



# Olink® MyData Cloud

## 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® Explore panels.



**NOTE:** *The information in the Olink® MyData Cloud 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

Olink MyData Cloud is a cloud-based data analysis software that is specifically designed for the Olink Explore analysis platform which, in turn, is intended to measure the relative concentration of proteins in liquid biopsies. Olink MyData Cloud and the accompanying pre-processing software are used for the generation and analysis of counts files for completed Olink Explore runs. Count files and metadata are uploaded to the Olink MyData Cloud service, where annotation of runs to projects, Quality Control, normalization, and export of the resulting NPX data is performed.

Olink MyData Cloud is intended for research use only. Not for use in diagnostic procedures. All trademarks and copyrights contained in this material are the property of Olink Proteomics AB, unless otherwise stated.

### 1.2.Intended target group

Olink MyData Cloud is intended to be used by trained staff certified to run the Olink Explore platform. Quality Control should be performed by trained users that determines whether data from a run can be approved for further analysis.

## 2. List of abbreviations

API	Application Programming Interface
BDM	Business Development Manager
CBCL	Concatenated Base Call
%CV	Coefficient of Variation
LOD	Limit of Detection
MAD	Median Absolute Deviation
MFA	Multi-factor authentication
NaN	Not a Number
NGS	Next Generation Sequencing
NPX	Normalized Protein eXpression
PC	Plate Control
QC	Quality Control
SD	Standard Deviation
TFA	Two-Factor Authentication

## 3. Associated documentation

### *Olink documentation*

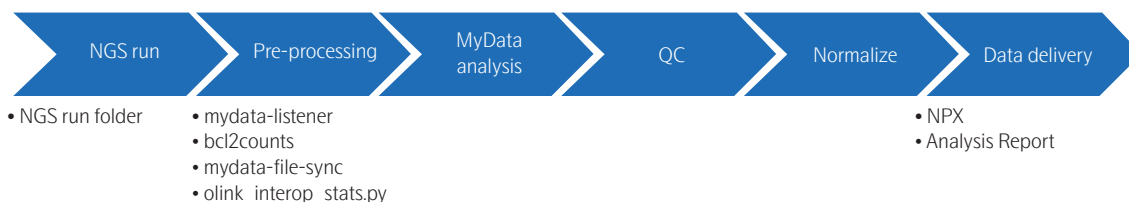
- Olink® Explore Overview User Manual
- Olink® Explore 384 User Manual
- Olink® Explore 4 x 384 User Manual
- Olink® Explore 1536 & Expansion User Manual
- Olink® Explore E3072 User Manual
- Olink® Explore Sequencing using NextSeq 550 User Manual
- Olink® Explore Sequencing using NextSeq 2000 User Manual
- Olink® Explore Sequencing using NovaSeq 6000 User Manual

All relevant Olink documentation is available from the Olink website. [www.olink.com/downloads](http://www.olink.com/downloads).

## 4. Technical support

For questions, guidance and support, contact Olink Proteomics at [support@olink.com](mailto:support@olink.com)

# 5. Process



## Process steps:

- NGS raw data in BCL or CBCL file format from NovaSeq™ 6000, NextSeq™ 550 or NextSeq™ 2000 is input.
- Pre-processing of the NGS raw data using the following software:
  - mydata-listener
  - bcl2counts
  - mydata-file-sync
  - olink\_interop\_stats.py
- Pre-processed data is analyzed in Olink MyData Cloud.
- The data is quality controlled (automatically Plate Control normalized data).
- The data is normalized (Intensity normalization).
- Data is exported.

## 5.1. Hardware and software requirements

### 5.1.1 Server requirements

Linux server with at least 32 GB memory and python. Recommended: 64 GB for S4 flow cell.

### 5.1.2 Pre-processing software

The following software is required for pre-processing of the NGS raw data:

- mydata-listener
- bcl2counts
- mydata-file-sync
- olink\_interop\_stats.py

Refer to section [5.4. Installing pre-processing software](#) for instructions on how to download and install this software.

## 5.2.Olink® MyData Cloud software requirements

Downloading or installing any software for using the Olink MyData Cloud service is not needed. Compatible web browsers (other browsers may work as well):

- Google Chrome
- Mozilla Firefox
- Microsoft Edge

For correct behavior in Olink MyData Cloud account, check the following:

- **Enable cookies**  
Make sure cookies are enabled in the browser so all parts of our application work as expected.
- **Enable JavaScript**  
JavaScript must be enabled in the browser.
- **Set the screen resolution**  
Minimum screen resolution: 1024 x 768. A higher resolution is recommended.
- **Check the web browser for add-ons**  
In some cases, browser add-ons, extensions, ad blockers, or plugins can interfere with our application's functionality. Consider disabling these extras or try a browser without them.
- **Other software requirements**  
For macOS users the System preferences settings under General for Show scroll bars should be set to Always for optimal presentation of scrollbars in some web browsers..

## 5.3.Required files and information

Files and information needed for the analysis:

- NGS raw data in BCL or CBCL file format
- Plate layout for the NGS run with well-id, sample name, and sample type, in csv-format (refer to section [14.1. Upload plate layout files](#))
- Panel Lot number of reagents used (provided on the Lot configuration insert delivered with the kit)

## 5.4.Installing pre-processing software

The NGS raw data needs to be pre-processed and uploaded to the Olink MyData Cloud service before analysis. First, the *bcl2counts* software must process the NGS run folder to generate the counts files and run the metadata file. Then these files must be uploaded.

To automate this process, the *mydata-listener* and *mydata-file-sync* software must be installed. This software monitors a specific folder where the NGS raw data is stored. When a new run is completed, *mydata-listener* will trigger *bcl2counts*, *bcl2counts* will call *olink\_interop\_stats.py* to extract data from the InterOp data to a meta data file. When *bcl2counts* has finished processing the run, *mydata-file-sync* will upload the produced counts files and meta data file to Olink MyData Cloud service.

### 5.4.1 Installation instruction

1. Download pre-processing software installation package from the Dashboard page of the Olink MyData Cloud Admin site, <https://eu.admin.mydata.olink.com> or <https://us.admin.mydata.olink.com>.
2. To install, please follow the instructions in the file `INSTALLATION_AND_USER_GUIDE.txt`, which is included in the installation package.

# 6. Account access and security

## 6.1.Account access

To access the Olink MyData Cloud account, go to <https://eu.mydata.olink.com> (Europe), or <https://us.mydata.olink.com> (US), depending on at which server the account is set up, and sign in using your email and password.

If an incorrect password is entered three times, the account is disabled and must be unlocked by an administrator. If the password needs to be reset, click **Forgot your password?** in the login window and follow the instructions.

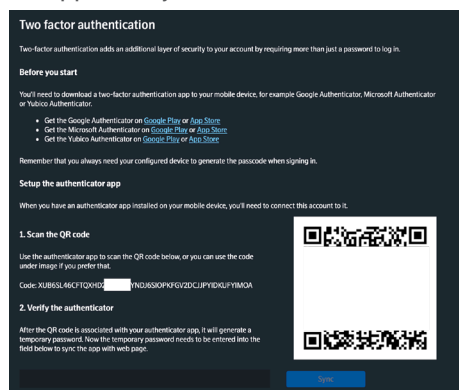
Contact the local IT administrator when having problems logging in. When logged in, the start page will display a list of all projects.

## 6.2.Account security

It is recommended to configure an additional security layer, also known as *Two-factor authentication* (TFA, sometimes referred to as *Multi-factor authentication* MFA). In this way, the account and data are much safer from unauthorized access.

To set up TFA:

1. Select **My profile** from the menu bar.
2. In the **My profile** view, instructions are found on how to set up and configure the TFA using different authenticator apps widely available.



After activation, the one-time code from the authenticator app needs to be entered, in addition to the password, every time signing in. If the Authenticator app is lost or help is needed with the TFA, contact the local IT administrator.



## 6.3.Administration

A separate administration application is available at <https://eu.admin.mydata.olink.com/admin> and <https://us.admin.mydata.olink.com/admin>.

The Lab's IT team should manage the Administration application to manage the Lab configuration and users.

### 6.3.1 Dashboard

In the **Home > Dashboard** pane, the Lab can manage settings for the Olink MyData Cloud account.

#### 6.3.1.1 API accounts

*Application Programming Interface* (API) accounts are used for authentication when uploading data to the Olink MyData Cloud service when using the mydata-file-sync application. To create a new API account, click the **Create an api account** button. A popup window displays the account key and secret, which is the only time the secret can be viewed. However, it is possible to generate a new secret at a later stage.

To generate a new secret for an API account, click the **Regenerate secret**. A popup window will display the API account. Click **Generate new secret** in the popup. A new secret is created and is shown by clicking the **Show/Hide secret** button.

#### 6.3.1.2 Pre-processing software

The latest version of the pre-processing software is available for download on this view. It is a zip archive containing the pre-processing software needed. The build number and the release date are shown next to the download link. Download the latest version by clicking the link and install it following the installation instructions included in the zip archive.

### 6.3.2 Home

In this menu, the Dashboard, Delete run function and lab audit log can be reached.

In the Delete run view, the runs flagged for deletion is listed. To delete a run, click the bin icon and confirm the deletion.


### 6.3.3 User

In this menu, new users are added to the system, roles are configured, and accounts are unlocked.

- To create a new user, select **User** menu, click **Create new user**. In the page displayed, enter the user information, select which Role the user should have, and click **Create user**. The user will be added to the list.
- To unlock a user that has been locked out, find the person in the list and click on it. On the **User information** page displayed, click **Enable user**.
- To disable a user and prevent them from logging in, find the person in the list and click on it. On the **User information** page displayed, click **Disable user**.

### 6.3.4 Roles

The roles defined in the system are listed in the table below. A user can have a combination of these roles in the system, except for the Contact role, which is limited to the end customer and cannot be combined with other roles.

Role	Description
Administrator	Administrator role with full access to managing the Lab and users in the Administration application, see section <a href="#">6.3. Administration</a> .  <b>NOTE:</b> <i>The Administrator cannot view projects in Olink MyData Cloud, to do that, additional roles need to be added to this user.</i>
Project Coordinator	Creates and manages projects and project types.
Reviewer	Manages projects and runs; manages normalized and QC data and generates NPX and Analysis Report files.
Approver	Has capability to approve NPX and Analysis Report files for delivery to end customer.
Project Auditor	Gives access to all projects created in the Lab.
Application Support	Role for Olink support staff. Views and manages projects, normalized and QC data; generates NPX and Analysis Report files.
Business Development Manager	Role for users that should be listed as BDM's. BDM's included in a project can be included on the Analysis Report cover page.
Contact	Customer Contact role. Cannot be combined with other roles. Contacts included in a project can be added to the Analysis Report cover page.

## Part 2: User interface

# 7. General



The Olink MyData Cloud user interface consists of five main menus:

- Projects menu, refer to [9. Projects](#).
- Runs menu, refer to [10. Runs](#).
- Contacts menu, refer to [11. Contacts](#).
- Settings menu, refer to [12. Settings](#).

The five main menus can be found in the upper right corner, together with the user account information and My Profile menu, refer to [8. My profile](#).

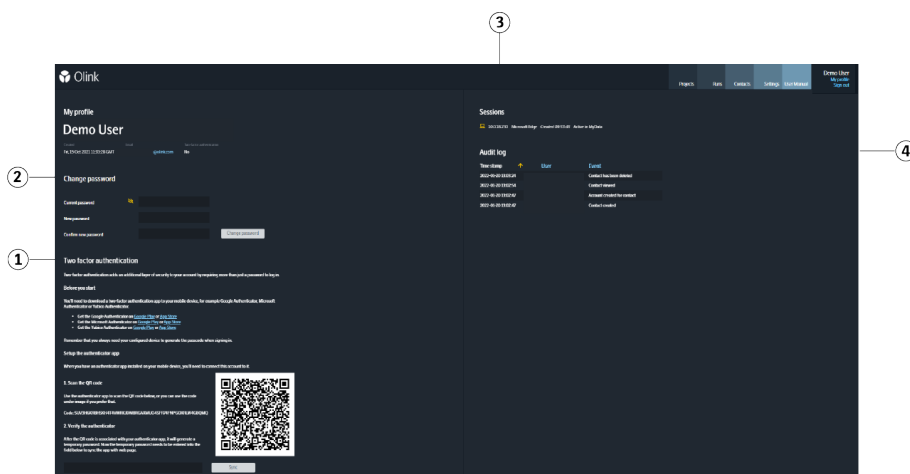
# 8. My profile

## 8.1. My profile view

To access the **My profile view**, click on the **My profile** menu in the menu bar. The view shows contact information entered by the administrator.

## 8.2. Change password pane

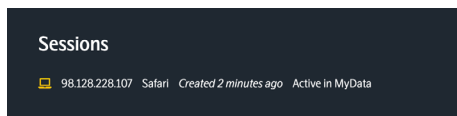
Password changes are managed from this pane.



