QuantideX® qPCR *ESR1* exoMutation Analysis Module

Version 1.0

Software User Guide

Research Use Only. Not for use in diagnostic procedures.

REF A01043

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Table of Contents

Overview 3

System Requirements/Prerequisites 3

Installation 4

Software Features 7

- Settings 7
- Batch Report 8
- Understanding Results 10
- Data Export 11

Uninstallation Instructions 11

Known Limitations 12

Quality Control Procedures 12

- Positive Control (CONP) 12
- Negative Control (CONN) 12
- Samples 13

Troubleshooting 14

Third Party Applications and Software Licenses 17

Disclaimers 18

Overview

The QuantideX® qPCR *ESR1* exoMutation Analysis Module (the Software) is used as a data analysis and visualization tool designed to facilitate the interpretation of qPCR instrument output files generated with the following Asuragen kitted products:

- REF A01031 QuantideX qPCR *ESR1* exoMutation Kit - *Research Use Only. Not for use in diagnostic procedures.*

The Software is currently compatible with the following Thermo Fisher instruments:

- 7500 Fast Dx Real-Time PCR System (7500 Fast Dx)
- QuantStudio 5 Dx Real-Time PCR System (QS5 Dx)
- QuantStudio 7 Pro Dx Real-Time PCR System (QS7 Pro Dx)

The Software is capable of analyzing outputs generated from these instruments using the following software:

Software	Instrument	File Type
SDS Software v1.4/v1.5	7500 Fast Dx	SDS
Design & Analysis 2	QS5 Dx / QS7 Pro Dx	EDS
Diomni Enterprise Software*	QS7 Pro Dx	EDS

*Diomni Enterprise Software is compatible with the QS5 Dx Instrument but has not been verified with the QuantideX qPCR *ESR1* exoMutation Analysis Module.

The QuantideX qPCR *ESR1* exoMutation Analysis Module is for Research Use Only (RUO) and not for use in diagnostic procedures.

System Requirements/Prerequisites

To download the QuantideX qPCR *ESR1* exoMutation Analysis Module, go to https://asuragen.com/myasuragen.

Minimum hardware and software requirements are as follows:

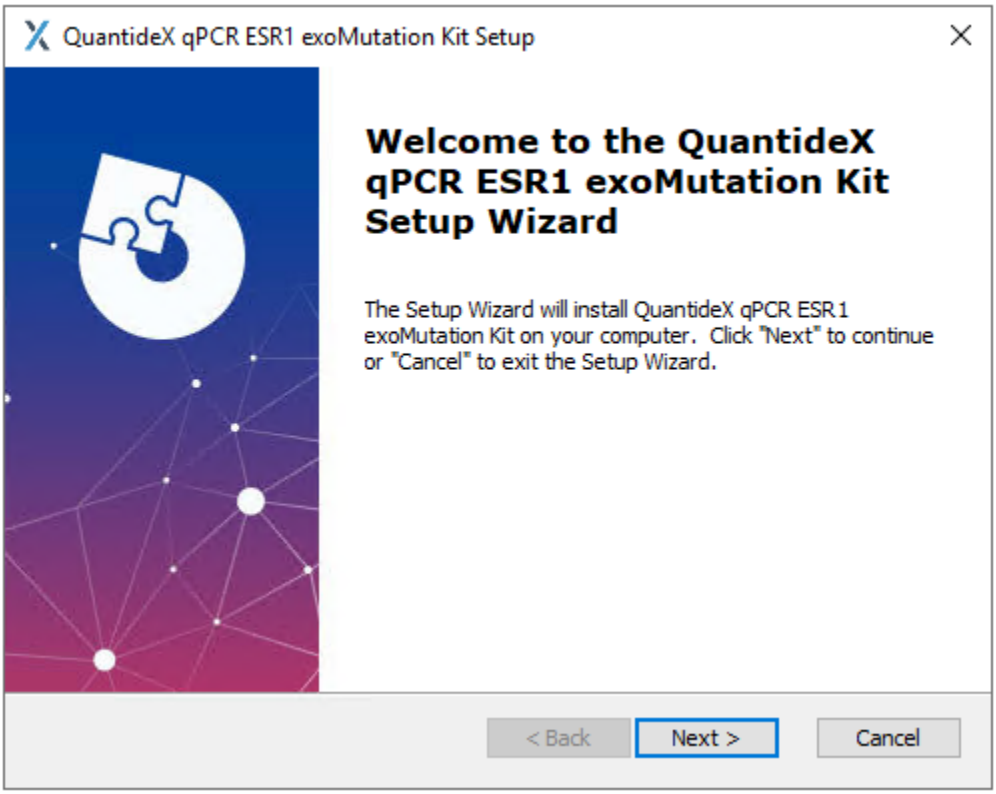
- **Operating System**: Windows 10 or Windows 11
- **Processor (CPU)**: Intel® Core i3 or better (Intel Core i5 or i7 recommended)
- **Memory (RAM)**: Minimum 4 GB (8 GB recommended for optimal performance)
- **Disk space**: Minimum 10 GB available disk space
- Access to the Internet is **not required**
- Port 5148 for localhost must be available

