Technical Information

NPX™ Signature Software version 1.x vs 2.0

User Interface

The UI is harmonized with NPX™ Map (successor of NPX™ Explore software).
The new UI is more intuitive and user friendly.

Projects

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Terminology change	• Study • Flagged • Table view • Assay QC view • Z-Score • Sample Type • Sample Type • Plate Name	• Project • Warned • Workspace view • Plate Data view • Plate Variation • Sample Matrix (urine, plasma, etc) • Sample Type (Sample, External Control) • Run Name
File Format	• Studies saved as single .npx files.	• Projects are stored as folders including a project file with the extension .osp (Olink® Signature Project).
Compatibility	• .npx study files can be opened. • .oaf (Olink® NPX Manager project file) can be opened in NPX™ Signature v1.x and saved as a .npx file. • .osp project files created in NPX™ Signature v2.0 cannot be opened.	• .osp project files can be opened • Studies created in NPX Signature 1.x, can be migrated in NPX Signature v2.0 ensuring a smooth transition. During migration a copy will be created and saved as an .osp project file. The original .npx or .oaf file is unchanged.
Notes	The user can enter internal notes on project and plate level.	The user can enter internal notes on project and plate level. Plate Notes can be entered in Plate Details view only.

Panel Data File and Archive

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Olink® Target Panel Data Archive concept	All Olink® Target 48 and Olink® Target 96 panel data files are integrated into the software. In order to analyze data from a newly released kit reagent lot, a new software version must be installed. The Olink® Target Panel Data Archive cannot be imported in NPX Signature v1.x.	Olink® Target Panel data files are not integrated in the software. An Olink® Target Panel Data Archive must be imported in NPX™ Signature 2.0 before any Olink® Target 48 or Olink® Target 96 data can be analyzed. In order to analyze data from a newly released kit reagent lot, an updated Olink® Target Panel Data Archive needs to be imported into the software. This archive will encompass all Panel Data Files for both Olink® Target 48 and Olink® Target 96. The **Panel Data Archive** is an encrypted **.dat file**. It is compatible exclusively with NPX™ Signature v2.0 and cannot be used with NPX™ Signature v1.x.
Panel Data Files for Olink® Flex and Olink® Focus	For Olink® Flex and Olink® Focus panels, the format of the Panel Data File must be format **NPXSv1**.	For Olink® Flex and Olink® Focus panels, the Panel Data File will have the suffix **_NPXSv2**.
Reagent Lot releases	In order to analyze data from a newly released reagent lot, a new software version must be installed.	During a reagent lot release for Olink® Target 96 or Olink® Target 48, a new Panel Data Archive file will be issued. This file will include **all previous panel data files** along with the newly added panel data file.

Sample Manifest

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Template	A generic template is available via the Plate Manifests page.	A product-specific template is available in the main application under **Project -> Sample Manifest**. Product-specific templates are also available from the **Project Creation** Workflow.
Requirement	The Sample Manifest is optional, and is not needed when run data is annotated with SampleID. The Sample Type is automatically inferred by the software based on SampleID. Incorrect automatic annotation must be manually corrected in the Plate Layout view. The Sample type can be assigned in the Plate Layout view manually.	The Sample Manifest is mandatory for all plates and must include Sample Type annotation per well. The Sample Types can only be set via the Sample Manifest. To change Sample Types, the Sample Manifest can be replaced via Plate Details view. There is no need to re-import the run data.
Sample Type	The Sample Manifest did not include Sample Type. Sample Type is based on the Sample ID. The Sample Type can be changed in the user interface.	The Sample Manifest now includes a new required column: Sample Type.
Sample ID	No restrictions when importing the manifest. Character "." (period) resulted in errors in the user interface.	Maximum 100 characters, allowed characters are: • A-Z • a-z • 0-9 • . • _ • - • # • space
Plate ID	The Plate ID which is included in the data export file is either the run name in the raw data file or set via the user interface.	The **Plate ID** which is included in the data export file can only be set in the Sample Manifest.
Duplicates	Duplicates are allowed.	All **sample IDs** within a single Sample Manifest must be **unique**. Sample IDs can be changed by replacing the Sample Manifest for the run.
Anonymization	It is possible to anonymize a study.	It is possible to create an anonymized copy of a project.

Run file

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Run Data location	Data can be imported directly from an Olink® Signature Q100 instrument with enabled remote connectivity.	Data cannot be imported directly from an Olink® Signature Q100 instrument.