No	Function	Description
1	Two factor authentication	Additional layer of security, refer to <a href="#">6.2. Account security</a> .
2	Change password	Fields for changing password.
3	Sessions	Lists active MyData sessions, refer to <a href="#">8.3. Sessions pane</a> .
4	Audit log	Lists changes that have been made to the account and by whom.

## 8.3. Sessions pane

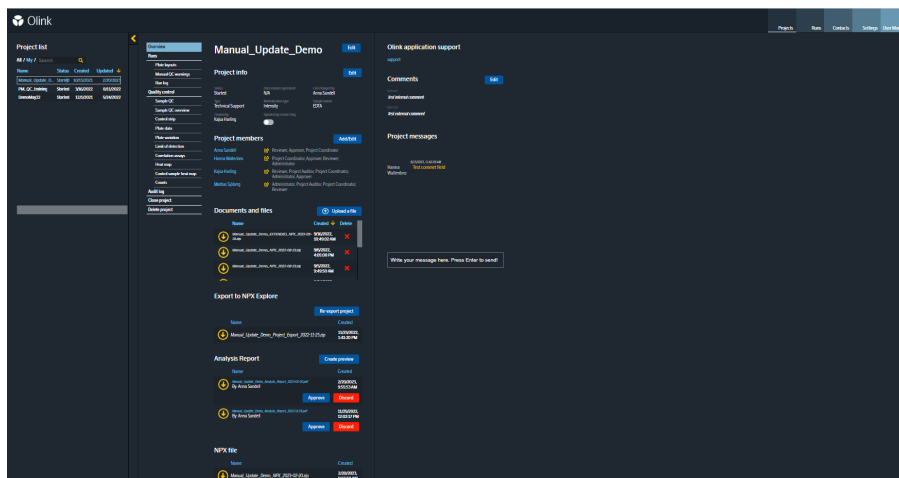
In the Sessions pane, you see which IP-address and web browser that is used for all currently active MyData sessions for the user, and when each session started. It also shows which site is used: *MyData* or *MyData Admin*. If sessions from different IP addresses is shown and the user has only signed in on one computer, the account may be compromised, and a password change is recommended.



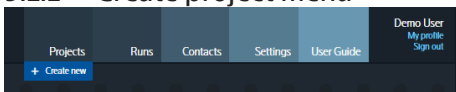
# 9. Projects

## 9.1. Overview

To access a project view, select the **Projects** menu and then select the project by clicking the project name in the list.

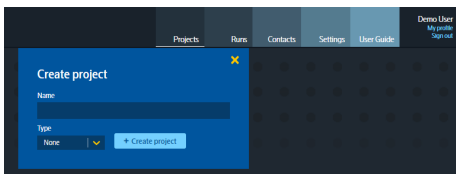


### 9.1.1 Create project menu



The **Create project** menu is accessed from the **Projects** drop-down in the menu bar.

In the popup window *Create project*: enter a project name, select type, and click **Create Project**.



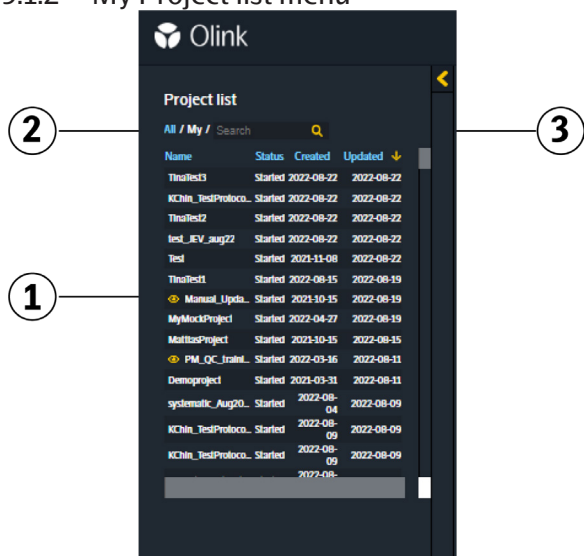
#### Project Name

The project name is used to identify the Olink MyData Cloud project and is displayed for the Contact in the Olink MyData Cloud service account, in the NPX data output file and the Analysis Report. The project name can be 3-40 characters long and contain the characters 0-9, a-z, A-Z, and the special characters - and \_.

#### Type

Type indicates the type of project, e.g., Olink Explore 1536, Olink Explore 384. The project types can be configured individually for each Lab. See section [12.1. Project types pane](#).

## 9.1.2 My Project list menu



No	Function	Description
1	Project list	Click on the name of the project to open that project page.
2	Folder	<b>All:</b> All projects that are available in the system for the Lab. <b>My:</b> All projects that are accessed.
3	Search	Type in the search box to filter the list.

The **Overview pane** contains information about the project.

- To add or edit information, click the Edit button next to the project info. Enter the changes and click Save to save the changes or close the popup window by clicking on the cross to cancel the changes.

## 9.2. Project specific information

To view the project specific information, choose a project from the Project list menu.

### 9.2.1 Project Info

To edit the project information, click the **Edit** button.

#### Status

There are two options for **Status**: *Started* and *Closed*. The status is set to *Started* when the project is created, i.e. when the quote for the project is accepted. The status is are uploaded to the actual Olink MyData Cloud account.

#### Type

**Type** indicates the type of project. See section [12.1. Project types pane](#).

#### Sample type

The **Sample type** field is used to describe the sample matrix, e.g., "Plasma" or "Serum". The information is displayed on the Analysis Report.

#### Normalization type

The **Normalization type** field indicates which normalization type used for the project. The two options are *Plate Control* and *Intensity*. For description of which normalization method to choose, see chapter [19. Normalization methods](#).

#### Updated by Contact

Set/Unset the flag indicating that a Contact user has made a change in the project.

#### Last changed by

Indicates who last changed the project and when.

#### Data Transfer Agreement

Use the dropdown to indicate whether a custom *Data transfer agreement* is in place for this project. This property is for information only and does not affect data transfer in MyData.

## 9.2.2 Project members

The Project members list shows which project members who has access. The Contact must be defined before adding to the project. See section [11. Contacts](#).

To send an email informing the member they have been added to the project, click the **Send** icon to the project members' right. To send the email, click **Yes**, click **No** to cancel the email.

Click on the member name to send a standard e-mail to the selected member. An e-mail window to the member will open up.

To manage the Project members list, click the **Add/Edit** button. A popup window is displayed listing all available Olink MyData Cloud users and Contacts. Click the **+** next to a person's name (the **+** will be visible when your cursor hover above the actual name) in the **Add new members** list to add it to the Current members list. Click on (the **cross** next to a person's name) in the Current members list to remove access from the project.

When edits are finalized, click **Save** to save the changes, click **Cancel** to cancel the changes.



**NOTE:** Any user with the role Project Auditor can access all projects in a Lab without being part of the project members list.

## 9.2.3 Documents and files

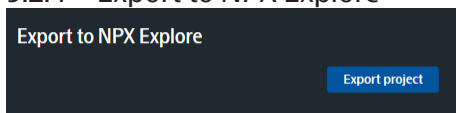
The Documents and files pane displays the files uploaded to the project by both the Lab and the Contact.

When a new project is created, all documents listed for the selected project type are added automatically and available for the Contact. Additional files to or from the Contact can be uploaded here.

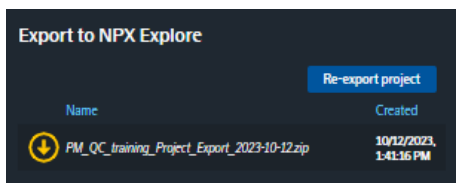
1. To upload a file, click on the **Upload a file** button. Drag and drop the file to the indicated field on the left. Alternatively, click on the field to browse and choose a file to upload. The maximum file size allowed for upload is 500MB.
2. Use the **Comments** field below the file area to comment the uploaded file(s).
3. Click **Upload**.



## 9.2.4 Export to NPX Explore



When the project is exported, the button text changes and the project file will appear in the download list below the button.



Export project to either use in NPX Explore or to store in a database for future use.

The following changes will be applied to the project:

- If a well has been manually set to NOT\_USED in MyData, it will not be possible to change it back in the exported project.
- Contact users have been added as a project comment and are not retained in the Analysis Report.
- Some run metadata values have been rounded to six significant figures.

The following information will not be included in the export:

- Run log annotations.
- Project messages
- Project type
- Regular plate layout files
- Export files (Analysis Report, NOX and extended NPX file) that have not been approved

## 9.2.5 Analysis Report

The Analysis Report can be generated, downloaded for review, and approved here. See section [16. Generate Analysis Report and NPX file](#), for description.

## 9.2.6 NPX file

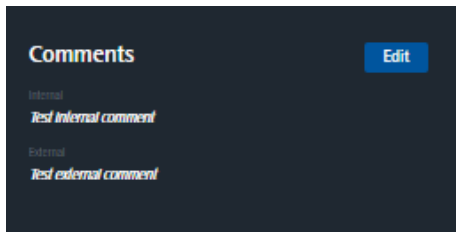
The NPX data file is automatically generated when the project is normalized. It can then be downloaded for review and approved here. See section [16. Generate Analysis Report and NPX file](#) for description.

## 9.2.7 Olink® application support

To request support from an Olink Application Support specialist, click the Support e-mail link. When a support specialist has been appointed, that person can be added to the project. Click the **Add** button under the Olink application support header and enter the e-mail of the appointed support person. The support specialist will then be able to access the project and help with troubleshooting.

## 9.2.8 Comments

There are two different comments fields, *Internal comments*, and *External comments*.



### Internal comments

This field can be used for any comments and is not included in Analysis Report or data export.

- To edit a comment, click the **Edit** button. Enter text and click **Save** when done or click the cross icon to cancel the changes.
- To remove a comment, click the **Edit** button. Delete the text and click **Save**.

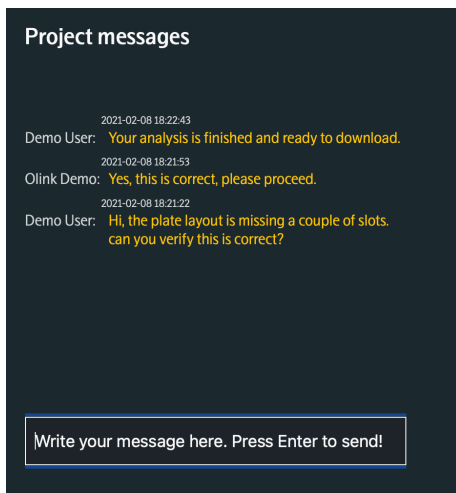
### External Comments

Comments added here will be displayed on the message board for the Contact and in the Analysis Report.

- To edit a comment, click the **Edit** button. Enter text and click **Save** when done or click the cross icon to cancel the changes.
- To remove a comment, click the **Edit** button. Delete the text and click **Save**.

## 9.2.9 Project messages

To add a message, write in the field shown in the figure, and press enter. The message will be added to the list. This messages are only for communication in the project, and will not be added to the Analysis Report.



## 9.3.Runs

In the **Runs** pane, plate layout files are uploaded, and runs can be added to the project and edited.

### Plate layout files

This pane contains the Plate layout files. Refer to section [14.1. Upload plate layout files](#), to describe how to upload plate layout files.

### Panels

This pane contains the panels added to the project. Refer to [14.2. Add panels](#), for a description of how to add panels.

- Restart an individual panel calculation by clicking the panel's **Status** icon.
- Display the plate layout of the panel by clicking the **Eye** icon next to the panel name.
- Delete the panel using the **Bin** icon.
- Restart all panel calculations by clicking the Refresh button in the pane's top right corner.

### Normalization

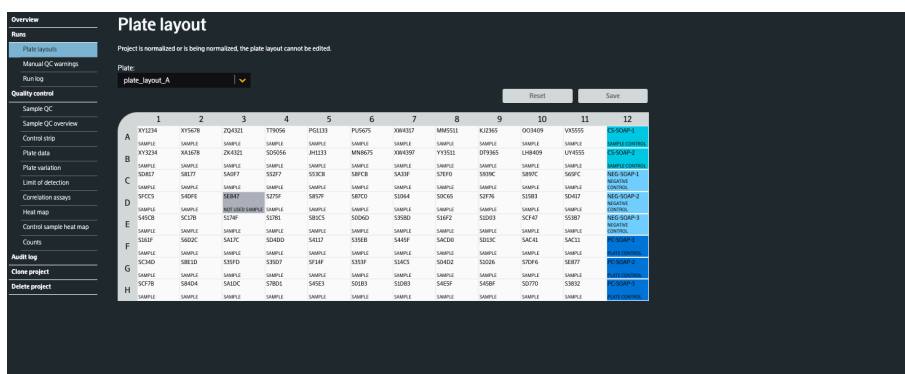
Click the **Normalize project** button to normalize data. Refer to section [15.3. Normalize the project](#) for a description of the project's normalization and how to redo normalization. When data is normalized, the **Generate extended NPX** button is disabled, and the **Clear normalization** button is enabled.

Set LIMS plate ID by clicking **Edit** and write of choose the predefined ID. The ID will be shown in the **Runs** pane, refer to [9.3.3 Run log](#). When a rerun is performed, the plate is connected to a new run and the LIMS plate ID can be changed but not deleted. The connection is lost when **Rerun of** in the **Run log** pane is set to *None*.

