Instrument Type from the drop-down menu and fill in type instrument ID. Click Add.

Instrument ID is used for traceability and will be present in downstream data, it doesn't necessarily have to be the correct ID if that information is not known.

It's important to set the correct Instrument Type since it affects how the software process the FASTQ files in question.

If uncertain please contact your service provider about what instrument was used in the sequencing.

Settings	
ADD INSTRUMENT	INSTRUMENT TYPE
123	Element Biosciences AVI 🗸 🖌
INSTRUMENT ID	ТҮРЕ
	Remove selected
Cancel	✓ Save

4. The added instrument(s) will be listed. When finnished, click Save.

Settings ADD INSTE	RUMENT	INSTRUMENT TYPE
Type inst	rument ID	Choose from dropdown V Add
	INSTRUMENT ID	ТҮРЕ
	123	Element Biosciences AVITI
		Remove selected
Cancel		Save

5. Click **New run**, and import compressed FASTQ files and select an output folder to save the run. Individual compressed FASTQ files can only be imported by drag and dropping them onto the FASTQ FILES field. Folders containing compressed FASTQ files can be imported both by drag and drop or with the file picker.

The experiment name field is optional but if set will appear as metadata for the processed run. Pick the product type used in the run, and also fill in the run identifier field. The run identifier field is mandatory to help identify the processed run, it does not have to be a correct ID if that information is not known. When all fields are filled, click **Run** and the processing of the data will start.

New Run				
FASTQ FILES				
	Select folder with FAS	TQ files or drop indi	vidual files	
OUTPUT FOLDER	Uploaded FASTQ	files will appear here		
	🕚 Select	output folder		
EXPERIMENT NAME				
PRODUCT TYPE				
Olink® Explore	3072 Olin	k® Explore HT	Olink	PReveal
INSTRUMENT Select instrument	~	RUN IDENTIFIER	3	
Cancel				Run

6. It is not possible to leave the Process FASTQ data view while the uploaded run is processed. The FASTQ batch jobs are listed in the Process FASTQ data main window.

Completed batch jobs are marked as either success or failure. Click on a batch job to view detailed information.

Click on the **Open output folder** button to open the output folder for the selected batch job.

🗳 NP3	(™ Мар									- 🗆	×
Pro	cess FASTQ d	ata									
	+ New run								C Open out	tput folder)
	Uploaded Runs	S	NAME PRO	DUCT	TOTAL NO. OF READS	NO. OF FILES	INSTRUMENT	INSTRUMENT ID	STATUS		
	123	HT_manual	Expl	ore HT	432 000	1	Element Biosciences AVITI	123	In process		
	Log										
	TIMESTAMP	LOG LEVEL	LOG EVENT								
	[10:37:01]	Inf	Process has starte	ed							
	[10:37:01]	Inf	Processing run "1	.23"							
	[10:37:01]	Inf	Instrument type i	s "Element B	iosciences AVITI"						Ì
	[10:37:01]	Inf	Input method use	ed is FastqFil	es						I
	[10:37:01]	Inf	Given product is	"Explore HT"							
										← Return to main n	nenu

Click Return to main menu to return to start page.

10.2 Upload Panel Data Archive

Before creating a project, a compatible panel archive must be imported into the NPX Map.

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

1. On the start page, click **Panel Data Archive.**

NPX™ Map		- 0
Welcome to NPX [™] Map		
What would you like to do today?		
Create a new project Set up your project by importing plate layouts, select reference IDs, and import an NGS run folder	Open an existing project Continue where you left off from a previously saved project	Panel Data Archive Upload a new Panel Data Archive for use in your projects Process FASTQ data Convert new sequencing FASTQ output to an NOS run folder with counts that can be imported into a project
Recent Projects		Last opened: 2025-01-19 at 15:51
New_Project		Last opened: 2025-01-08 at 10:53
Explore_HT		Last opened: 2024-12-18 at 10:42
0 test		Last opened: 2024-12-17 at 15:55
New_Project		Last opened: 2024-12-16 at 09:37
D Reveal_manual		Last opened: 2024-12-16 at 08:52

2. The Panel Data Archive view shows latest version and minimum compatible version. Click **Upload archive**.

У™ Мар							- 0
NPX™ Мар							
Panel Data Archiv	re						
CURRENT PANEL D	ATA ARCHIVE VERSION						Upload archive 🐧
CARDIOMETABOLIC	INFLAMMATION	NEUROLOGY	Explore 3072	Explore HT Reveal		NEUROLOGY II	ONCOLOGY II
E70009	E50010	E80009	E60009	E71008	E51008	E81008	E61009
E70008	E50009	E80008	E60008	E71007	E51007	E81007	E61008
E70007	E50008	E80007	E60007	E71006	E51006	E81006	E61007
E70006	E50007	E80006	E60006	E71005	E51005	E81005	E61006
B23407	E50006	B23408	B23406	E71004	E51004	E81004	E61005
E70005	B23405	E80005	E60005	B22605	B22603	B22606	E61004
							Done

3. Browse for files to upload. Click **Open**.

The uploaded panel data files will be listed. Click **Done** to get back to the start page.

™ Map							- 0
NPX™ Map		\odot	Success Panel Data Archive has	been loaded.	×		
Panel Data Archiv	e						
CURRENT PANEL D	ATA ARCHIVE VERSION		Explore 3072	Explore HT Reveal			Upload archive 🐧
CARDIOMETABOLIC	INFLAMMATION	NEUROLOGY	ONCOLOGY	CARDIOMETABOLIC II	INFLAMMATION II	NEUROLOGY II	ONCOLOGY II
E70009	E50010	E80009	E60009	E71008	E51008	E81008	E61009
E70008	E50009	E80008	E60008	E71007	E51007	E81007	E61008
E70007	E50008	E80007	E60007	E71006	E51006	E81006	E61007
E70006	E50007	E80006	E60006	E71005	E51005	E81005	E61006
B23407	E50006	B23408	B23406	E71004	E51004	E81004	E61005
E70005	B23405	E80005	E60005	B22605	B22603	B22606	E61004
							Done

10.3 Create a new project or open an existing project

10.3.1 Create a new project

NOTE: Some of the viws differs between the products.