Installation

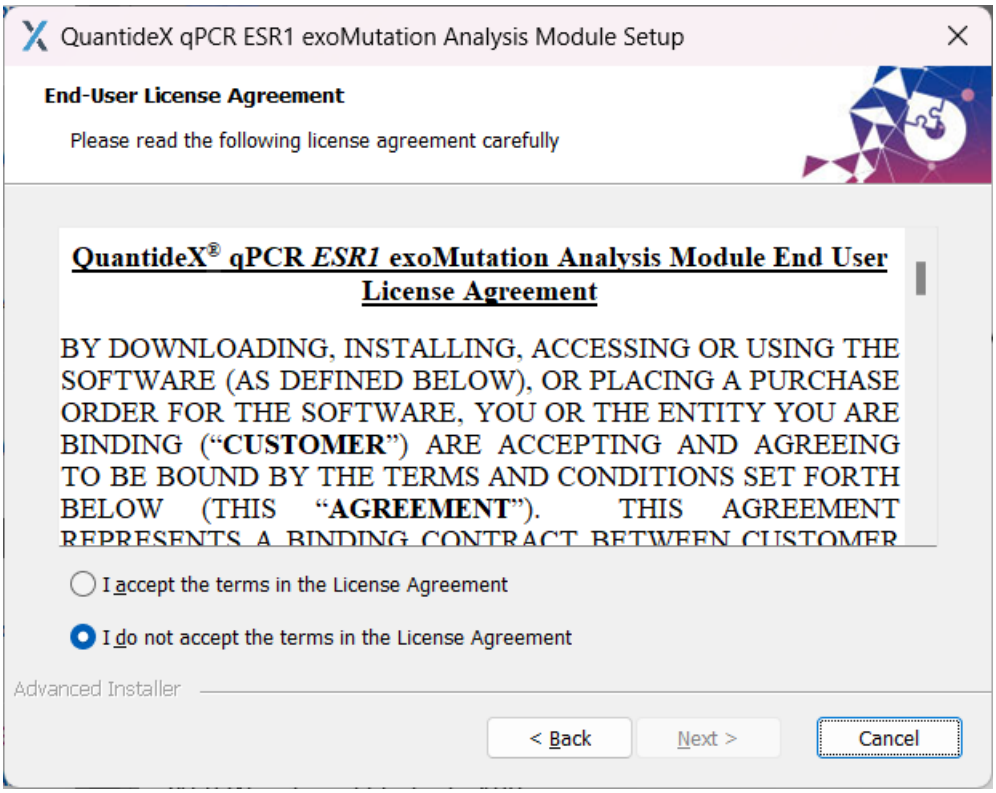
Note: The installer must be run on the same computer where the Software will be installed.