To prepare for rerun, fill in preferred information and click the rerun box.

Runs set to not be included (check box for Include is not selected) are excluded from the calculations and results.

### 9.3.1 Plate layout



**Plate layout** pane displays the plate layouts used in the project when layouts have been uploaded and are connected to a lane. Each plate position is annotated to one of the following content types, based on the information given in the plate layout file:

- SAMPLE
- CONTROL
- NEGATIVE\_CONTROL
- PLATE\_CONTROL
- NOT\_USED

The standard plate layout has samples in columns 1-11 and a control strip in column 12. In the control strip, A and B are Sample Controls in duplicate (plasma samples) used to calculate %CV, C to E are Negative Controls, and F to H are Plate Controls (pooled plasma samples) used for between plate normalization.

Proceed as follows if a position has been annotated incorrectly:

1. Click on preferred sample well. To select more than one, click and drag over preferred sample wells.

	2	3	4	5	6
.Y5678	ZQ4321	TT9056	PG1133	PU5675	
AMPLE	SAMPLE	SAMPLE	SAMPLE	SAMPLE	
A1678	ZK4321	SD5056	JH1133	MN8675	
AMPLE	SAMPLE			SAMPLE	
8177	SA0F7			S8FCB	
AMPLE	SAMPLE			SAMPLE	
4DFE	SE847	S275F	S857F	S87C0	
AMPLE	SAMPLE	SAMPLE	SAMPLE	SAMPLE	
C17B	S174F	S1781	SB1C5	S0D6D	

2. Right-click on the selection and select status.
3. To save the changes, click **Save**. To remove the changes, click **Reset**.



**NOTE:** The sample type cannot be changed when the project is normalized. Before changing, clear the normalization on the Runs pane. Normalize the project again after the sample type change.

Not used samples are not included in the total number of samples in the Analysis Report.

When a change for a sample in Used/Not used mode is saved, the panel calculations are performed again automatically and QC views are updated with Plate Control normalized data. QC must be performed again after the update, refer to section [15.2. Optional data handling steps](#).

### 9.3.2 Manual QC warning

The **Manual QC Warning** pane displays the plate layout with automatic QC warnings (red flag) and manual QC warnings (yellow flag).

All marked, not saved, manual warnings are listed above the plate layout, and the saved below.

All manual warnings for the selected plate panel combination is shown above the plate layout both previously saved and unsaved ones. The list below the plate layout contains all manual warnings for the whole project.

**Manual QC warnings**

Plate: **plate\_layout\_C** Panel: **Cardiometabolic**

Current manual QC warnings

Plate	Panel	Sample	Sample type	Well	Block	Rationale
plate_layout_C	Cardiometabolic	SC832	Sample	C8	A	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	B	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	C	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	D	Int
plate_layout_C	Cardiometabolic	SE83C	Sample	E7	A	Int
plate_layout_C	Cardiometabolic	SE83C	Sample	E7	B	Int
plate_layout_C	Cardiometabolic	SE83C	Sample	E7	C	Int
plate_layout_C	Cardiometabolic	SE83C	Sample	E7	D	Int
plate_layout_C	Cardiometabolic	SC837	Sample	G4	A	Int
plate_layout_C	Cardiometabolic	SC837	Sample	G4	B	Int
plate_layout_C	Cardiometabolic	SC837	Sample	G4	C	Int
plate_layout_C	Cardiometabolic	SC837	Sample	G4	D	Int

plate\_layout\_C Cardiometabolic

Project is normalized or being normalized, cannot add manual QC warnings.

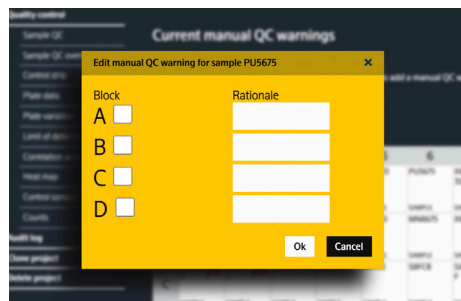
	1	2	3	4	5	6	7	8	9	10	11	12
A	SC807	SC837	SC841	SC838	SC816	SC803	SC839	SC803	SC837	SC808	SC808	SC808
B	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
C	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
D	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
E	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
F	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
G	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816
H	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816	SC816

All manual QC warnings

Plate	Panel	Sample	Sample type	Well	Block	Rationale
plate_layout_C	Cardiometabolic	SC832	Sample	C8	A	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	B	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	C	Int
plate_layout_C	Cardiometabolic	SC832	Sample	C8	D	Int

To add or edit a manual QC warning:

1. Click on preferred sample well.
2. In the pop-up menu, select preferred block(s) by clicking the checkbox(es).
3. Fill in rationale information.



4. Click **Ok**. A yellow flag will appear on the sample well(s).
5. Repeat step 1-4 until all affected wells are marked.
6. Click **Save**.

### 9.3.3 Run log

The run log displays the runs added into the created projects. If the list is empty, a calculation must be performed, refer to [9.3. Runs](#).

Sequencing date	LIMS plate ID	Rerun of	Panel	QC warn	PC warn	NC warn	Manual assessment	Primary cause	Step failed	Rerun	Include
2021-01-22		None	CAR	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	CAR	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	CAR	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	CAR	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	INF	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	INF	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	INF	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	INF	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	NEU	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	NEU	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	NEU	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	NEU	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>
2021-01-22		None	ONC	!	!	!	None	Not applicable	Not applicable	<input type="checkbox"/>	<input type="checkbox"/>

The following information can be filled in for each run to evaluate the run quality and assess whether rerun is needed:

Rerun of	<ul style="list-style-type: none"> <li>None</li> <li>Available LIMS plate ID</li> </ul>
Manual assessment	<ul style="list-style-type: none"> <li>None</li> <li>Pending</li> <li>Good</li> <li>Bad</li> </ul>
Primary cause	<ul style="list-style-type: none"> <li>Not applicable</li> <li>Unknown error</li> <li>Other error</li> <li>Sample related</li> <li>Instrument error</li> <li>Human error</li> </ul>
Step failed	<ul style="list-style-type: none"> <li>Not applicable</li> <li>Incubation</li> <li>Extension</li> <li>PCR1 pool</li> <li>PCR2 setup</li> <li>PCR2 pool</li> <li>Purification</li> <li>Sequencing</li> </ul>
Rerun	Click box for rerun
Include	Click box to include run in project
Comment	Free text
Include reason	<ul style="list-style-type: none"> <li>Manual assessment good</li> <li>Not applicable</li> <li>Business reason</li> <li>Sample volume</li> <li>Sample quality</li> </ul>

## 9.4. Quality control

The **Quality control** pane displays an overall quality assessment for the selected plate. A green checkmark indicates that the block fulfilled the corresponding QC criteria, a yellow exclamation that the block is flagged and may need further investigation and red cross indicates that the block does not meet the specification and further assessment is needed. Refer to sections [18.1. Sample QC](#), [18.2. Control strip QC](#) and [18.4. Run QC](#) for description of the QC criteria.

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Plate:  
plate\_layout\_A

Cardiometabolic B04413

Block	Warnings ≤ 1/6 of samples	MAD INC control ≤ 0.3 NPX	MAD AMP control ≤ 0.3 NPX	MAD Z-score Plate control ≤ 1.5	Median Z-score Plate control absolute value ≤ 5	Median Z-score Negative control ≤ 3
A	34	0.17	0.15	1.44	5.62	6.67
B	36	0.16	0.13	1.32	1.13	9.41
C	33	0.08	0.13	0.95	0.99	14.90
D	34	0.18	0.08	0.76	1.28	14.24

Cardiometabolic II B13805

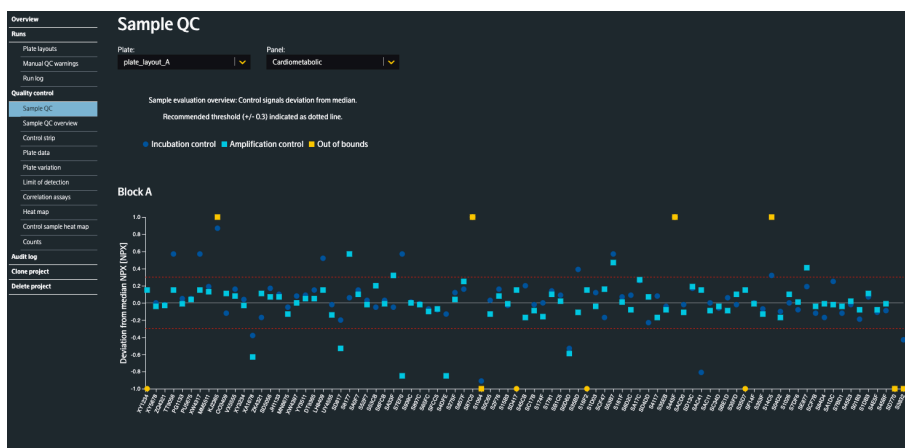
Block	Warnings ≤ 1/6 of samples	MAD INC control ≤ 0.3 NPX	MAD AMP control ≤ 0.3 NPX	MAD Z-score Plate control ≤ 1.5	Median Z-score Plate control absolute value ≤ 5	Median Z-score Negative control ≤ 3
A	1	0.07	0.14	0.34	-1.29	-1.08
B	1	0.11	0.12	0.19	-0.10	-0.59
C	0	0.06	0.06	0.35	-0.39	-1.03
D	4	0.17	0.05	0.28	-0.43	-1.02

Inflammation B04411

Block	Warnings ≤ 1/6 of samples	MAD INC control ≤ 0.3 NPX	MAD AMP control ≤ 0.3 NPX	MAD Z-score Plate control ≤ 1.5	Median Z-score Plate control absolute value ≤ 5	Median Z-score Negative control ≤ 3
A	29	0.14	0.13	1.55	4.31	4.05

### 9.4.1 Sample QC

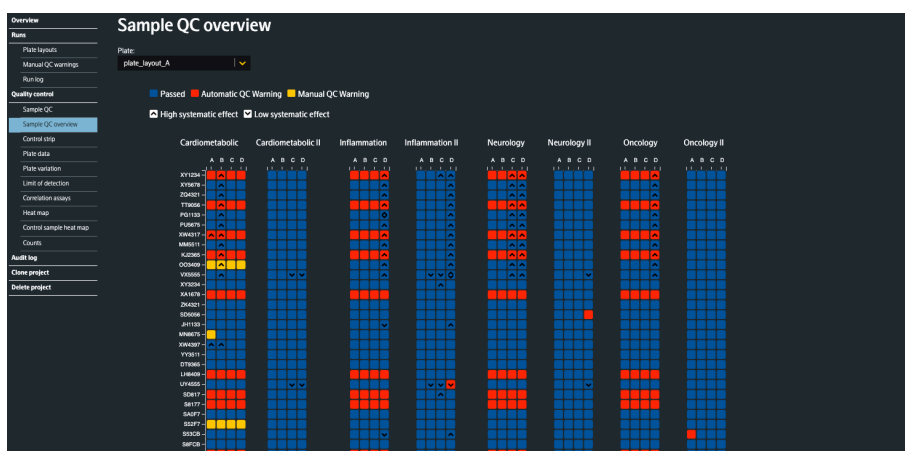
The **Sample QC** pane displays the deviation from the median (zero, light gray line) in all samples for Incubation Control (dark blue circles) and Amplification Control (light blue squares). Yellow color denotes deviations that are out of bounds of the graph. Red dotted lines depict the QC criteria cutoff of 0.3 NPX deviations from the median. All blocks are displayed on the same page. The x-axis is ordered by row, so the sample at A1 is far left, followed by the sample at A2.



- In the left drop-down menu, select the plate to view and then select the panel to view in the right drop-down menu.
- Hovering over a data point in the plot will display the sample name and the deviation from the median.

### 9.4.2 Sample QC overview

**Sample QC overview** displays a heatmap with passed (blue), automatic QC warning (red), or manual QC warning (yellow) status for each sample in all blocks and panels.



- Select the plate to view in the drop-down menu.
- Hovering over the cells will give information about the sample, block, and what criteria have resulted in a QC warning status.



### 9.4.3 Control strip

The **Control strip** pane displays z-scores for Negative Controls (dark blue circles) and Plate Controls (light blue squares) in a scatterplot per block. Yellow color denotes deviations that are out of bounds of the graph.

The median absolute deviation (MAD) of the Z-score of Plate Controls must not exceed 1.5, the absolute value of the median Z-score of Plate Controls must not exceed 5 and the median Z-score of Negative Controls must not exceed 3.