1. Start NPX Map and click on **Create a new project**.

IPX™ Map		- 0
Welcome to NPX™ Map		
What would you like to do today?		
Create a new project Set up your project by importing plate layouts, select reference ID and import an NGS run folder	Open an existing project Continue where you left off from a previously saved project	Panel Data Archive Upload a new Panel Data Archive for use in your projects
		Process FASTQ data Convert new sequencing FASTQ output to an NGS run folder with counts that can be imported into a project
Recent Projects		
Reveal_training		Last opened: 2025-01-19 at 15:51
Reveal_training		Last opened: 2025-01-19 at 15:51 Last opened: 2025-01-08 at 10:53
New_Project		Last opened: 2025-01-08 at 10:53
New_Project Explore_HT		Last opened: 2025-01-08 at 10:53 Last opened: 2024-12-18 at 10:42
New_Project Explore_HT test		Last opened: 2025-01-08 at 10:53 Last opened: 2024-12-18 at 10:42 Last opened: 2024-12-17 at 15:55

2. Select Project Type and enter Project name and Sample matrix (optional).

🖏 N	NPX™ Map				- 🗆 X
Ę	New Project				STEP 1 OF 4
		PRODUCT TYPE Olink® Explore 3072	Olink® Explore HT	Olink [®] Reveal	
		PROJECT NAME	SAMPLE MATRIX		
		Name your project here	Enter Sample Matrix		
	E Back				Data Analysis Reference IDs >

3. Click Data Analysis Reference IDs.

4. Select Data Analysis Reference IDs for the blocks/panels in the product from the drop-down menu.

125	Olink	Explore	нт
	UIIIK	Explore	п

Olink Reveal

C MP* Mp	- 0 X	C Nin Me			- 0 X	C M/C Map		- 0 X
Set Data Analysis Reference IDs	STEP 2 OF 4	Set Data Analysis Reference IDs			5767.2-06-4	D Set Data Analysis Reference IDs		58272-074
Generalis, Monado Rocky Docky Conto V (2000 V (2000 V (2000 Generalis) Monadol Normal Docky Conto V (2000 V (2000 V (2000	•	(2005 v) R33 (2005 v)	Not Not Gran v Boar Not Baar (nom v Sore)	Best 5	8		Bad and as at an an	
		• fail			Real Importante -	+ Bek		Next ReportDate +
< fax	Next Import Data							

5. Click Next: Import Data.

Olink Explore 3072/384

Clicking CreateProject will create a project without plate layoyts and NGS runs. They can be imported later, from the Modify Project Data menu, refer to *12.2 Modify Project Data menu*.

6. Import plate layouts and NGS run folder.

It is not possible to import a zipped NGS run folder.

It is also possible to create a project without plate layouts and NGS runs by clicking **Create project**. It is not possible to create a project when only either plate layout or NGS run folder is uploaded.

X™ Map		- 0
Import Data		STEP 3 OF
NGS RUN FOLDER		
	ی Select or drop folder	
PLATE LAYOUTS		
	Select or drop files (csv)	
	Uploaded plate layout files will appear here	
Back		Create project

7. When the files are uploaded, click **Select NGS Runs.**

S NPX	™ Мар		- 0	×
\bigcirc	Impo	rt Data	STEP :	OF 4
	NGS RUN F	DLDER		
	6	IMPORT SUCCESSFUL Validation - Experiment 1 - Replicate 1	Remove	
	PLATE LAY	DUTS		-
		Select or	drop files (.csv)	
		IMPORT SUCCESSFUL Q-Plex1_SS112233_SP1000012	Remove	
		IMPORT SUCCESSFUL Q-Plex1_SS112233_SP10000121	Remove	
		IMPORT SUCCESSFUL Q-Plex1_SS112233_SP10000122	Remove	
< Ba	ack		Select NGS Ru	ns →

8. Import NGS run.

Bolink Explore 3072/384	Olink Explore HT	Olink Reveal
Import RGS Runs	Brennet NGS Runs Starters	Greene - 0 ×
RUA EXPLANE RESEARCH REPART OF REPART.	RANCONTRER REFERENCE 0 REFERENCE PRE DESTRUCTION DESTRUCTION <thdestruction< th=""> <thdestruction< th=""></thdestruction<></thdestruction<>	N.R. DELYY ME. NOTRAMIN'D ADDRESN'T PER PROVIDENCES SHIT LOTRAMIN' MAK MIN'NOD F257 Runtankovine(300) 36.53400C boliconiweitere
MOLENNAT POD 7/2011 POD 7/201	VERTILIZE PECETURE Ju C 510 RECOUNTER/C 07100 Ming1 V Min Annu V Min Annu V Ming V Min Annu V Min Annu V Min Annu V	URINING V MAX.LONG MAX.LONG MAX.LONG MAX.LONG URINI V MAX.LONG MAX.LONG MAX.LONG MAX.LONG
1 Newslay V same (Sectional)	1 A Book1 00007 (Nec50000)	A Read Rates (National
I 1 Pode - saw Silling	I Aud Inter GRADU	
		Ro MPSTDG (TBM) (Bakhad 2

Click Create Project.

The project is created and the Project view is displayed.

10.3.2 Open an existing project

1. Click on **Open an existing project**.

PX™ Map		- 0
Velcome to NPX [™] Map		
What would you like to do today?		
Create a new project Set up your project by importing plate layouts, select reference IDs, and import an NGS run folder	ct by importing plate layouts, select reference IDs, Continue where you left off from a previously saved project	
		Process FASTQ data Convert new sequencing FASTQ output to an NGS run folder with counts that can be imported into a project
Recent Projects		Last opened: 2025-01-19 at 15:51
New_Project		Last opened: 2025-01-08 at 10:53
Explore_HT		Last opened: 2024-12-18 at 10:42
test		Last opened: 2024-12-17 at 15:55
New_Project		Last opened: 2024-12-16 at 09:37
Reveal_manual		Last opened: 2024-12-16 at 08:52
Manual test		I act opened: 2024-12-12 at 11-38

- 2. Browse for project to open.
- 3. Click **Open**.

or:

Open an existing project from the **Recent Projects** list.

It is possible to migrate an old Olink Explore 3072/384 project using this function. Refer to *12. Migration of Olink® Explore 3072/384 projects* for more information.

10.4 Save project

1. Go to the **Project Actions menu -> Save Project**.

New Project	Ctrl+N
Open Project	Ctrl+O
Save Project	Ctrl+S
Clone Project	Ctrl+Shift+C
Close Project	Ctrl+F4
Help	F1
About & Licensing	
Quit NPX™ Map	Alt+F4

- 2. Browse for preferred folder to save the project.*
- 3. Click Save.