Note: Administrator privileges are required to install the Software.

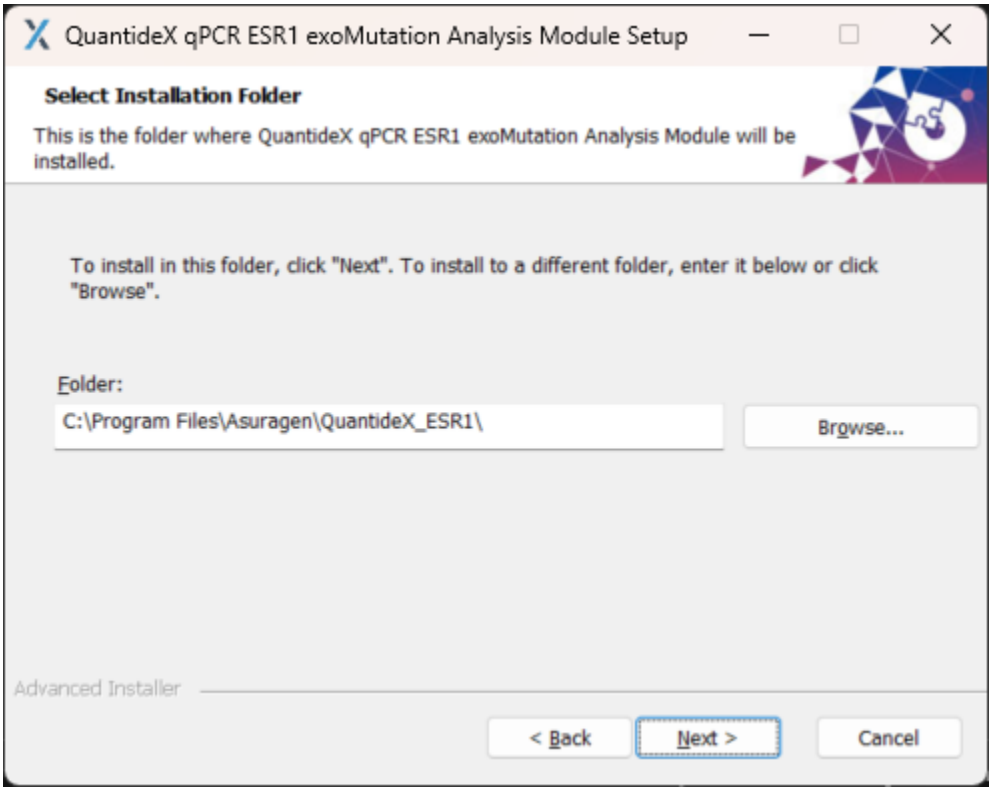
1. Save the QuantideX qPCR *ESR1* exoMutation Analysis installer file to the desired location on the target PC.
2. Browse to the file location and double click the QuantideX qPCR ESR1 exoMutation Analysis Module.exe installer file.
3. Follow the prompts to install the Software on the target PC. If prerequisites needed for the Software are not found on the target PC, you may utilize the Prerequisites Setup Wizard as seen below with an example of an item that may need to be installed.
4. Click **Next** button to continue.