QC and Normalization

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Manual QC	Possible Manual QC: – Manual sample fail – Manual assay fail – Manual datapoint fail	Possible Manual QC: – Manual sample fail – Manual assay fail – Manual datapoint fail Effects of manual QC emulates automatic QC: e.g. manual sample fail is equivalent to automatic sample fail. During migration, if all datapoints in an assay were individually manually failed in NPX Signature v1.x, NPX Signature v2.0 will treat the assay as manually failed.
Pre-Normalization adjustments	• Background cap applied to all sample types in Olink® Target 48, Olink® Flex, and Olink® Focus, meaning that all measurements undergo a background correction to limit non-specific, irrelevant signals that may interfere with the accurate measurement of the intended target. In customer samples this might artificially mask actual variation with the risk of false positive associations. • Background cap is not applied for Olink® Target 96. • Detect line-in-chip: >5 assays with no detectable signal (**used for all Sample Types including Negative Controls**). All data for sample is considered failed.	• Background cap applied only to Negative controls in Olink® Target 48, Olink® Flex and Olink® Focus. Customer samples with very low signal (Ct >24) will have different NPX and Quantified value in NPX Signature v1.x and v2.0, due to the actual Ct value not being replaced with Ct 24. • No change for Olink® Target 96 (Background cap is still not applied for Olink® Target 96, due to different chemistry). • Detect line-in-chip: >5 datapoints within an assay that are manually failed or with no detectable signal (**not used in Negative Control**). All data for sample is considered failed. No normalization possible, Sample QC set to Fail.
Normalization	• Ct values are rounded to 5 decimals prior to normalization.	• No rounding of Ct-values. Using full precision from input data can lead to small numeric differences. • Calibrator / IPC Normalization calculation stays as before.
Olink® Target 96 Intensity Normalization	• Intensity normalization is done differently compared to Olink® Explore 3072, Olink® Explore HT, and Olink® Reveal. • Intensity normalized NPX changes when plates are added or removed from the project. • Median NPX across all plates is used as the reference value during Intensity normalization.	• Intensity normalization is done in the same way as for Olink® Target 96 as for Olink® Explore 3072, Olink® Explore HT, and Olink® Reveal. • Intensity normalized NPX is unchanged when plates are added or removed from the project. • 0 (zero) is used as the reference value during Intensity normalization. • Intensity normalized NPX values are different compared to NPX Signature v1.x, however, there will be no changes to statistical results.
Data	NPX data above ULOQ and below LLOQ will be provided and included in the data export.	NPX and Absolute quantification (pg/ml) data above ULOQ and below LLOQ will be provided and included in the data export with the information that the data is above ULOQ / below LLOQ in extra columns.

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Sample QC	• In Olink® Target 96 all samples are QC:ed relative to internal control assay medians calculated based on all sample types. This can cause false QC failure for external controls when the regular samples are from a different sample matrix such as CSF or urine. • In Olink® Target 96 Incubation Control 2 is used. • Olink® Focus calculates separate plate medians for customer samples and sample controls vs. negative controls and calibrators.	• In Olink® Target 96 external controls and regular samples are QC:ed relative to internal control assay medians calculated separately for the two groups. This removes the risk for false QC failure for external controls when the regular samples are from a different sample matrix such as CSF or urine. • Incubation Control 1 is used in Olink® Target 96 which is the same antigen as the only Incubation Control in other products. Olink® Focus calculates separate plate medians for customer samples and external controls, where external controls are sample controls, negative controls and calibrators.
Assay QC	Assay QC warning criteria: • Sample Control precision (%CV), Calibrator/IPC precision (%CV): – is calculated based on absolute quantification. (Exception: Sample Control precision not used for QC for Olink® Target 96). • Sample Control Accuracy (for panels with absolute quantification only): – Absolute quantification data (pg/ml) including above/below LOQ used. Mean as point estimate, which may be sensitive to extreme outlier values. – Less than two sample controls above LQL and below ULOQ. Less than two calibrators above LQL and below ULOQ. Assay QC fail criteria: • Minimum 2 Sample Control replicates with Sample QC “Pass” and no instrument warning. • Inter-Plate Control replicated. No Negative Control ctrl in QC for 1.x. For Olink® Target 96: no automatic Assay QC checks	Assay QC warning criteria: • Sample Control precision (%CV), Calibrator/IPC precision (%CV): – is calculated based on NPX. (Exception: Sample Control precision not used for QC for Olink® Target 96). • Sample Control Accuracy (for panels with absolute quantification only): – Absolute quantification data (pg/ml) including above/below LOQ used. Median as point estimate, which is less sensitive to outliers. Assay QC fail criteria: • Minimum 2 valid (Sample QC “Pass” and no instrument warning) Calibrator / IPC replicates. • Minimum 2 Sample Control replicates with Sample QC “Pass” and no instrument warning. • Minimum 2 Negative Control replicates with Sample QC “Pass”. For failed assays only Ct values will be provided. For Olink® Target 96: New Assay QC check based on precision (%CV) of Interplate Controls.