* 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 work is complete, it can be uploaded or synced to a network location after disconnecting from the VPN or when network stability improves.

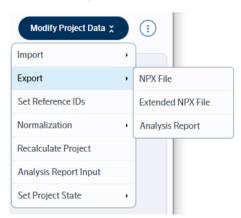
10.5 Perform quality control

Refer to 13. Views for instructions on how to use the different views to perform quality control.

10.6 Export data and Analysis Report

Three different files can be exported from NPX Map: NPX file, Extended NPX file and Analysis Report. For more information about the files and file formats, refer to *11. Export result files.*

For smaller projects, CSV format is available.



To export the file, go to the **Modify Project Data -> Export**, and select preferred file type to export.

Before an Analysis Report an be exported, the Analysis Report input form must be entered.

- 4. Go to Modify Project Data -> Analysis Report input.
- 5. Fill in the fields and click either **Save without exporting** or **Save & Export**.

Customer Information		
NAME	EMAIL	
Input	Input	
Analysis Lab		
NAME	EMAIL	
Input	Input	
Business Development		
NAME	EMAIL	
Input	Input	
COMMENTS FOR REPORT		

10.7 Finalize a project

	(;)
,	
,	
,	
•	Send to Review
	Reopen
	Finalize
	•

Before a project can be finalized, it need to be sent for review.

- 1. Save the project.
- Go to Modify Project Data -> Set Project State -> Send to Review.
 The project is set to status Review, and no changes can be done. To cancel the review, go to
 Modify Project Data -> Set Project State -> Reopen.
- 3. When the review is done, **Modify Project Data -> Set Project State ->Finalize.** The project cannot be reopened, and no more changes can be made to the project.

11.Export result files

11.1 NPX file

The NPX file is a Parquet or CSV-file that can be generated from NPX Map. It contains the following information about the project in table format:

Column	Description	Туре	Typical value
SampleID	The annotated sample ID	String	
Sample Type	Type of sample	String	PLATE_CONTROL, NEGATIVE_ CONTROL, CONTROL, SAMPLE
WellID	ld for well	String	Capital letter A–H followed by number 1–12
PlateID	Name of the plate the sample was run on	String	
DataAnalysisRefID	Reference ID for data analysis	String	
OlinkID	OlinkID for assay	String	
UniProt	UniProt ID for assay	String	
Assay	Gene name for assay	String	
AssayType	Type of assay	String	Amp_ctrl, inc_ctrl, ext_ctrl
Panel	Panel name	String	Reveal
Block	Name of the block the sample was run on	String	1, 2, 3, 4, 5, 6, 7, or 8
Count	The total number of counts	Integer	Greater than or equal to 1
ExtNPX	Intermediate value between count and NPX: log2 of the ratio between data-point Count value and the count for the Extension Control assay for the same sample.	Double	-1.94701
NPX	NPX value	Double	
Normalization	Type of normalization used in project	String	Plate control, Intensity or EXCLUDED
PCNormalizedNPX	NPX value displayed if plate control normalization has been chosen.	Double	1.735509
AssayQC	Overall QC status for an assay	String	NA, PASS, WARN
SampleQC	Overall QC status for a sample in a block	String	NA, PASS, WARN, FAIL
MapVersion	Software version of the module in NPX Map used for panel calculations and normalization	String	

11.2 Extended NPX file

The Extended NPX file is a Parquet or CSV-file generated by the NPX Map CLI and NPX Map. It contains all the columns in the NPX file, plus the set of additional columns listed below.

- IntraCV
- InterCV
- SampleBlockQCWarn
- SampleBlockQCFail
- BlockQCFail
- AssayQCWarn

For more information, refer to the NPX[™] Map CLI Technical Information.

11.3 Analysis Report

The analysis report is a pdf file generated by the NPX Map CLI and NPX Map. It summarizes the quality analysis of the project. It contains basic information and descriptions of the project. It also contains a summary of the Quality control including sample passed QC.

The Analysis Report contains the following:

- Project information
 - Sample matrix
 - Project specific comments
- Quality control
 - QC summary
- Data output
- Software version information
- Appendix A table including the following columns
 - Olink Panel
 - Block
 - UniProt
 - Assay Olink ID

11.4 Parquet file format

NPX data is exported from NPX Map in either CSV or Parquet file format. Parquet is a free open source file format built to handle columnar storage data.

Parquet files provide several advantages compared to CSV, such as

- Much more efficient data compression with enhanced performance to handle complex data in bulk.
- Improved data integrity through binary encoding (files can't be opened and changed using a text editor).

The binary encoding also means the file cannot be opened in Microsoft[®] Excel. If required, Parquet files can be exported to CSV format. The CSV format is available for projects with approximately 1 million datapoints.

Parquet is available in multiple languages including Java, C++, Python, C# etc. You can find further details at these locations

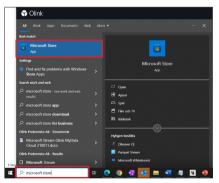
- https://parquet.apache.org/docs/
- https://en.wikipedia.org/wiki/Apache_Parquet

11.4.1 How to interact with a Parquet file

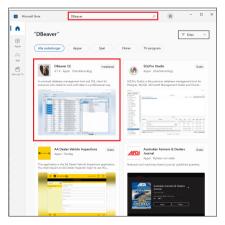
There are many different solutions to interact with a Parquet file, for example DBeaver. Olink do not recommend any specific solution.

Installing DBeaver

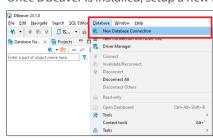
1. Download DBeaver for free from the Microsoft[®] Store by clicking on the **start menu** and type "Microsoft store".



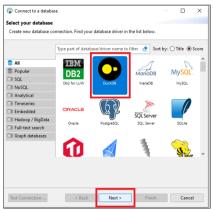
2. Type "DBeaver" in the search field, and press Enter.



- 3.
- 4. Once DBeaver is installed, setup a new database connection.



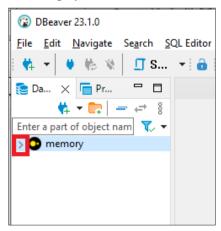
5. Choose **DuckDB** and hit the **Next** button.