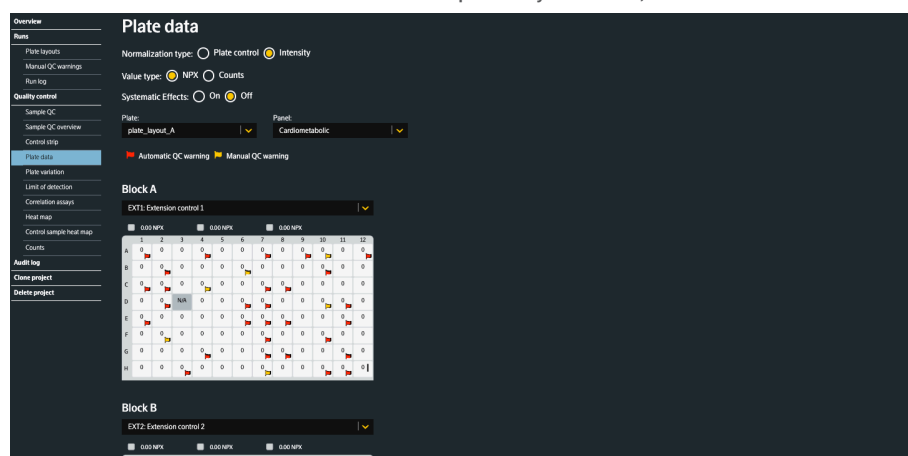
- In the left drop-down menu, select the plate to view and then select the panel to view in the right drop-down menu.
- Hovering over a data point in the plot will display the assay name and the deviation from the median.



### 9.4.4 Plate data

The **Plate data** pane shows the plate layout with automatic and manual QC warnings for one assay from each block. The plate layout can be shown with NPX or count as value type and with and without systematic effects.

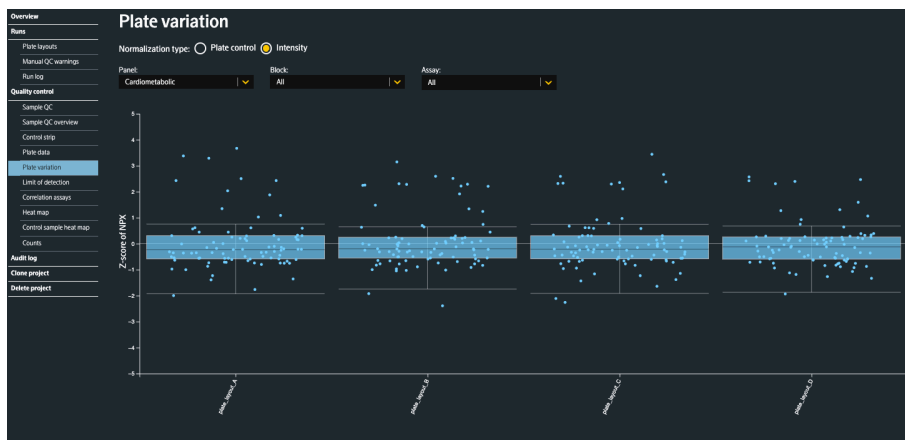
- The default assay is the assay Extension Control.
- The NPX values are shown above each plate layout: Max, Min and Median values.



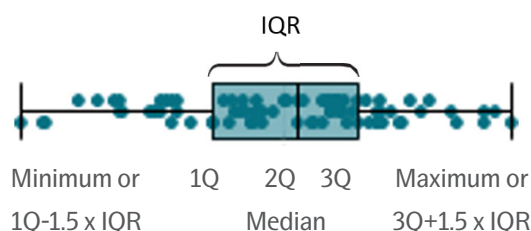
- Select the plate to view in the top-left drop-down menu, and the panel in the top-right drop-down menu.
- For each block section, switching assays is possible by selecting an assay from the drop-down menus. Fill in the assay name in the drop-down menu box to find it in the list or use the scroll function. To quickly flip through the assays in the image, use the left (scrolls down) or right (scrolls up) arrow keys on the keyboard.
- The flags in the sample wells indicates automatic (red) and manual (yellow) QC warnings.
- Hover over a well to display the sample name, counts and deviation from the median.
- Option for intensity normalized projects: Choose which data to view by selecting *Plate control* or *Intensity*. *Intensity* is set by default.

## 9.4.5 Plate variations

The **Plate variations** pane displays a box plot, which represents the deviation of total intensity of each sample from the median. In the default setting, each sample's average z-score is displayed as a points and boxplots, one plot per plate. Use this plot to detect systematic variance in total intensity between plates or to find samples with different total intensities.



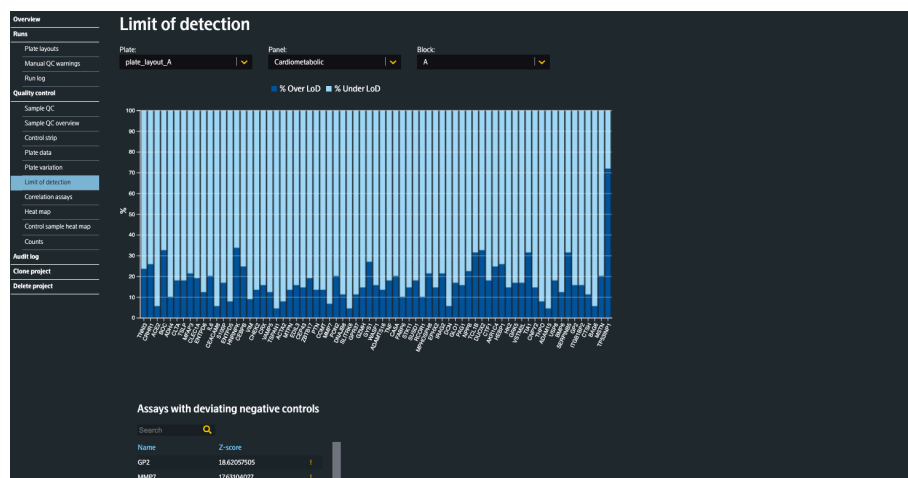
A box plot is a graph that gives a good indication of how the data values are spread out. It shows outliers, symmetry, how tightly the data is grouped, and if and how the data is skewed. The box plot splits the data set into quartiles. The first quartile (1Q) is the 25<sup>th</sup> percentile and the third quartile (3Q) is the 75<sup>th</sup> percentile. A line is drawn at the median, the second quartile (2Q). The interquartile range (IQR) is a measure of where the bulk of the data set values lie, and how they cluster around the mean. The explanatory picture below shows a box plot that is rotated 90 degrees relative to the box plots in [Figure 25](#).



- The sum of NPX for each sample is calculated and converted to Z-scores. This means that a sample at +1 on the plot has one Standard Deviation (SD) higher NPX values overall than the average of all samples.
- Use the drop-down menus at the top to select specific blocks, panels, and assays for a targeted view of the z-score. Type the name of an assay in the drop-down menu box to find it in the list.
- Option for intensity normalized projects: Choose which data to view by selecting *Plate control* or *Intensity*. *Intensity* is set by default.

## 9.4.6 Limit of detection

The **Limit of detection** (LOD) pane contains a bar chart with the percentage of data over LOD (dark blue), data under LOD (light blue), no data (yellow) and data for assays for which LOD could not be calculated (light gray), for all assays in the selected plate, panel, and block.



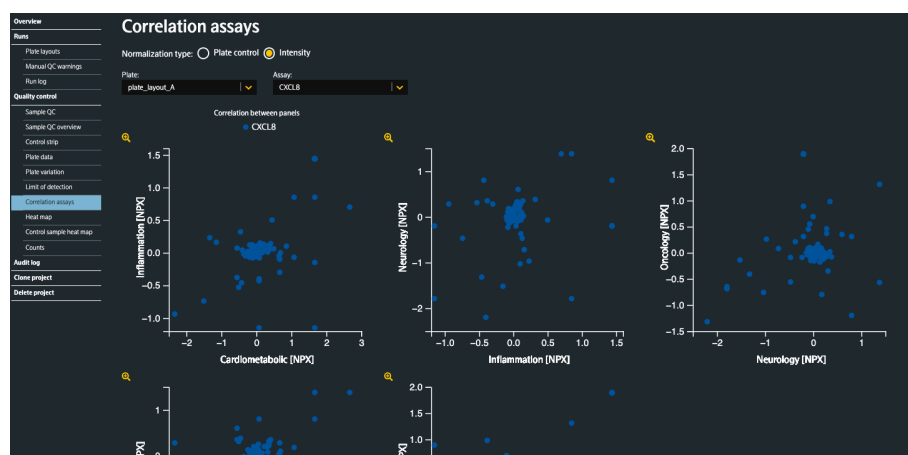
- Use the drop-down menus to change the plate, panel, or block displayed.
- Assays with deviating Negative Controls +3 SD in Z-score are listed below the graph. In this list, assays with Z-scores > 5 SD that receive an assay warning in the results file are indicated with a yellow exclamation mark.

## 9.4.7 Correlation assays

On following Explore panels: Cardiometabolic, Inflammation, Neurology and Oncology, there are three assays present on all panels: IL6, CXCL8 and TNF.

On following Explore panels: Cardiometabolic II, Inflammation II, Neurology II and Oncology II these three assays are present: LMOD1, SCRIB and IDO1.

These overlapping assays will be measured four times each in studies where all panels are included. The **Correlation assays** pane shows data for these overlapping assays.

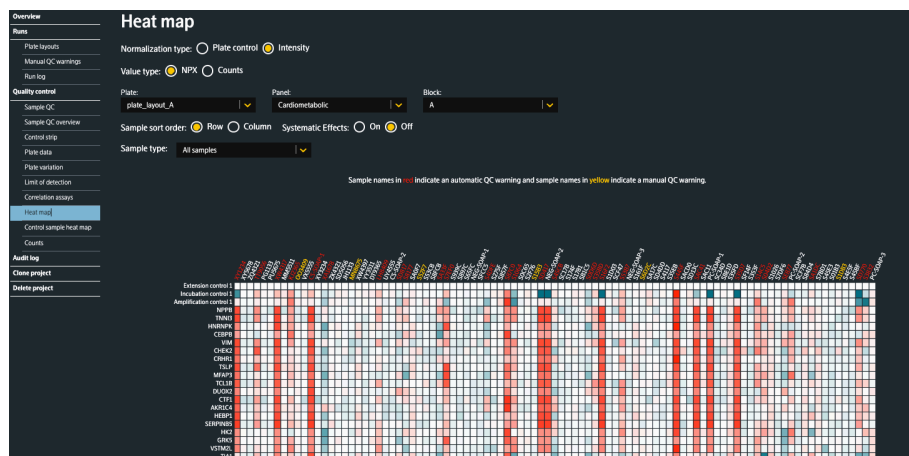


- To display a specific correlation plot, use the drop-down menus to select a combination plate versus assay.
- Option for intensity normalized projects: *Plate control* or *Intensity*. *Intensity* is set by default.
- Hover over a data point to show the name and NPX values.

## 9.4.8 Heat map

The **Heat map** pane contains a heat map of all samples and assays using the deviation from each assay's median of the plate. The heat map shows the deviation from median values on a red-white-green color scheme per assay. Red wells indicates NPX values higher than the median, and green indicates values lower than the median.

Sample names in red indicate an automatic QC warning and sample names in yellow indicate a manual QC warning.

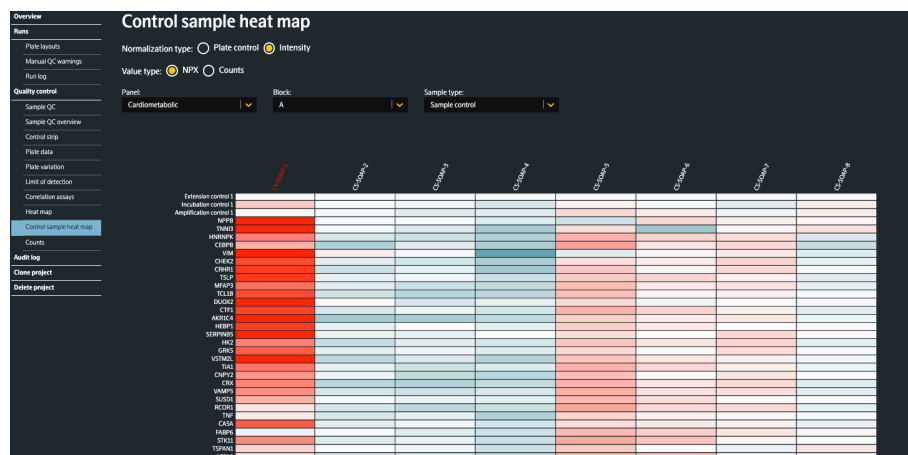


- Use the drop-down menus to change the plates, panels, or blocks displayed.
- Choose how to sort the map by selecting *Row* or *Column* for *Sample sort order*.
- Use the Sample type drop-down menu to filter the heat map. Available options are: All samples, Sample, Sample Control, Negative Control, Plate Control.
- Option for intensity normalized projects: Choose which data to view by selecting *Plate control* or *Intensity*. *Intensity* is set by default.
- Hover over a cell to display the assay, sample name, deviation from assay median, QC warnings and NPX value for that cell.

### 9.4.9 Control sample heat map

The **Control sample heat map** shows one specific sample type from all the plates with the same selected panel, one block at the time. Red wells indicates NPX values higher than the median, and green indicates values lower than the median.