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Run QC	Run QC warning criteria: • Information is displayed under Panel Metrics • Variation in internal controls: Standard deviation uses NPX values and is calculated for customer samples and external controls, is calculated for the same sample groups as Assay QC. The standard deviation is sensitive to extreme outliers. • Definition of the thresholds for the number of non-passing samples varies between panels. • Above 5 (Olink® Target 48)/ 3 (Olink® Flex) assays with too few sample control or calibrator replicates within LOQ. • Minimum 1 Sample Control replicates (Only used in products that are sold with Sample Controls, not used for Olink® Target 96) with Sample QC "Pass". • Minimum 1 Negative Control replicates with Sample QC "Pass", else error with import. • No minimum defined for Calibrator / IPC replicates with Sample QC "Pass" for Olink® Target 48 and Olink® Target 96. • Minimum 2 Calibrator replicates (for each Calibrator type) with Sample QC "Pass" for Olink Focus.	Run QC warning criteria: • Information is displayed on new view Project Metrics. • Variation in internal controls: Median absolute deviation uses NPX values and is calculated for separate groups; customer samples vs. external controls. The median absolute deviation is less sensitive to outliers than standard deviation. • Definition of the thresholds for the number of non-passing samples varies between panels. Number of warned or failed customer samples is more than 1/6. • Number of warned or failed assays (not INC, EXT, AMP) above 1/9. • Minimum 2 Sample Control replicates (Only used in products that are sold with Sample Controls, not used for T96) with Sample QC "Pass" • Minimum 2 Negative Control replicates with Sample QC "Pass". • Minimum 2 Calibrator / IPC replicates with Sample QC "Pass".
Project Metrics	• %CV (Inter) displayed under Panel Metrics is calculated as the total variability of all Sample Controls on all plates. This is not according to common practice in the scientific community. • Range of internal control Ct values is not presented.	• Inter-plate CV displayed on Project Metrics is calculated as the variability across plates of the typical Sample Control value. This is according to common practice in the scientific community. • Range of internal control Ct values – Only data from customer samples is considered.
Bimodal assays	Olink® Target 96 assays TDGF1 in panel Neuro Exploratory are handled as bimodal, meaning that IPC normalization is applied even in studies where Intensity normalization has been selected on a study level.	No assay is set as bimodal by default. It is recommended to define in the Plate Variation view if an assay has a bimodal profile. In this case the extended data export will provide both the Intensity normalized (NPX) and IPC normalized NPX values in one file.

Data output

Summary	NPX™ Signature v 1.x	NPX™ Signature v2.0
Export file	Data Export file format: – .xlsx – .csv Layout: – Wide format – Long format Columns: – A subset of the NPXS v2.x columns. A new export file/ three separate files are required for exporting Ct, NPX and Quantified values is required.	Data Export file format: – .csv Layout: – Long format Two variants of the data export file are available: standard and extended. Both variants are presented in a long format only (text based and semi-colon separated columns) and provide: – Ct data – NPX data – Quantified data (for absolute quantification panels) All numeric values are presented with five decimals. Please note that due to increased precision in intermediate calculations in the software, the NPX and Quantified values are expected to differ between NPX Signature v1.x and v2.0. This will not impact the biological relevance of the data. Additional columns are included: – The Sample Type is indicated in a separate column, allowing for easy separation between external controls and samples. – The Limit of Detection (LOD) is included in the data export file, and a separate column indicates whether each datapoint is above or below LOD.
Analysis Report	Customized company name & logo can be added via Settings -> Analysis Report Configuration.	The procedure for including a customized company name & logo in the Analysis Report has changed. For detailed instructions on how to implement the logo, please refer to the NPX™ Signature Software User Manual.

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