6. Type ":memory:" in the **Path** input field.

-				
😨 Connect to a database				×
Generic JDBC Connection Settings				
DuckDB connection settings				Ъ,
				-
Main Driver properties				
General				
Connect by: Host URL				
JDBC URL: jdbc:duckdb::memory:				
Path :memory:		Open	Create	
① You can use variables in connection para	neters.	Connection details	(name, typ	e,)
Driver name: DuckDB		Driver Setting:	Driver li	ense
Test Connection < Back	Next >	Finish	Canc	el

7. Click the gray arrow.



8. Download driver files.

Driver settings		×
Download driver files		
Download DuckDB driver files		
DuckDB driver files are missing. Force down Force downloaded automatically.	load / over	write
Files required by driver		_
File	Version	De
org.duckdb:duckdb.jdbc:RELEASE:RELEASE T https://raw.githubusercontent.com/duckdb/duckdb/master/LICENSI	0.8.0 E	LA
<		>
You can change driver version by clicking on version column.		
Then you can choose one of the available versions.		
Or you can obtain driver files by yourself and add them in driver editor.	ad configu	ratio
Or you can obtain driver files by yourself and add them in driver editor.	ad configu	ration

9. Open a new script window.



10. Type a PostgreSQL query such as

SELECT * FROM 'C:/npxfile.parquet'. Make sure that the path in double quotes refers to the NPX file you want to query.

11. Click the "Execute SQL query" button

Deever 23.1.0 - <memory> S</memory>	oipt-2
File Edit Nevigete Search S	QLEditor Detabase Window Help
🗱 🔹 🕸 🏀 😻 🛄 S	* []; Cemmit []; Rollback 🍸 * 🔒 🛛 🗛 💿 * 😋 memory * 🛢 memory * 🙆 🚔 * Q. *
🖹 Da X 🚡 Pr 🤗 🗖	
🐔 + 🗇 🖉 🚅 😫	
Enter a part of object name 🐮 💌	3
v 💽 memory	8
> 🚍 memory	
> 🚍 system	
> 🚍 temp	2

12. View the result.

Г

Export data to a CSV file

1. Click Export data.



2. Highlight CSV and click Next.



3. Click Next.

😮 Data Transfer		- 0 X
xtraction settings		
Database table(s) extraction :	settings	
Export target Export target Extraction settings Formal settings Output Cenfirm	Progres Maximum Harved 1 Expendent size 10000 Opper net consticution) Select the count Fehr kize 10000 Selected columns only Selected columns only	
Save task		
	< Back Next> Pro-	rend Cancel

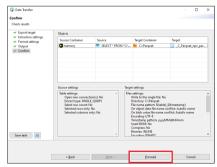
4. Click Next.



- 5. Set the "Directory" field to a location on your hard drive.
- 6. Click Next.

							×
rtput							
onfigure export output per	ameters						
 ✓ Export target ✓ Extraction settings 	General	d					
 Format settings Output 	Directorys					Global Setting	
Confirm	File name pattern	File name pattern: \${table};\${timestamp}					
	Encoding	UTF-8 ~	Timestamp pattern:	yyyyMMddHHmm		Insert	BOM
	On object data f <u>You can use val</u> Results Show finish met Show exported t Execute process	file in system explorer Configure	ame; On blob value fi	le name conflict: Autofi	ix name		
	See how you can go	port files to external storage	en our wiki				

7. Click **Proceed**.



11.4.2 Quick-check parquet-viewer

To quick-check the parquet, perform the following steps:

- 1. Download ParquetViewer from the GitHub wepage: <u>https://github.com/mukunku/ParquetViewer/</u> releases.
- 2. Install ParquetViewer and open it.
- 3. Drag and drop the parquet file to the application window.
- 4. Use the query input field to filter out desired datapoints, or use the Record Offset filed to jump in the file.
- 5. For more information or help, refer to the GitHub homepage: <u>https://github.com/mukunku/</u> ParquetViewer/wiki/Basics.

12.Migration of Olink[®] Explore 3072/384 projects

Olink Explore 3072/384 projects created in NPX Explore can be migrated into NPX Map to utilize the new QC procedure and features.

12.1 Migrate a project

1. Click on Open an existing project. NPX[™] Map Welcome to NPX[™] Map What would you like to do today? Create a new project Open an existing project Panel Data Archive a new Panel Data Archiv Process FASTQ data nvert new sequencing FASTQ output to an NGS run folder with ints that can be imported into a project **Recent Projects** ÷ Reveal training Last opened: 2025-01-19 at 15:51 New Project Last opened: 2025-01-08 at 10:53 Explore HT Last opened: 2024-12-18 at 10:42 Last opened: 2024-12-17 at 15:55 New Project Last opened: 2024-12-16 at 09:37 Last opened: 2024-12-16 at 08:52 Reveal_manual Manual test wed- 2024-12-12 at 11-39

- 2. Browse for project to migrate
- 3. Click Open.

12.2 Migration information

Not all data will be copied over into the migrated project such as data specific to the old QC and normalization. Some project data fields may also be trimmed due to newer constraint requirements on length and content. Data such as counts, plate layouts and imported run units are imported as they were in the old project. The migration of the project leaves the old project and files intact and can be opened in the old software as usual.

13.Different projects or sample matrices on the same plate

When running different projects or sample matrices on one plate, an extended plate layout must be created.

1. Manually create a single plate layout file with two extra columns: Project Name and Sample Matrix, according to the following order:

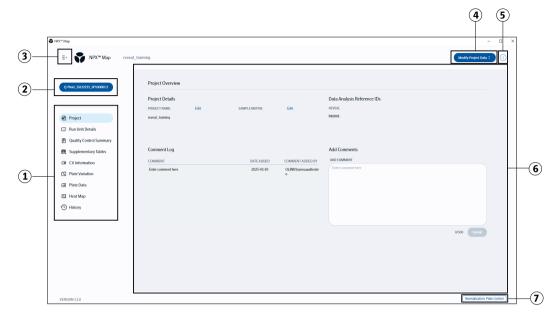
well_ID; sample_ID; sample_type; sample_matrix; project

- 2. Create one project per project/sample matrix, according to 10.3 Create a new project or open an existing project.
- 3. Upload the manually created plate layout in both projects. The following view will be displayed:

Plate ID: 20200279-003	PROJECT	SAMPLE MATRIX
Set new Plate ID:	proj1	blood
20200279-003 Apply	proj2	saliva
Select samples belonging to the project. The selection will only be applied to samples of type sample.		