- The project must be normalized.

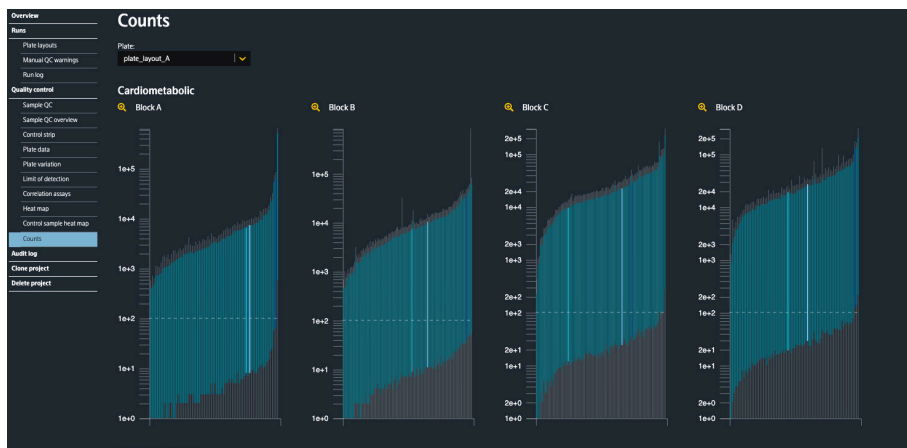


- Select the panel to view in the top-left drop-down menu, and the sample type in the top-right drop-down menu.
- The value displayed in each well is the NPX value for that sample and the selected assay.
- Hover over a well to display the sample name, how much each well deviates from the median, and the NPX value.
- Option for intensity normalized projects: Choose which data to view by selecting *Plate control* or *Intensity*. *Intensity* is set by default.
- The automatic and manual QC warnings are shown when Sample Control is chosen as sample type.

### 9.4.10 Counts

The **Counts pane** contains boxplots of the counts for all assays. A dashed line at the bottom indicates 100 counts and is there to facilitate troubleshooting.

- Use the drop-down menu to choose which plate to view.
- Click the **Zoom** icon for the counts plot to open a new window showing that plot. The colors of the box plots represent assays (green), Incubation Control (dark blue), Amplification Control (light green), Extension Control (light blue). Yellow represents a box plot out of bounds.
- Hover over the box for one assay in the plot to show assay name, mean-, max- and min-counts values, counts values for 25<sup>th</sup> and 75<sup>th</sup> percentile, and the Number of wells referring to the number of samples.



Counts pane with box plots for one panel



Counts pane zoom function, showing plot for one block. Hovering over a box shows assay specific information

## 9.5.Audit log

The **Audit log** pane contains all events for the current project, including who accessed the project, downloaded files, and other events. See [Table 1](#) for a description of the information presented.

Overview	Audit log			
Home	Time stamp	User	Event	Data
Plate layouts				
Manual QC writings				
Run log				
Quality control	2022-05-31 10:27:08	IT Support	New intensity normalized heat map view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Samples QC	2022-05-31 10:27:08	Hanna Wallenberg	The normalization has been completed	({"service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png"})
Control strip	2022-05-31 10:27:05	IT Support	New intensity normalized plate variation view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Plate data	2022-05-31 10:27:05	IT Support	Unknown event	({"service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png"})
Plate variation	2022-05-31 10:27:05	IT Support	Control sample heat map has been produced	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Limit of detection	2022-05-31 10:27:03	IT Support	A control sample heat map has been produced	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Correlation assays	2022-05-31 10:27:03	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Heat map	2022-05-31 10:27:03	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Control sample heat map	2022-05-31 10:27:03	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Counts	2022-05-31 10:27:00	IT Support	An extended NPX file has been produced	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Exporting	2022-05-31 10:26:59	IT Support	The Analysis Report data is now available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Clone project	2022-05-31 10:26:52	IT Support	New intensity normalized heat map view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
Delete project	2022-05-31 10:26:52	IT Support	An NPX file preview has been created	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
	2022-05-31 10:26:50	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
	2022-05-31 10:26:49	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")
	2022-05-31 10:26:47	IT Support	New intensity normalized correlation assays view data available	("service","web-console","bln","labdata@304-304-4311-9440-57963576d6d6project/963a412-1619-44aa-9c89-8aebd4975aheatmap-intensity-normalized_e45102aa-60a7-482a-85ac-8b0a5c511131_903a67cc-27ff-4375-88b0-272c385d067a.png")

Column	Description
Date	The date and time of the event
User	The user responsible for the event
Event	The type of event
Data	Optional additional information about the event

## 9.6.Clone project

In this pane, the user can create a copy of the project.

- To create a clone of the project, enter a name for the clone in the *Give the clone a name* field, then click **Create**.

The copy will include runs, plate layouts, comments and messages from the project but will not include files and is not normalized. Panel calculations must be started manually in the cloned project, by clicking the Refresh button (Start all panel calculations) on the Panels pane of the Runs pane. Only the person who created the clone will be a project member for the cloned project. Other members can be added.

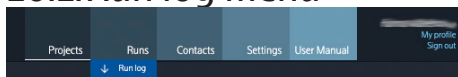
## 9.7.Delete project

In this pane, the user can delete the current project completely. The runs will not be deleted, only the project referencing the runs. It is not possible to undo this action.

- To delete the project, enter the project name and click **Delete**.

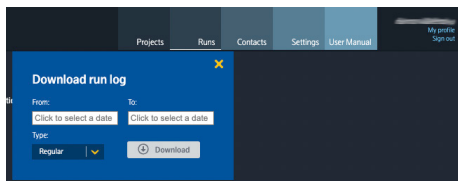
## 10. Runs

## 10.1.Run log menu

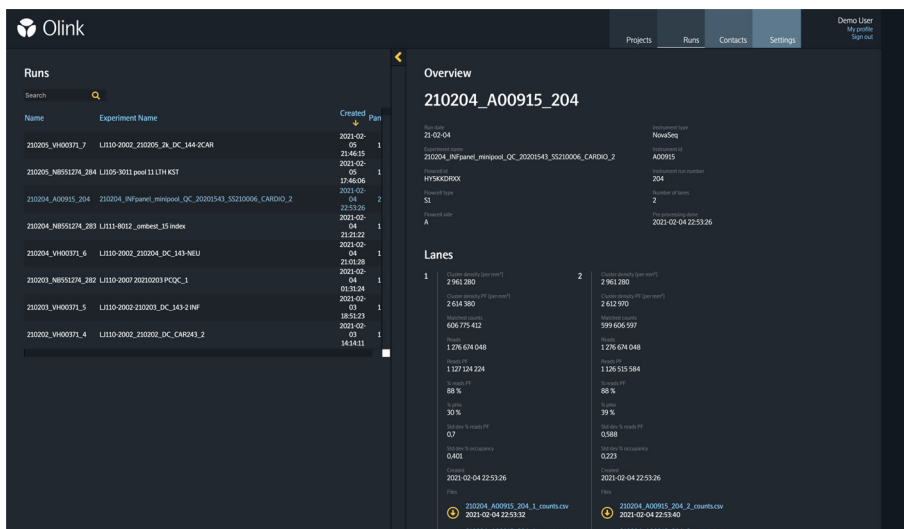


The **Run log** menu is accessed from the Run drop-down in the menu bar.

In the popup window Downloading run log, select from and to date and type. Click Download. The log will list all runs made during this time range.



The popup window will remain open when changing panes.



In the **Runs** menu, all NGS runs uploaded to the MyData system are listed. Type in the search box to filter the list according to the search word. Click on the run name to display more information about the run.



### 10.1.1 Delete a run

This function is made in two role based steps: flag a run and delete a run. To avoid deletion by mistake, the steps must be performed by two different persons.

The following roles have permission to flag a run for deletion:

- Application support
- Approver
- Project auditor

Only the administrator can delete a flagged run.

To delete a run:

1. Click on the flag in the middle of the screen.

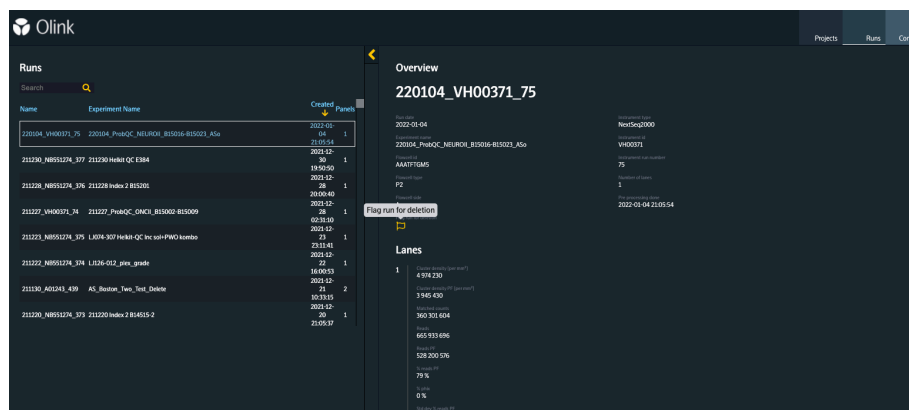


Figure 1. Runs menu with the delete run flag

**NOTE:** A run cannot be flagged if it is included in a project. If the marked run is included in a project, a warning will be prompted, containing information about which project the run is included in. Go to the runs pane of the project and remove the run from the project.

2. Edit reason for flagging in the pop-up window and press **Confirm**. The flag will be yellow.
3. The run will be deleted by an administrator in the administrator application.

**NOTE:** The deletion is terminal and can not be undone.

# 11. Contacts

## 11.1. Create contacts menu

Select the **Contact menu** in the menu bar and select **Create new**.

Enter a title (optional), first name, last name, and e-mail address and click **Create**. The account will be created for the Contact immediately, and an e-mail is sent to the entered e-mail address with login information.

## 11.2. Contacts list

Select the **Contacts menu** in the menu bar to display all accessible Contacts created. A search function is available together with a filter.

## 11.3. Overview

Select a Contact in the Contacts list to view details about the Contact in the overview pane. This pane displays Contact information, projects associated with the Contact, and account status.

- To edit the Contact information, click the **Edit** button. Type the new text in the displayed popup window and click **Save** or click the cross icon to cancel the changes.

- Below the Contact information, there are buttons to manage the Contact Olink MyData Cloud account.

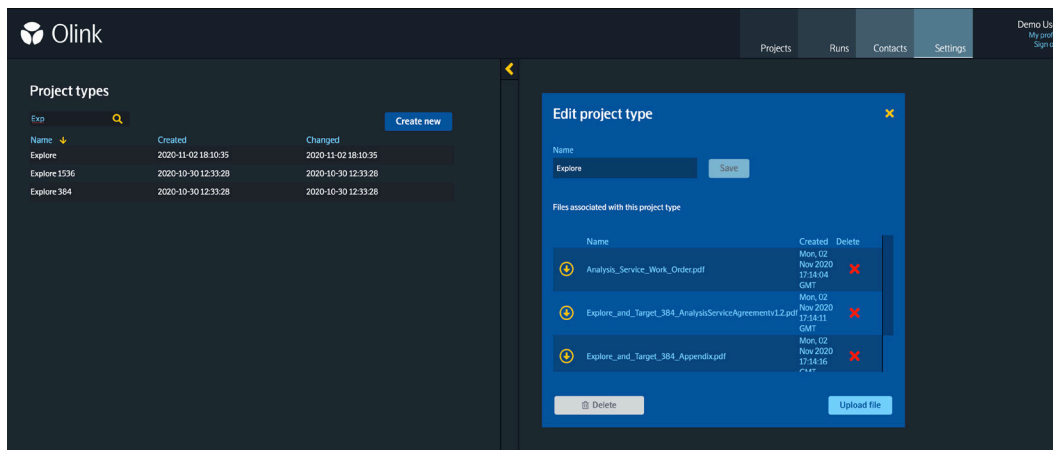
**Resend invitation** – Click this button to send a new invitation by e-mail to the Contact. This feature will only be active if the Contact has not yet signed in to Olink MyData Cloud.

**Enable contact** – Click this button to unlock the system's Contact account if it has been locked due to too many failed login attempts.

**Delete contact** – Click this button to delete the Contact from the system. Please note that this action cannot be undone.

# 12.Settings

This menu will let the user change project type settings for the Lab.



## 12.1.Project types pane

A person with the Project coordinator role can configure *Project types*. Go to the **Settings** menu in the menu bar to view a list of the currently available *Project types*. To edit a project type, click the project type name in the list. To create a new project type, click **Create new**, enter a name for the project type, and click **Save**.

A project type can be associated with specific documents added to each project within a project type when created. In the **Edit project type** pane, click the **Upload file** button, select the file to associate with this project type, and click **Upload**. To edit the project type name, edit the name and click **Save**. To delete the project type, click the **Delete project type** and click **Delete**.