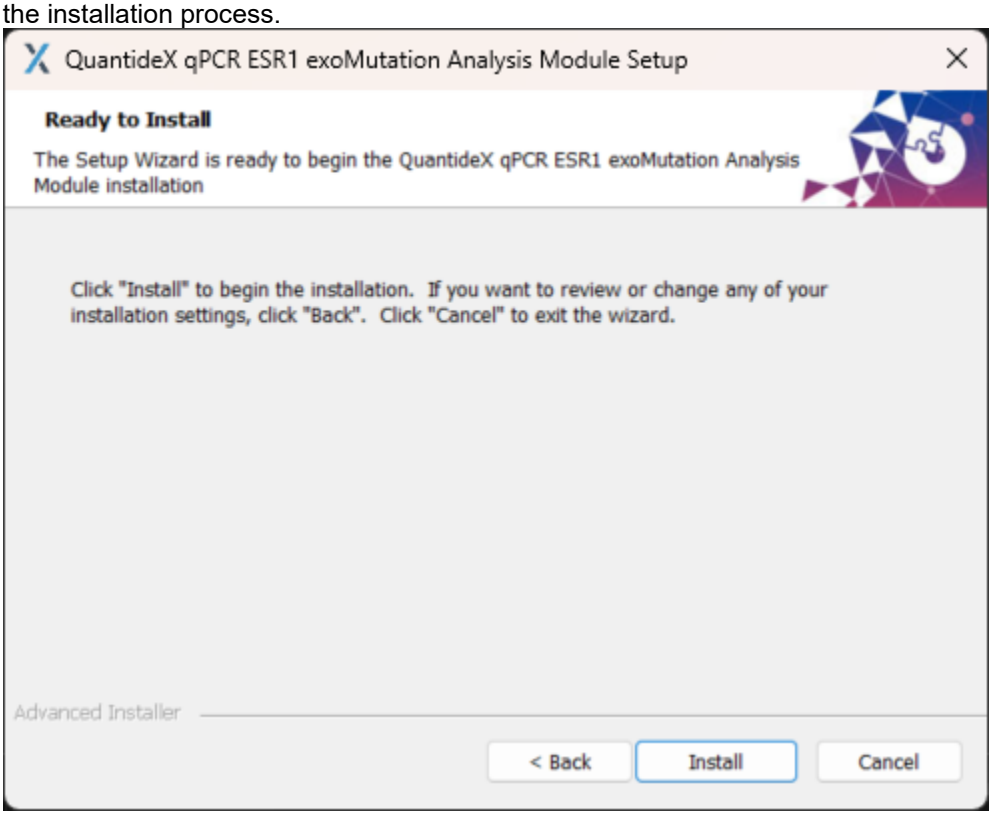
5. Review and accept the terms in the End-User License Agreement. Click **Next** to continue.



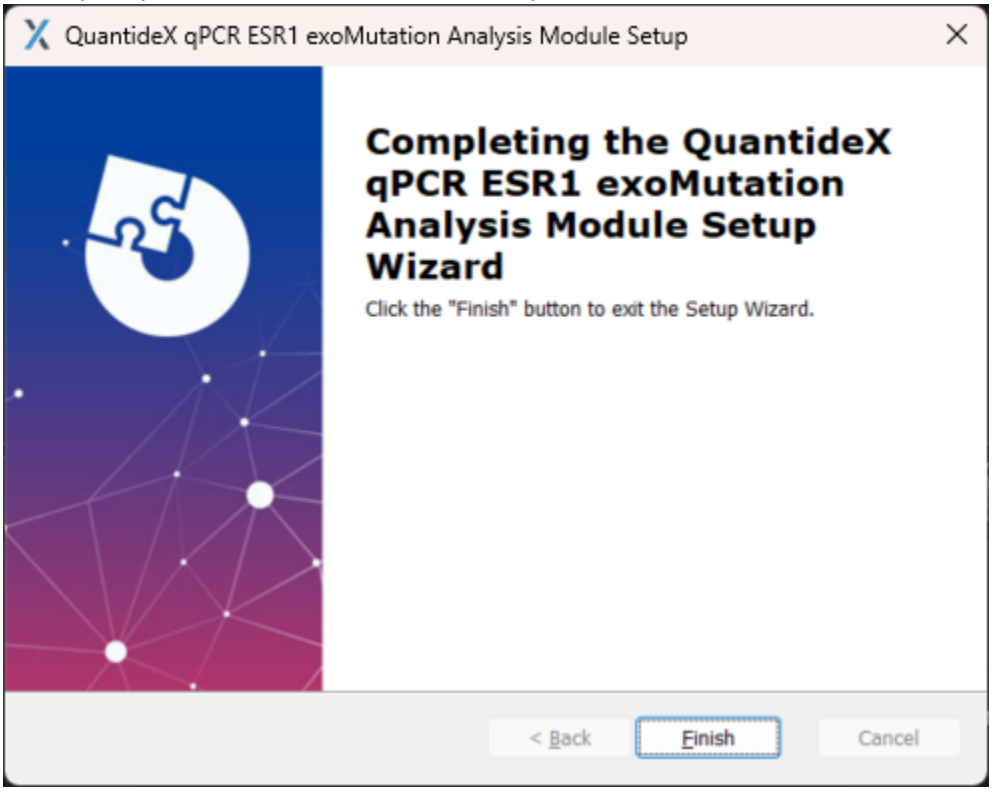
6. Select installation folder for the location of the Software. Click **Next** to continue.