- 4. Fill in new Plate ID and select Project and Sample Matrix.
- 5. Click Apply.
- 6. Continue according to 10.3 Create a new project or open an existing project.

Part 4: User Interface 14.General



No	Part	Description	
1	View menu	For information about the different views, refer to 15. Views	
2	Plate Selection	Select plate and panel to display (For Olink Reveal and Olink 3072/384) Select plate and block to display (For Olink Explore HT)	
3	Minimize View menu	Minimaie or maximize the View menu	
4	Modify Project Data menu	Menu for modifying the project data, such as Set Reference IDs, Normalization etc. Refer to <i>14.2 Modify Project Data menu</i>	
5	Project Actions menu	Menu for handling the project, such as Open Project, Save Project, Clone Project etc. Refer to <i>14.3 Project Actions menu</i> .	
6	Main view	Shows the selected view from the View menu.	
7	Normalization	Shows selected normalization method. To change normalization method, refer to 14.2 Modify Project Data menu.	

14.1 Plate Selection

	Project UVerview		
Q-Plex1_SS112233_SP100001 21	Q-Plex1SS112233_SP1000012		
Reveal	✓ Q-Plex1SS112233_SP10000121 →	✓ Reveal	

Use this menu to select which plate and panel/block to view.

NOTE: Olink Reveal consists of only one panel, called Reveal.

14.2 Modify Project Data menu

Modify Project Data 💲

Menu	Expansion	Description
Import	Plate Layouts Import NGS Run Folder Import Zipped NGS Run File	Import Plate layouts and NGS run folders to the current project.
Export	NPX File Extended NPX File Analysis Report	Export the data files. Refer to 11. Export result files.
Set Reference IDs	-	Set reference IDs for the current project.
Normalization	Plate Control Intensity	Change normalization method for the project. Refer to <i>7.4 Normalization</i> .
Recalculate Project	-	Recalculate the project.
Analysis Report Input	-	Enter information to the Analysis Report. Refer to <i>11.4 Analysis Report.</i>
Set Project State	Send to Review Reopen Finalize	Final stages for the project. Refer to <i>10.7 Finalize a</i> project.

14.3 Project Actions menu

Menu	Description	
New Project	Create a new project. Refer to 10.3 Create a new project.	
Open Project	Open a saved project.	
Save Project	Save the current project. Refer to 10.4 Save project.	
Clone Project	Create a clone of the current project.	
Close Project	Close the current project.	
Help	Open the NPX [™] Map User Manual as a pdf file.	
About & Licensing	Show information about the software and licensing information.	
Quit NPX™ Map	Close the software.	

15.Views



Project

Overview of the project, showing project details, comment log etc.



Run Unit Details

The Run Unit Details shows information about the selected plate and panel/block.



Quality Control Summary

The Quality Control Summary tab displays a summary of the QC criteria per block.



Supplementary Tables

Contains tables that display sample IDs and assays that failed or warned based on any of the QC criteria. It helps identify the possible root cause for sample and block failure.



CV Information

Assess the precision of the data based on a group of pre-selected assays across different blocks on the Sample Controls.



Plate Variation Display differences in sample distributions within and between plates.

..11

Plate Data

Shows the values of NPX and Counts for internal controls and assays for each sample per block. Assess if there are patterns in the plate representing a possible technical error.



Correalation Assays (Only for Olink Explore 3072/384) Assess the correlations of each overlapping assay between blocks. Skewed data correlation in any of the plots can indicate the technical error in the lab workflow.



Heat Map

Search for extreme outlier samples and systematic patterns that reflect a possible technical error.



Sample QC Overview (Only for Olink Explore HT and Olink Explore 3072/384) View and evaluate patterns of failed/warned samples within and across the blocks in each

plate and view the criteria that have resulted in failure or warning status.



History

Shows the changes made in the project.

15.1 Project

General:

The Project view shows the following information about the project:

- Project Details
- Comment Log
- Data Analysis Reference IDs
- Add Comments

Project Overview				
Project Details PROJECT NAME Reveal_training	Edit	SAMPLE MATRIX	Edit	Data Analysis Reference IDs REVEAL R10001
Comment Log		DATE ADDED	COMMENT ADDED BY	Add Comments ADD COMMENT Enter comment bere
				0/500 Submit

Useful for:

Add comments regarding the QC. The comments will not be included in the Analysis Report.

- Change Project Name or Sample Matirix by clicking on Edit and write in the field. Click Done.
- Add comments for the project by writing in the **Add Comment** field. The comment will be shown in the Comment Log field. The comments will be included in the Analysis Report.

15.2 Run Unit Details

General:

The Run Unit Details shows information about the selected plate and panel.

			1	2	3	4	5	6	7	8	9	10	11	12
reprocessing Date	2024-09-11	A					EMPTY5	A6-3				Ref_1-3	Ref_1-2-3	Ref_1-3-3
lun Identifier	223JGNLT3	в												
ibrary	1	с				C4-3	C5-3							
tun Name	My Experiment	D										D10-3	D11-3	D12-3
anel	Reveal	E	E1-3	E2-3	E3-3	E4-3	E5-3	E6-3	E7-3	E8-3	E9-3	E10-3	E11-3	E12-3
ata Analysis Reference ID	R10001	F	F1-3	F2-3	F3-3	F4-3	F5-3	F6-3	F7-3	F8-3	F9-3	F10-3	Notused	Not used
ndex Plate	Sample 1-96	G	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	Not used	PLATEO.	PLATEO	PLATEO
nstrument ID	LH00156	н										NEGATIO	NEGATIO	NEGATIO
Count File	counts_2022-10-01_A223JG				Sample	Negat	tive control	Sa	mple control	F	Plate control	E E	npty	Not used
legative Control	Fail													
Plate Control	Pass	MANUAL ASSESSMENT None Pending Good Bad							RUN STATUS					
lowcell Type	10B	PL	TE LAYOUT					ADD COMMENT						
lowcell Side	A		20200279-			~			ter commen					
		RE	RUN OF											
			No rerun				~							
		PR	IMARY CAU	JSE										
			Not applica	ble			~							
		ST	EP FAILED											
		ľ	Not applical	ble			~							
		ING	CLUDE REA	SON										

Useful for:

- Reviewing failed blocks/samples.
- Finding systematic effects.
- Include, exclude, or delete plate.