# Part 3: Operation

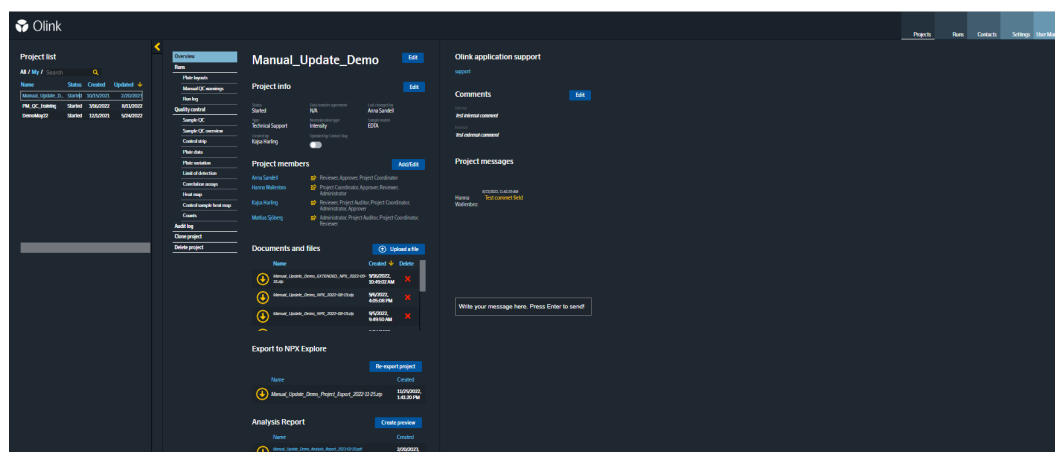
## 13.General

This chapter describes how to analyze the pre-processed NGS raw data in Olink MyData Cloud. The pre-processing operation itself is not subject to this manual but is briefly described section [20. Pre-processing software](#).

Input data for MyData are counts files uploaded from a local server where the pre-processed data is stored. After quality control of data automatically normalized against the Extension Control and Plate Control, a normalization step (intensity normalization) is performed, and the output data produced are obtained in Normalized Protein eXpression (NPX). NPX is an arbitrary unit on a  $\log_2$  scale, where a high NPX value corresponds to high protein concentration.

The following steps are included in the standard operating procedure:

1. Create a project
  - 1.1. Upload plate layout files
  - 1.2. Add panels
2. Perform Quality Control
  - 2.1. Additional quality assessment
  - 2.2. Optional data handling steps
  - 2.3. Normalize the project
3. Generate Analysis Report and NPX file



# 14. Create a project

1. Select **Create new** from the **Projects** menu in the menu bar.
  - a. Enter a **Project name**. It will be displayed in the data export file and the Analysis Report.



**NOTE:** No spaces are allowed in the Project name.

- b. Assign "Project Type" as Explore 1536 or Target 384
2. If there is an account for the Contact, go to step 3. If it is a new Contact, see the [11.1. Create contacts menu](#) to create a Contact and an Olink MyData Cloud account for the customer.
  3. Click the **Overview button** in the navigation menu on the project view.
    - a. In the Overview pane, click the **Edit** button next to **Project info** and enter information in all fields. Click **Save**.
    - b. Click the **Add/Edit** button next to **Project members** and add Lab users, Contacts, and business development managers to the project by clicking on the **+** next to a person's name in the **Add new members** list to add it to the **Current members** list. Use the *Search* box to filter the list for a specific user. The user's role will indicate what privileges they have once added to the project. When all project members are added, click **Done**. Click on the **Send** icon to the right of the project member's name to send an email informing the member they have been added to the project. To send the email, click **Confirm**. Click **Cancel** to cancel the email.



**NOTE:** BDMs must have their account configured by the System administrator. BDM's is a special role with no data access but can be included on the Analysis Report cover page.

- c. Internal and external comments can be added by clicking **Edit** next to **Comments**. Enter the comment and click **Save**.

Information from the **Overview pane** displayed in the Analysis Report:

- Sample Type
- Normalization Type
- External Comments
- Contacts
- Business Development Manager

Optional: **Internal Comments** will only be visible within Olink MyData Cloud.

## 14.1. Upload plate layout files

Before linking any data to the project, upload the plate layout files to the project in the **Runs** pane.

1. Create plate layout files:
  - a. Plate layout files must be uploaded in .csv format using semicolon as delimiter.
  - b. Upload one file per sample plate with a file name that corresponds to the plate ID.
  - c. Files must contain columns with the following format:

```
well_id;sample_id;sample_type
A1;S0426;SAMPLE
A2;S0447;SAMPLE
A3;S0500;SAMPLE
...
A12;Control_1;CONTROL
B12;Control_2;CONTROL
C12;Neg_1;NEGATIVE_CONTROL
D12;Neg_2; NEGATIVE_CONTROL
E12;Neg_3;NEGATIVE_CONTROL
F12;PC_1; PLATE_CONTROL
G12;PC_2; PLATE_CONTROL
H12;PC_3; PLATE_CONTROL
```

- Well IDs must be unique and all plate wells must be included in the CSV file.
- Sample IDs must be unique within the project, 1-50 characters long and the characters a-z, A-Z, 0-9 and the special characters - and \_ should be used. Do not use , ; or /n (new line).
- The CSV file must include at least one NEGATIVE\_CONTROL and at least two PLATE\_CONTROL.
- Acceptable and available samples types: SAMPLE, NOT\_USED, CONTROL, PLATE\_CONTROL, NEGATIVE\_CONTROL.
- For wells that are not used, sample\_id column should be empty, e.g. H11;NOT\_USED.



**NOTE:** No spaces are allowed in any field. Headers are case-sensitive.

2. Upload plate layout files using the following instruction:
  - a. Select **Runs** in the left menu in the **Project view**.
  - b. In the **Plate layout files** pane, click the **Upload a file** button to upload a CSV file of the plate layout. Drag and drop the file(s) to the indicated field to the left or click the field to browse and choose a file to upload.
  - c. Comment the uploaded file(s) using the *Comments* field to the right.
  - d. Click **Upload selected file**, then click **Close**. Only one plate layout file at a time can be uploaded.
3. Verify that the plate layout is correct by selecting the **Plate layout** pane from the left menu. To change the plate layout, select one or several wells and right-click to change the well type.
  - a. Make sure you are in the Edit sample type mode see section [9.3.1 Plate layout](#), select one or several wells and right-click to change the well type.
  - b. When you have made the changes, click **Save**.

## 14.2. Add panels

**Add panels from a run** Search ✕

Run name	Experiment name	Flowcell type	Flowcell side	#Lanes
211130_NB551274_359	LJ074-300 pwo TITRERING	High	A	1
211130_A01243_439	20211647_LJ126-009_NEUII_ONCII	SP	A	2

**Add/Edit project lanes** ✕

**Panel lot number:**

Cardiometabolic	Inflammation	Neurology	Oncology
B04413	B04411	B04414	B04412
Cardiometabolic II	Inflammation II	Neurology II	Oncology II
B13805	B13803	B13806	B13804

**Lanes:**

Experiment name	Position	Index plate	Panel type	Lot nr	Plate layout	
20211647_LJ126-009_NEUII_ONCII	Lane 1	Sample 1-96	Neurology II	B13806	None	<span>▼</span> <span>Add</span>
20211647_LJ126-009_NEUII_ONCII	Lane 2	Sample 1-96	Oncology II	B13804	None	<span>▼</span> <span>Add</span>

Run name	Experiment name	Flowcell type	Flowcell side	#Lanes
211130_A01157_415	20211647_LJ126-009_CARII_INFII	SP	A	2

All the run files are added and linked to the appropriate plate layout file in the next step.

1. Select **Runs** in the left menu. In the **Panels** pane, click **Add**.
2. Find the appropriate item in the runs list in the **Add panels pane** by scrolling or using the **Search** feature on the top right and select the run to be added by clicking on that row.



**NOTE:** Each run represents results from one flow cell. SP1.5 flow cells have two lanes where one sample plate can be analyzed in each lane, while S4 flow cells have four lanes where four sample plates can be analyzed in each lane. One panel type can be analyzed in each SP1.5 or S4 lane.

3. Select the panel lot numbers for the components used in the run. The panel lot number must be the same for all runs of each panel run in the project.
4. Add a lane to the project by assigning the correct panel and plate layout in the drop-down menus and verify that the correct panel lot number is displayed. The correct panel type will be pre-selected for all lanes where the pre-processing software has successfully detected the panel type.
5. Click **Add**.
6. Repeat from step 2 until all the runs for the project have been added. When all lanes have been added, close the runs list.
7. The added panels will appear in the **Runs > Panels** pane. By clicking the icons to each panel's right, the plate layout can be viewed, and panels can be removed. The first icon to the panel's right, a **cross**, a **question mark** or a **check mark**, indicates if the panel calculation has been successful or not. Click the icon to redo the calculation. When all panels have check marks, proceed to Quality Control. If the check mark does not appear for a panel, remove the panel and add it again. If this does not solve the problem, verify that the plate layout is correct.



**NOTE:** Each flow cell run will need to be added one at a time. Each Plate Layout will be used four times (once per panel type) for an Explore 1536 study.

# 15. Perform quality controls

Select **Quality control** in the left menu, review the overall QC for each block per panel and plate separately. Perform quality assessment for all plates using the views described in [15.1. Additional quality assessment](#). Then make an overall assessment. For acceptance criteria for passing QC, refer to [Part 4: Quality Control](#) and summarize in the table below.

Criteria	Recommended value
Sample QC	
Average counts per sample	> 500 counts
Run QC	
MAD of Plate Control	$\leq 1.5$ NPX
Median of Z-score Plate Control	$\leq 5, \geq -5$
Median of Z-score Negative Control	$\leq 3$
Number of samples with warning flags	$\leq 1/6$ of total number of samples on plate (15/88 samples on a full plate)
Assay QC	
Median of the triplicates of the Negative Control	< 5 SD from the predefined values set for each assay.



**NOTE:** If a run fails to meet the acceptance criteria, refer to [21. Troubleshooting](#) or contact [support@olink.com](mailto:support@olink.com).

When a rerun is needed, **Remove** the original lane from the project:

1. Select the **Runs** pane.
2. Click the delete icon to the right of the lane.
3. Add the rerun lane using the same plate layout (if used) or upload a new plate layout for the rerun.



## 15.1. Additional quality assessment

Click the following panes in the left menu to assess the quality of the data in each pane and further determine if any reruns are required (Panels are further depicted in [9.4. Quality control](#)):

1. **Sample QC:** View deviation from the median in all samples per plate and panel. Each block is displayed. Change the plate and panel using the drop-down menus and assess samples deviating  $> 0.3$  NPX from the median.
2. **Sample QC overview:** View and evaluate patterns of flagged samples, hovering over each flagged sample to view the criteria that have resulted in a warning.
3. **Control strip:** View the z-score deviation for selected Plate Controls and Negative Controls for each assay.
4. **Plate data:** View the values of internal controls and assays (change assay in the drop-down menu above the plate layout) for each sample plate and panel. Each block is displayed. Assess if there are patterns in the plate (i.e. row or column) representing a technical error. Change the plate and panel using the drop-down menus at the top of the page.
5. **Plate variation:** Display differences in sample distributions within and between plates.



**NOTE:** For intensity normalized projects, the assessment of this view should be performed using the *Intensity* option for Normalization type after normalization has been performed, refer to [15.3. Normalize the project](#).

6. **Limit of detection:** Evaluate detectability for each plate, panel, and block. Assays with a high limit of detection are listed below the graph. Assess if there are deviations between sample plates within the same block.
7. **Correlation assays:** Assess the correlations of each overlapping assay between panels.
8. **Heat map:** Search for extreme outlier samples.
9. **Sample heat map:** Search for outlier external control samples or plates.
10. **Counts:** View boxplots of counts distribution per assay, block, panel, and plate. Each plot shows assays for one block sorted by average counts from low to high.



**NOTE:** All assays for each Olink panel will be visible in NPX and extended NPX file. Assays that do not pass QC will not be included in the calculations. Please reach out to your contact person at Olink for further information.

## 15.2. Optional data handling steps

If a sample is identified as an extreme outlier or a missing sample, then the sample can be removed from the analysis by manually remove the sample. Change the sample to **Not used** in the **Plate layout pane** (refer to [9.3.1 Plate layout](#)) and then perform the data QC again. A not used sample will not be included in the export, will be marked with Not used sample in the QC\_warning column and the NPX column will have empty cells.

## 15.3. Normalize the project

When all panels have been added to the project and quality controlled:

1. In the **Runs** pane: click the **Normalize project** button.
2. Click **Confirm** in the popup window to normalize, click **Cancel** to cancel. This procedure may take a few moments to process.

**Result:** NPX values are calculated for all added lanes.



**NOTE:** Plate Control normalization should be used for single plate or non-randomized studies. For randomized studies of more than one plate, intensity normalization should be used. The normalization method can be viewed in the project **Overview** pane (refer to section 14. [Create a project](#)) The normalization method can, if required, be changed by clicking the **Edit** icon to the right of **Project info** and selecting the correct method from the drop-down menu for Normalization type. If the normalization method is changed, the normalization step will need to be performed again. For more information, please contact [support@olink.com](mailto:support@olink.com).



**NOTE:** If additional runs are added later, this step will need to be performed again. Click **Runs** in the left menu to redo the normalization, then click the **Clear Normalization** button below **Normalization**. Add any additional runs and, if required, change **Normalization type** in the **Overview** pane and click **Normalize project**.

# 16. Generate Analysis Report and NPX file

After data has undergone Quality Control and been normalized, the NPX data files (.csv format) are automatically generated, and a Analysis Report is generated in the **Overview** pane.

The Analysis Report gives an overview of the Quality Control of the project and includes the following headlines:

- Project information
- Quality Control (including QC summary, intra-and inter-assay coefficient of variance %CV)
- Protein detection results

To generate Analysis Report:

1. In the **Analysis Report pane**, click **Create Preview**. Make sure that the preview contains the following:
  - Complete and correct project information
  - Correct number of plates and samples
  - Acceptable values for intra and inter-plate %CV
  - Acceptable detectability per panel based on sample type
2. Under **NPX file**, click the file to download and review the file.
3. When the inspection is done, click **Approve** to the right of each file generated to create a file available for download in the **Files** pane. The NPX-file is exported as a semi colon-delimited .csv-file. The content is described in .
4. Optional: If the project requires additional QC parameters to be exported, an Extended NPX file can be generated. In the **Runs > Normalization** pane, click the **Generate extended NPX** button. The generated Extended NPX file will appear on the **Overview > NPX file** pane. Click the file to download and review the file. When the inspection is done, click **Approve** to the right of the file to make it available for download in the **Documents and files** pane. The NPX-file is exported as a semi colon-delimited .csv-file. It contains the same information as the extended NPX file and in addition, a column for Well-Id and data for Incubation- and Amplification Controls.

Specification for column content in the NPX extended file.

Column	Description	Type
SampleID	The annotated sample ID	String
Sample_Type	Type of sample	String PLATE_CONTROL, NEGATIVE_CONTROL, CONTROL, SAMPLE
Index	Well index in 96 plate	Integer 1-96
OlinkID	OlinkID for assay	String
UniProt	UniProt ID for assay	String
Assay	Gene name for assay	String
MissingFreq.	Frequency of missing data (below LOD or NaN)	Float
Panel	Panel name assay belongs to	String
Panel_Lot_Nr	Lot number for the panel	Integer
PlateID	Name of the plate the sample was run on	String
WellID	Id for well	String Capital letter A-H followed by number 1-12
QC_Warning	Indicates whether the sample passed QC or not	String PASS, WARN, MANUAL_WARN
LOD	LOD value for assay	Float
NPX	NPX value	Float
Normalization	Type of normalization used in project	String Plate control or Intensity
Assay_Warning	Indicates whether the assay passed QC or not	String PASS or WARN
Intra CV	Intra CV for sample	Float
Inter CV	Inter CV for sample	Float
Processing_StartDate	Start date for processing	Timestamp
Processing_EndDate	End date for processing	Timestamp
AnalyzerID	The analyzer ID	String
ExploreVersion	Software version of the module in Olink NPX Explore used for panel calculations and normalization	String

Specification for column content in the NPX file.

Column	Description	Type
SampleID	The annotated sample ID	String
Sample type	Type of sample	CONTROL, SAMPLE
Index	Well index in 96 plate	Integer 1-96
OlinkID	OlinkID for assay	String
UniProt	UniProt ID for assay	String
Assay	Gene name for assay	String
MissingFreq.	Frequency of missing data (below LOD or NaN)	Float
Panel	Panel name assay belongs to	String
Panel_Lot_Nr	Lot number for the panel	Integer
PlateID	Name of the plate the sample was run on	String
QC_Warning	Indicates whether the sample passed QC or not	String PASS, WARN, MANUAL_WARN
LOD	LOD value for assay	Float
NPX	NPX value	Float
Normalization	Type of normalization used in project	String Plate control or Intensity
Assay_Warning	Indicates whether the assay passed QC or not	String PASS or WARN
ExploreVersion	Software version of the module in Olink NPX Explore used for panel calculations and normalization	String

## Part 4: Quality Control

# 17. Converting counts to NPX

The Explore 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.

### 17.1. NPX generation

The NPX values are calculated in two main steps, followed by an optional additional step between-plate-normalization. First, the assay counts of a sample are divided with the Extension Control for that sample and block. The resulting scale has increasing values with increasing concentration for each assay. A  $\log_2$  transformation is applied and the final step is subtracting the median of the Plate Controls. The optional additional step is intensity normalization which sets the median level of all assays to the same value for all plates. The different steps are described below.

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.

$$1. \text{ExtNPX}_{i,j} = \log_2(\text{counts}(\text{sample}_j \text{Assay}_i) / \text{counts}(\text{ExtCtrl}_j))$$

- Relate counts to known standard (Extension Control).
- For all assays and all samples, including Negative Controls, Plate Controls, and Sample Controls.
- $\log_2$  transformation gives more normally distributed data.

$$2. \text{NPX}_{i,j} = \text{ExtNPX}_{i,j} - \text{median}(\text{ExtNPX}(\text{Plate Controls}_i))$$

- Perform plate standardization.
- For all assays and per plate of samples.

$$3. \text{NPX\_Intnorm}_{i,j} = (\text{ExtNPX}_{i,j} - \text{plate median}(\text{ExtNPX}_i))$$

- Between plate normalization (Optional but default for multi plate projects).
- For each assay, for all plates in project.

### 17.2. LOD and CV calculation

LOD is defined as being three SDs above the median NPX of Negative Controls. The median is set using all samples annotated as Negative Controls per plate. A predefined SD is used (fixSD). Detectability is calculated per assay and plate and is defined by the percentage of samples above the LOD threshold. The overall detectability of the project is generated and reported in the Analysis Report.

The CV is calculated per assay (i) using the assumption of a log-normal distribution. The average CV is then calculated across panels and included in the Analysis Report output:

$$CV_i = 100 \sqrt{e^{S\ln_i^2} - 1}, \text{ where } S\ln_i = \ln(2) \times SD_i$$

# 18. Controls

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 in [17.1. NPX generation](#), and the other two are used in Quality Control (described below).

## 18.1. Sample QC

Each sample is given the status *pass* or *warning* in each block. For a complete Explore 1536 run, this means that each sample is evaluated 16 times. There are three sample QC criteria per block. Exceeding specifications in any one of them will result in a QC warning status for that sample. The three criteria are listed below. Data from samples with warning should be treated with caution.

1. Incubation Control deviation from the median may not exceed 0.3 NPX.
2. Amplification Control deviation from the median may not exceed 0.3 NPX.
3. Average counts for a sample may not fall below 500 counts.

For acceptance criteria for passing a sample, refer to .

## 18.2. Control strip QC

The Plate Controls have specific QC criteria for precision, measured as median absolute deviation (MAD) of the Z-score, and accuracy, measured as median of the Z-score. The Negative Control is only measured as median of the Z-score. The Z-scores are computed using predefined values set for each assay.

## 18.3. Assay QC warning

Assays where the median of the triplicates of the Negative Control deviate more than 5 SDs (predefined) from the predefined values set for each assay, will get a QC warning in the results NPX file. Data from these assays should be treated with caution.

For acceptance criteria for passing an assay, refer to .

## 18.4. Run QC

In addition to specific samples that exceed specifications, the entire block can be considered failed if it exceeds criteria 1 and 2 below. For practical reasons, this will most likely result in the rerun of a panel. If criteria 3 below is exceeded the panel is failed.

For acceptance criteria for passing a run, refer to .

# 19. Normalization methods

The two between-plate-normalization methods are called *Plate Control (PC) normalization* 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 [Randomization FAQ](#) on the Olink website.

For randomized studies with more than one plate, intensity normalization is the appropriate method to use. In this setting, the median for a random selection of samples is more stable across plates than the three Plate Control samples on each plate. For projects where complete randomization of samples cannot be guaranteed, or where there is only one plate, Plate Control normalization should be used.

## 19.1. Bimodal distribution

Plate Control normalization must be used for assays with bi-modal distribution. The following assays use bimodal distribution:

- TDGF1 (Teratocarcinoma-derived growth factor 1, Uniprot number: P13385)
- FOLR3 (Folate receptor gamma, Uniprot number: P41439)
- PNLIPRP2 (Pancreatic lipase-related protein 2, Uniprot number: P54317)



## 20. Pre-processing software

Before analysis in Olink MyData Cloud is possible, some pre-processing steps need to be performed. For this purpose, three software applications are installed on a local server, monitoring the folder where the NGS runs are stored.

### 20.1.bcl2counts

The *bcl2counts* software generates count files for each lane in a NGS run. A run metadata file, including run information and InterOp data, is also created by running the `olink_interop_stats.py` script. The application is started by *mydata-listener* as described below. The application will output messages and errors to `/var/log/olink/bcl2counts.log`.

The application can be run manually by the command:

```
bcl2counts <NGS_RUN_FOLDER>
```

For troubleshooting, it is possible to get more debug logs by passing arguments to the command:

```
bcl2counts -vv --stdout <NGS_RUN_FOLDER>
```

This command will output more logs to standard output.

Use the `--help` option to get a list of options for *bcl2counts* (note two dash signs):

```
bcl2counts --help
```

### 20.2.mydata-listener

The *mydata-listener* application scans a folder for new runs. When a new run has been found, the application triggers *bcl2counts* to generate counts files for all lanes together with a run metadata file. When done, it will trigger *mydata-file-sync* to upload the counts and run metadata files to the Olink MyData Cloud service. After processing the NGS run, the software outputs a file called *mydata-listener.done*.

The application logs output and errors to `/var/log/olink/mydata-listener.log`. The application is started via a timer polling the monitored folder every 5 minutes.

### 20.3.mydata-file-sync

The *mydata-file-sync* software uploads count files and run metadata to the cloud service, and registers the run there. The application logs output and errors to `/var/log/olink/mydata-file-sync.log`. The application is started by the *mydata-listener* service as described above. The application can also be run manually by the command:

```
mydata-file-sync <NGS_RUN_FOLDER>
```

For troubleshooting, it is possible to get more debug logs by passing arguments to the command:

```
mydata-file-sync -vv --stdout <NGS_RUN_FOLDER>
```

This command will output more logs to standard output.

### 20.4.mydata-listener.timer and mydata-listener.service

The *mydata-listener.timer* is a system service that is configured when installing the pre-processing applications with the *install.sh* script. The *mydata-listener.timer* runs the *mydata-listener.service* every five minutes to scan for new NGS runs. The *mydata-listener* is started or stopped using the *systemctl* command:

```
sudo systemctl start|stop|status mydata-listener.timer
```

The configuration files for the timer and service are found in the `/etc/systemd/system/mydata-listener.timer` and `/etc/systemd/system/mydata-listener.service` folders.

# 21. Troubleshooting

This chapter includes a trouble shooting guide, describing possible root causes and recommended actions for obtained non-expected or erroneous results. Reruns are not recommended due to failed individual samples, but only if a project has failed to pass formal QC.

The chapter describes issues that may occur using the Olink MyData Cloud software. The issues are described as well as the UI panes where they occur. The panes are described in detail in section [9.1. Overview](#), in this manual. The table columns *Possible causes* and *Actions* can, for example, refer to instruments and steps in the laboratory work (described in detail in the applicable Olink Explore User Manual).

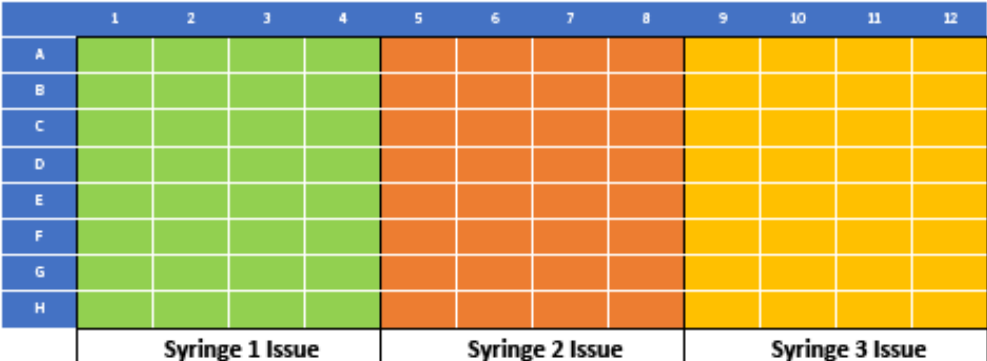

## 21.1. Low counts / Low NPX values


No	Issue	Possible cause	Recommended action
1	For one sample, only Internal Controls have counts above background in all blocks for all panels. Instead, the sample is like the Negative Control with very low NPX levels, visible in the <i>Heat map</i> or in the <i>Plate data</i> pane.	<ol style="list-style-type: none"> <li>1. The original sample was diluted.</li> <li>2. The sample was missing from the sample plate.</li> <li>3. The sample was present in the sample plate but was not transferred to the sample source plate.</li> </ol>	Set manual warning for the sample in all panels.
2	Same as issue no 1 but affecting only diluted blocks for one sample. The undiluted blocks are unaffected.	The sample was present in the Sample source plate (containing undiluted samples) but was not transferred to the Sample dilution plate (containing serial sample dilutions). A Mosquito® tip may have caused the fail in a position when pipetting the dilution plate.	Set manual warning for the sample in all panels.
3	Same as issue no 2 but affecting diluted blocks for all samples.	Sample dilution protocol on the Mosquito® was not performed. The samples are present in the Sample source plate (containing undiluted samples) but not in the Sample dilution plate (containing serial sample dilutions).	Make sure to dilute the samples before a possible rerun.
4	In the <i>Quality control</i> pane, all samples have warnings. Plate Control and Negative Controls, Amplification Control and Incubation Control criteria fail for all blocks and panels. In the <i>Plate data</i> pane, there are very low NPX levels or NaN for all samples and controls.	No counts or very few counts for both samples and controls when sequencing. If the Library QC analysis showed a correct Library peak at 150bp, it could have been caused by an over-dilution of the library (wrong volumes pipetted), or the library was not added at all in the dilution step. If there are few or no counts/reads, this should also be visible when reviewing the quality metrics in Illumina's Sequencing Analysis Viewer.	Repeat the Library preparation before sequencing, starting with the purified Olink Library that passed in QC. A possible rerun of the sequencing.