Functions:

- For each plate, the following information is displayed:
 - Preprocessing Date
 - The date when instrument data for this run unit was pre-processed.
 - Run Identifier
 The unique identifier of the instrument run this run unit belonged to.
 - Library

The assay library of this run unit.

- Run name
 The name of the instrument run containing this run unit.
- Block (Only for Olink Explore HT) The block of this run unit.
- Panel (Only for Olink Reveal and Olink Explore 3072/384)
 The panel of this run unit.
- Data Analysis Reference ID
 The specific Data Analysis Reference ID this run unit was calculated with.

- Index Plate
 - The index range of the samples in this run unit.
- Instrument ID
 The ID of the instrument that performed the run.
- Count File
 The name of the file that contained the counts data for this run unit.
- Negative Control
 The status of the negative controls in this unit; "Fail" if any negative control is failed, otherwise "Pass"
- Plate Control
 The status of the plate controls in this unit; "Fail" if any plate control is failed, otherwise "Pass"
- Flowcell Type
 The type of flow cell that was used in the corresponding run (only applicable to Illumina instruments).
- Flowcell Side The side of the instrument that was used in the corresponding run (only applicable to Illumina instruments)
- Select Manual Assessment: None/Pending/Good/Bad.
- Set **Run Status** to Include run/Exclude run or Rerun needed.
- Use the drop-down menues to do the following changes:
 - Select Plate
 - Annotate a **Rerun of**
 - Set Primary error cause
 - Set Step fail reason
 - Set Include reason
- Add comments in the comment field. Comments will not be included in the Analysis Report.

NOTE: The Counts presented in this view is expected to differ from the sum of counts for individual data points presented in the NPX File. The counts presented in the NPX File is the value that shall be used in down stream analysis.

15.3 Quality Control Summary

General:

The Quality Control Summary view displays a summary of the QC criteria per plate.

											ACTIONS	
											Choose	Apply
PLATEID	BLOCK	FAILED SAMPLES	WARNED SAMPLES	FAILED PC	FAILED NC	FAILED SC	WARNED ASSAYS	BLOCK QC	SYSTEMATIC EFFECTS	COUNTS	RUN STATUS	
Q-Plex1_SS112233_SP100001	1	0/63	0/63	2/15	0/9	0/9	0/742	Pass	Row effect	16 639 861	Included	
Q-Plex1_SS112233_SP100001	2	0/63	0/63	0/15	0/9	0/9	0/1312	Pass	Column effect, Row effect	82 013 682	Included	
Q-Plex1_SS112233_SP100001	3	0/63	0/63	7/15	0/9	0/9	0/1195	Pass	Column gradient, Diagonal gradient type 1, Diagonal gradient type 4	167 740 439	Included	
Q-Plex1_SS112233_SP100001	4	0/63	0/63	0/15	0/9	0/9	0/1099	Pass	Column effect	543 210 526	Included	
Q-Plex1_SS112233_SP100001	5	0/63	0/63	0/15	0/9	0/9	2/581	Pass	Column effect	885 817 943	Excluded	
Q-Plex1_SS112233_SP100001	6	0/63	0/63	1/15	0/9	0/9	2/270	Pass	Column effect	631 112 948	Excluded	
Q-Plex1_SS112233_SP100001	7	0/63	0/63	1/15	0/9	0/9	2/134	Pass	Row effect	536 684 528	Included	
Q-Plex1_SS112233_SP100001	8	0/63	0/63	0/15	0/9	0/9	8/68	Pass	None	324 539 929	Included	

Useful for:

- Reviewing status of the selected block.
- Finding systematic effects.

- For each plate, the following information is displayed:
 - Plate ID
 - Panel (Only for Olink Reveal and Olink Explore 3072/384)
 - Block (Only for Olink Explore HT)
 - Failed samples
 - Warned samples
 - Failed PC
 - Failed NC
 - Failed SC
 - Warned assays
 - Block QC
 - Systematic effects
 - Counts
 - Run status
- Use the actions drop-down menu to include, exclude or remove the selected items. Click Apply.

15.4 Supplementary Tables

General:

The supplementary Tables view contains five tables displaying samples that have failed or have warnings based on QC criteria. All data for one sample source plate is displayed at a time.

Only tables that contain data will be displayed.

upplementary Tables									
ssay Warnings									
Plate ID	Panel	Assay O	Olink ID						
Q-Plex1_SS112233_SP10000121	Reveal	NPM1 0	OID50794						
Q-Plex1_SS112233_SP10000121			OID50067						
Q-Plex1_SS112233_SP10000121	Reveal	WASF3 0	OID51004						
1-3 of 3								1 v of 1 pages	Previous Next 🕨
1-3 of 3								1 v of 1 pages	Previous Next 🕨
1-3 of 3								1 v of 1 pages	Previous Next 🕨
								1 v of 1 pages	Previous Next >
eviating Plate Controls	Panel	WellID	Sample ID	Amplification	Extension	Incubation		1 v of 1 pages	Previous Next >
eviating Plate Controls			Sample ID PC_EESBIIS-33	Amplification	Extension	Incubation		1 v of 1 pages	Previous Next >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of 1 pages (Previous Next)
eviating Plate Controls Plate ID Q-Plext_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33					1 v oflage (Previous Next >
eviating Plate Controls Plate ID Q-Plext_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v oʻlpays (Previous Net >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of 1 pages (Previous Next >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of 1 pages (Previous Next >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v oʻlpays (Previous Next >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of larges (Vrevious Next >
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v oʻlpays (Yesioan (Next)
eviating Plate Controls Plate ID Q-Plex1_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of 1 pages (Yreion. Net I
eviating Plate Controls Plate ID Q-Plext_SS112233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of 1 pages (Yrrion (Net)
1-3 of 3 eviating Plate Controls Plate 0 QPeta_\$\$112233_\$P10000121 QPeta_\$\$112233_\$P10000121	Reveal	E12 F	PC_EFSBUS-33	+	+	+			
eviating Plate Controls Pate to Q-Plect_SSI12233_SP100001 21 Q-Plect_SSI12233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+		1 v of pages (
eviating Plate Controls nae ID Q-Pled_SSI12233_SP100001 21 Q-Pled_SSI12233_SP100001 21	Reveal	E12 F	PC_EFSBUS-33	+	+	+			