No	Issue	Possible cause	Recommended action
5	In the <i>Quality control</i> pane all samples in a block have a warning and a failed Plate Control for that block (Negative Control might also fail). The sample warning is caused by a failed counts criterion. It is visible in the <i>Sample QC overview</i> pane when hovering over cells with a warning. High variation for Amplification Control can be observed in the <i>Sample QC</i> pane. In the <i>Plate data</i> pane, all samples will have low NPX values or NaN for the block controls and assays.	Reverse probes for a block were not added when the incubation mixes were prepared. Then there are no counts for the block (both assays and controls).	This issue can be discovered during the laboratory steps and corrected: The probe incubation mix contains 90 µL instead of 100 µL, and after transferring the mix to the reagent source plate, there is only 10 µL left instead of 20 µL.
6	In the <i>Quality control</i> pane there is a failed Plate Control criterion in a block. In the <i>Plate data</i> pane very low NPX values will be seen for all assays and Incubation Control.	Forward probes for a block were not added when the incubation mixes were prepared. This means that the block has counts only for the Extension and Amplification Controls.	This issue can be discovered during the laboratory steps and corrected: The probe incubation mix contains 90 µL instead of 100 µL, and after transferring the mix to the reagent source plate, there is only 10 µL left instead of 20 µL.
7	In the <i>Quality control</i> pane, a sample has a warning for one or more blocks. The sample warning is caused by a failed counts criterion. It is visible in the <i>Sample QC overview</i> pane, when hovering over cells with a warning. Incubation Control, Amplification Control, and all assays will show low levels or NaN in these samples in the <i>Heat map</i> pane and the <i>Plate data</i> pane.	An epMotion® tip failure during the PCR1 pooling, implying there will be no counts for one or several blocks in a sample.	Contact <a href="mailto:support@olink.com">support@olink.com</a> for support
8	In the <i>Quality control</i> pane, a failed counts criterion is indicated as a warning for a sample for all blocks in one panel. In the <i>Sample QC</i> pane, Amplification and Incubation Controls are out of specifications for a sample for all blocks in one panel. The QC failures will also be seen in the <i>Sample QC overview</i> pane. These samples will have NaN for all assays and controls in the <i>Plate data</i> pane and <i>Heat map</i> pane, and several samples can be affected.	An epMotion® tip failure in 10µl tool during the PCR2 setup. The addition of PCR1 pool for one or more samples fails, which causes that no Library is created for one or more samples, resulting in no counts for either the sample or the controls. This can be compared to the case with missing samples in the sample plate, when there are no counts for the controls.	Contact <a href="mailto:support@olink.com">support@olink.com</a> for support

No	Issue	Possible cause	Recommended action
9	In the <i>Quality control</i> pane, there is a failed counts criterion for all blocks in a panel in a sample/index, indicated as a warning for a sample. This issue can also be seen in the <i>Sample QC overview</i> pane, indicating counts criteria as the cause for the warning. In the <i>Plate data</i> and <i>Heat map</i> panes, Incubation and Amplification Controls, and all assays, will show low levels or NaN in these sample(s) with high variation compared to the rest of the plate. Potentially in several panels for the same index. If several samples are affected, then row or column effects could be seen on the <i>Plate data</i> pane.	<ol style="list-style-type: none"> <li>1. An epMotion® tip failure in 10 µl tool during PCR2 set up during Index primer adding to a single sample. This issue can affect several samples. There was no or too low volume index primer added in the PCR2 reaction, affecting one or several samples.</li> <li>2. Forgot to centrifuge index plate.</li> <li>3. One or several wells of the index plate were empty.</li> </ol>	<ul style="list-style-type: none"> <li>• Contact <a href="mailto:support@olink.com">support@olink.com</a> for guidance.</li> <li>• Make sure to centrifuge the Index Plate before starting the EpMotion® protocol.</li> <li>• Ensure liquid in every well of the Index Plate before starting the EpMotion® protocol (20 µL). If a well is empty or contains a lower volume, contact Olink for a free charge replacement Index Plate.</li> </ul>
10	In the <i>Quality control</i> pane, a counts criterion is failed for one or more (up to 12 in a row) sample(s) for all blocks in one panel, indicated as a warning for these samples. If Plate Control is affected, this can be detected in the <i>Control strip</i> pane QC. If several samples are affected, the <i>Plate data</i> pane will show systematic effects for Amplification Control, Incubation Control, and other assays with very low levels or NaN. There are no reads for one or up to 12 samples in a row in a panel. If several samples are affected, row effects can be seen in the <i>Plate data</i> pane.	An epMotion® one tip failure in 10 µl tool during PCR2 pooling, affecting one to 12 samples in a row in a panel. The samples will be missing from the library pool, and there will be no counts generated.	This issue can be avoided by checking the volume after PCR2 pooling in the PCR2 Pooling plate (should be 36 µL).
11	In the <i>Quality control</i> pane, a failed counts criterion is indicated as a warning for several samples in a row. In <i>Sample QC</i> pane, Incubation and Amplification Controls are failed for a block (one panel) in several sample in a row. Up to 12 samples in a row or several rows have failed criteria. The <i>Plate data</i> pane show systematic row effects for Amplification Control, Incubation Control, and other assays with very low values or NaN. Rows of samples will have a warning for Amplification Control, Incubation Control, and counts criteria in the <i>Sample QC overview</i> pane. Row effects can be seen in the <i>Plate data</i> pane.	One or several Mosquito® tip(s) fails in a position when dispensing incubation mix, resulting in a missing block for up to 12 samples in a row of samples (12 samples). One or more rows of samples can be affected and lacks both assays and controls for a block.	This issue can be avoided during the Mosquito® protocol: make sure to verify that there is a volume in all incubation plate wells after adding the Incubation mix (0.6 mL).

## 21.2. Inconsistent results

No	Issue	Possible cause	Action
1	<p>Systematic column effects in every 4 columns in all blocks, column 1-4, column 5-8 and column 9-12. The effects are visible on the Plate data pane and vary in NPX values between columns compared to the rest of the plate for all assays and Incubation Control. Refer to schematic figures below.</p>  <p>Figure 1. Pattern that would be observed in Olink MyData Cloud if there is an issue with one syringe.</p> <p> <b>NOTE:</b> Any Syringe Issue will be visible on all blocks with same pattern.</p>	<p>During PCR1 setup, the Dragonfly will dispense PCR1 Mix using three different syringes. If the dispensation from one of the syringes fails (lower volume), this could affect the results column-wise, as shown by the figures below.</p>	<p>Make sure the Dragonfly is calibrated correctly and functional before possible rerun. After the Dragonfly protocol, always inspect the PCR1 plate. Every well should contain the same amount of liquid (19.8 <math>\mu</math>L).</p>
2	<p>In the <i>Plate data</i> pane, all assays show a pattern with lower or higher values than the samples in the rest of the plate. A systematic effect is repeated on the left and right sides of the plate. Refer to the schematic figure 3 below.</p>	<p>The samples in some wells are less diluted than intended. This issue is likely to be caused by uneven vortexing of the sample dilution plate between dilutions. As a result, the assays will be higher, and controls will be lower than expected in affected samples. This causes systematic effects that are repeated on the left and right side of the plate.</p>	<p>This sample plate needs to be rerun for affected panels. Make sure to vortex the sample dilution plate thoroughly between dilutions during the Mosquito® protocol.</p>

No	Issue	Possible cause	Action																																																																																																																														
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	<p>Figure 2. Example pattern that would be observed in all blocks of same dilution MyData if vortexing was skipped or insufficient.</p> <p> <b>NOTE:</b> If the issue is in 1:10 you would also see a pattern at further dilutions, but it would fade with each dilution if vortexed.</p>																																																																																																																																
3	Systematic effects between plates are visible in the <i>Plate variations</i> pane.	The samples were not randomized between plates. Instead, different sample groups were systematically placed on different plates, causing plates to deviate from each other.	Change from Intensity normalization to Plate Control normalization.																																																																																																																														
4	Systematic effects between rows and columns are visible in the <i>Plate data</i> pane.	The samples were not randomized on the plate. Instead, different sample groups were systematically placed in different wells, causing rows/columns to deviate from each other.	Contact <a href="mailto:support@olink.com">support@olink.com</a> .																																																																																																																														

## 21.3. Deviating External Controls

No	Issue	Possible cause	Action
1	In the <i>Quality control</i> pane, plate control criteria fail on all blocks in all panels. Systematic effects in Plate Control normalized data in the <i>Plate variations</i> pane if several plates are used.	The External Controls/Control strip was not added or added at the wrong positions (Plate Control and Negative Control switched places). This issue will cause incorrect normalization and calculation of NPX.	If the Plate Control was not added, a rerun is necessary. If the Plate Control and Negative Control switched places, this issue could be corrected in the Plate layout.

No	Issue	Possible cause	Action
2	In the <i>Quality control</i> pane, Plate Control criteria fail on diluted blocks. In the <i>Controls strip</i> pane, the Plate Control will exceed the upper limit while the Negative Control's confidence interval will be OK. Undiluted blocks are unaffected.	The sample dilution plate was not vortexed between dilutions. This issue will result in samples less diluted than intended: The Mosquito® will add a sample on top of the sample diluent in each well and take a sample from the bottom of each well during the protocol. Thus, the assays will be higher, and the controls will be lower than expected for all samples in the plate.	This sample plate needs to be rerun for all panels. Make sure to vortex the sample dilution plate thoroughly between dilutions during the Mosquito protocol.

## 22. Revision history

Version	Software version	Date	Description
5.5	23.10.19.01	2023-10-19	<a href="#">17.1</a> added. <a href="#">4.1.1</a> , equation 3 updated. Editorial changes
5.4	23.01.18.01	2023-01-21	<a href="#">16</a> , all tables updated. <a href="#">18.4</a> updated.
5.3	22.11.29.01	2022-11-29	<a href="#">Figure 19</a> updated. <a href="#">15.1</a> Note added. <a href="#">18</a> equation 3 updated.
5.2	22.09.29.01	2022-09-29	<a href="#">5.1.1</a> updated.
5.1	22.08.15.01	2022-08-16	<a href="#">Figure 14</a> , <a href="#">Figure 24</a> , <a href="#">Figure 25</a> , <a href="#">Figure 27</a> , <a href="#">Figure 28</a> and <a href="#">Figure 29</a> updated.
5.0	22.05.23.01	2022-05-23	Changed name of Certificate of Analysis to Analysis Report. <a href="#">9.3</a> and <a href="#">Figure 14</a> updated. <a href="#">9.3.3 Run log</a> added. <a href="#">10.1. Run log menu</a> added. <a href="#">22</a> software version column added. <a href="#">Figure 9</a> , <a href="#">Figure 12</a> , <a href="#">Figure 20</a> and <a href="#">Figure 42</a> updated.
4.1.1		2022-03-16	<a href="#">Table 3</a> updated QC criteria.
4.1		2022-03-14	MAD negative control removed.
4.0		2022-01-31	<a href="#">10.1.1</a> added. <a href="#">9.4.3</a> and <a href="#">18.2</a> information about z-score updated. <i>Inconsistent results</i> changed information in Issue No 1.
3.0		2021-12-13	<a href="#">9.3.2 Manual QC warning</a> added. <a href="#">9.4.9 Control sample heat map</a> added. Failure function removed. Not used function added.
2.0		2021-08-31	Adopted information for data processing when running additional four new Explore panels, Cardiometabolic II, Inflammation II, Neurology II and Oncology II. Editorial changes.
1.2		2021-05-07	<a href="#">1.6.2</a> Updated software requirements. <a href="#">16</a> Table 3 and 4 updated with Normalization. <a href="#">15</a> Updated QC criteria for samples. <a href="#">18.4</a> Updated QC criteria for samples. <a href="#">22</a> Revision history added.

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