Useful for:

- Reviewing flags of all samples and assays in tables, divided into five different flag reasons:
 - Assay warnings
 - Samples with low total counts
 - Samples with low internal control count
 - Deviating plate controls
 - Failed negative controls

Functions:

• The following information will be displayed in the different tables:

Assay warnings	Samples with low total Couns	Samples with low internal control counts	Deviating Plate Controls	Failed Negative Controls
Plate ID	Plate ID	Plate ID	Plate ID	Plate ID
• Panel	• Panel	• Panel	• Panel	• Panel
Block*	Block*	Block*	Block*	 Block*
• Assay	• Well ID	• Well ID	• Well ID	• Well ID
Olink ID	Sample ID	Sample ID	Sample ID	Sample ID
	Sample Type	Sample Type	Amplification	Sample Type
		Total Count	Extension	
			Incubation	

* Block only for Olink Explore HT and Olink Explore 3072/384.

• The Failed Negative Controls table includes empty wells that show too high signal.

15.5 CV Information

General:

The CV Information view shows the CV information, one row per block. CV is calculated based on the data points of Sample Controls that passed the QC criteria.



Useful for:

• Getting an overview of the CV values per block, and which blocks that have missing CV values.

Functions:

- The following information will be displayed:
 - Panel (Only for Olink Reveal)
 - Block (Only for Olink Explore HT and Olink Explore 3072/384)
 - Plate
 - Mean CV value
- The CV Table shows the following information:
 - Intra-Assay %CV Mean
 - Inter-Assay % CV Mean
 - Number of plates with missing intra CV mean

Reference values:

The reported %CV is the mean %CV over all pre-selected assays. A high %CV does not fail a run automatically but could indicate a technical issue or poor sample randomization and can be a cause for further investigation.

- Inter %CV: < 25%
- Intra %CV: < 15%

15.6 Plate Variation

General:

The Plate Variation tab displays a box plot with NPX values from the Sample wells. Failed run units will be left empty, refer to the left run in the picture below.

;RF2 V		
	Assay : ADGRF2	
-		
· · · · ·		
A State of the second s		
· · · ·		

Useful for:

• Detecting systematic variance in total intensity between plates.

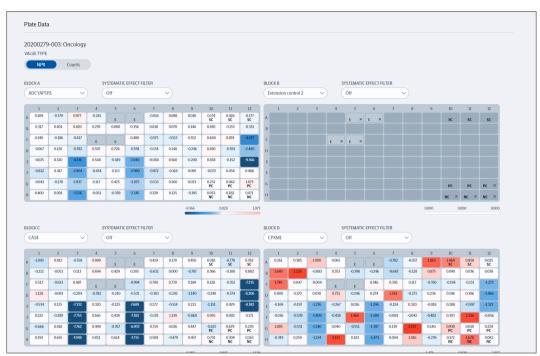
- Use the Assay drop-down list to show specific control or assay.
- Select All in the Assay drop-down list to view the summary of all assays and controls for each sample.
- Select **block** to view (Only for Olink Explore 3072/384)

15.7 Plate Data

General:

The Plate Data view shows heatmaps of one unit a time, including the internal controls: extension control, incubation control and amplification control. Empty wells show counts but not NPX.





Useful for:

- Inspecting counts and NPX across a plate for each assay.
- Discovering systematic patterns.
- Discovering plates swap based on data pattern across the plate (If the plate has any empty well).

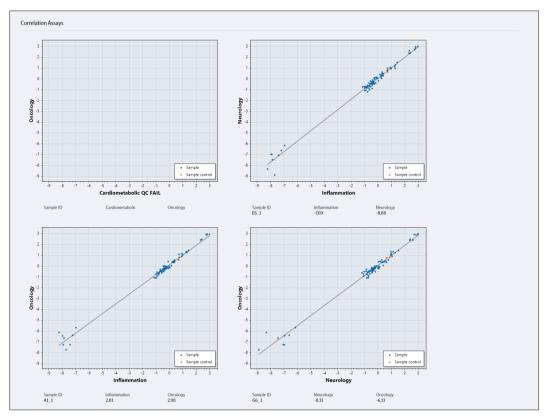
- Use the **assay** drop-down menu to change which assay to display.
- Change value type by selecting either NPX or Counts.
- Use the **Systematic effect filter** to select type of systematic effect, or swith it off. (Only available systematic effects types are shown in the drop-down menu)

15.8 Correlation Assays (Only for Olink[®] Explore 3072/384)

General:

The Correlation Assays tab shows correlation assays that overlaps across the blocks per sample plate.

- A plate is chosen: all included run units are shown.
- An excluded run unit is chosen: the text "No data display" is shown.
- Failed samples: no data points will be shown.
- Failed blocks: an empty graph with red text "Panel QC failed [NPX] "will be shown.



Useful for:

• Getting an overview of correlation assays.

Functions:

• Use the Assay drop-down list to switch between correlation assays.

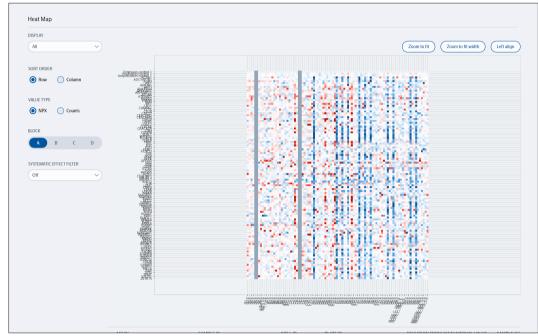
15.9 Heat Map

General:

The Heatmap view contains a color-coded view for all assays and samples in the chosen block. The assays are displayed in the horizontal direction and samples in vertical direction. Empty wells show counts but not NPX. A failed block is marked by a red banner.

The heatmap displays deviations from the plate median for the selected assay using the colors blue and red:

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



Useful for:

- Finding systematic patterns.
- Finding outlier samples for specific assays.

- Select sample type to display. (All External Controls, Sample, Plate Control, Negative Control, Sample Control)
- Switch Sort order between row and column.
- Change Value type by selecting either NPX or Counts.
- Select Block to view (Only for Olink Explore 3072/384)
- Use the **Systematic effect filter** to select type of systematic effect, or swith it off. (Only available systematic effects types are shown in the drop-down menu)
- Hover over a well to show:
 - Assay
 - Sample ID
 - Well ID
 - Plate ID
 - Deviation from assay median
 - Value
 - Sample QC

• Zoom functions

- Zoom in and out with scroll wheel.
- **Zoom to fit**: will adjust the heat map to fit in the view.
- **Zoom to fit width**: will adjust the heat map to fit vertically.
- **Left align**: will adjust the heaat map to align on the left of the view.
- Move the map to see different assays by clicking and holding the left mouse button and drag the heatmap.

15.10 Sample QC Overview (Only for Olink® Explore HT)

General:

The Sample QC Overview displays a color-coded heatmap showing status for each sample in all blocks.

- Blue: Pass
- Red: Fail
- Yellow: Warning

If a sample has both a fail and a warning, it is shown as fail.



Useful for:

• Finding systematic errors across different blocks.

- Switch Sort order between row and column.
- Hover over the cells provides information on:
 - Sample ID
 - Block
 - Well ID
 - For failed and warned samples: reason for failure or warning.

15.11 History

General:

The History view displays all events for the current project.

History			
Working Changes			
STATUS	REASON	TIME	USER
Pending	Updated the field ProjectName to 'Reveal_training'	01/19/2025 16:31:37	OLINK\hanna.wallenbro
Pending	Selected Data Analysis Reference ID R10001	01/19/2025 16:31:37	OLINK\hanna.wallenbro
Pending	Imported plate layout Q-Plex1_SS112233_SP100001 22	01/19/2025 16:31:37	OLINK\hanna.wallenbro
Pending	Imported plate layout Q-Plex1_SS112233_SP100001 2	01/19/2025 16:31:37	OLINK\hanna.wallenbro
Pending	Imported plate layout Q-Plex1_SS112233_SP100001 21	01/19/2025 16:31:37	OLINK\hanna.wallenbro
Pending	Imported run for run unit fcd9d2b8-c0f0-4bc9-8af3-937667a89427 with Data Analysis Ref ID R10001 and plate layout Q-Plex1_SS112233_SP10000121	01/19/2025 16:31:37	OLINK\hanna.wallenbro
History			
		TIME	USER

Useful for:

• Seeing what actions that has been done on the project.

- The upper table shows changes not yet saved.
- The lower table shows changes that has been saved.

Part 5: Troubleshooting 16.Troubleshooting

This chapter includes a trouble shooting guide, describing QC issues, affected level (sample, block, plate) and possible root causes for some potential non-expected results. Reruns are not recommended except for the projects that have failed to pass formal QC. Please contact <u>support@olink.com</u> for further investigations and receiving appropriate guidance for any of these cases.

QC issue	Affected samples/blocks/Sample plates	Possible cause
Plate Controls and/or samples fail		
Failed Plate Controls: Deviation from reference values (±)	Multiple blocks (1-4 or 5-8) Both sample plates plates	 Dilution plates or Sample Plate swapped positions on Mosquito Wrong dilution protocol was used
Plate Controls fail: Deviation from reference values (Inc & Ext -, AMP +) (Inc, Ext & AMP -)	Multi blocks in both Sample plates	Poor PCR enzyme efficiency due to not adding enough Enzyme A or Enzyme B
Plate Controls fail: Deviation from reference values (Inc -, Amp & Ext +) – Low counts for Incubation Controls – Low counts for assays	The same single block in both Sample Plates (e.g. Block 2 in SP1 and SP2)	1A: Forward probe was not added to the incubation reagent mix1B: Not enough incubation solution was added while preparing the reagent mix
 High counts for Amplification and External Controls 	The same two blocks in both Sample Plates (e.g. Blocks 2 and 5 in SP1 and SP2)	2A: Forward probes were mixed up for the two blocks when preparing incubation mix (wrong pair of probes) 2B: Not enough incubation solution was added while preparing the reagent mix
Samples and/or external Controls fail: Too low counts	1. One or multi samples in a block One or both Sample Plates.	1. Incubation reagents mix or index not added properly due to Mosquito pipetting error
	2. All or multi samples in multi blocks (Likely blocks 1-4) Both Sample Plates.	2. Poor sequencing if %PF is low
Samples and Plate Controls fail: Too low counts – Low counts for assays	1. One same block in both Sample plates (e.g. Block 2 in SP1 and SP2)	1. Reverse probe was not added into incubation reagent mix
 Low counts for all internal controls 	2. The same two blocks in both Sample plates (e.g. Blocks 2 and 5 in SP1 and SP2)	2. Reverse probes were mixed up for the two blocks when preparing incubation mix (wrong pair of probes). Amplification Control might have some counts.

Negative Control fail				
Negative Controls fail (Plate Controls might fail too)	1. Multi blocks in one Sample Plate (likely blocks 5-8)	 1A. The plate layout does not match with the uploaded run. 1B. NCs related to one of the Sample Plates were pipette into wrong wells on Sample Source Plates. 1C. Negative Controls are contaminated 2A. Sample Source Plates was rotated in the workflow 2B. Samples added instead of Negative Controls in Sample Source Plates 2C. Index plate A and B were swapped positions on mosquito between the two stages of adding indexes 2D. Negative Controls are contaminated 		
	2. The same multi blocks in both Sample Plates (blocks 1-4 or 5-8)			
Systematic effect				
Alternating column effect: NPX value deviates in every other column	One or multiple blocks in one or both Sample Plates	Mosquito pipetting error while dispensing reagent mix into incubation plate		
A B C C C C C C C C C C C C C C C C C C	11 12 Two blocks in both Sample Plates 11 12	 Dragonfly faulty syringe during dispensation of PCR mix Not well vortex of PCR mix 		
E F G H NPX values deviate between samples in one	Same row in the same block in both Sample Plate	 EpMotion pipetting error when pooling the samples 		
NPX values deviate between samples in one row and samples in all other rows	11 12	 Pooling the samples Human error missing one well during pooling step 		

(row NPX	ı/col (Val oss tł	t pat umn ues c he pl	/dia devi	igon ate <u>g</u>	grad	ually	/ in s			al		ne oi ates	 Not well vortex of reagent plates Not well adjusted/calibrat instruments
	1	2	3	4	5	6	7	8	9	10	11	12	I
A													
в													
С													
D													
E													
F													
G													

17. Revision history

Version	Software version	Date	Description
1.0.1	1.0.1	2025-01-27	New

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