7. Review the setup details and confirm that the installation is ready to begin. Click **Install** to start the installation process.



8. When prompted, click **Finish** to exit the Setup Wizard.



9. Navigate to your desktop and double-click the QuantideX qPCR ESR1 exoMutation Kit icon to launch the Software.

Software Features

Settings

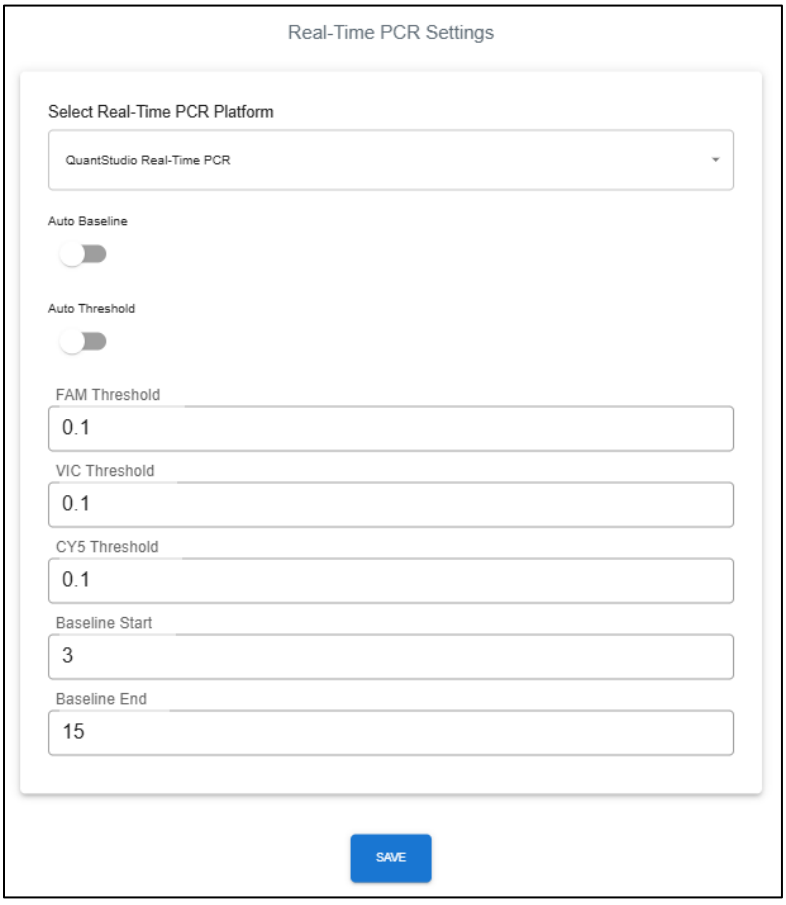
The Software allows end users to customize baseline and threshold settings that were used in the instrument software, allowing settings that differ from the recommendations in the QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide.

Default settings for each supported instrument are as follows:

Setting	7500 Fast Dx	QuantStudio Real-Time PCR (QS5 Dx / QS7 Pro Dx)
FAM / VIC / CY5 Threshold	0.2	0.1
Baseline Start	3	3
Baseline End	15	15

To adjust the settings, follow these steps:

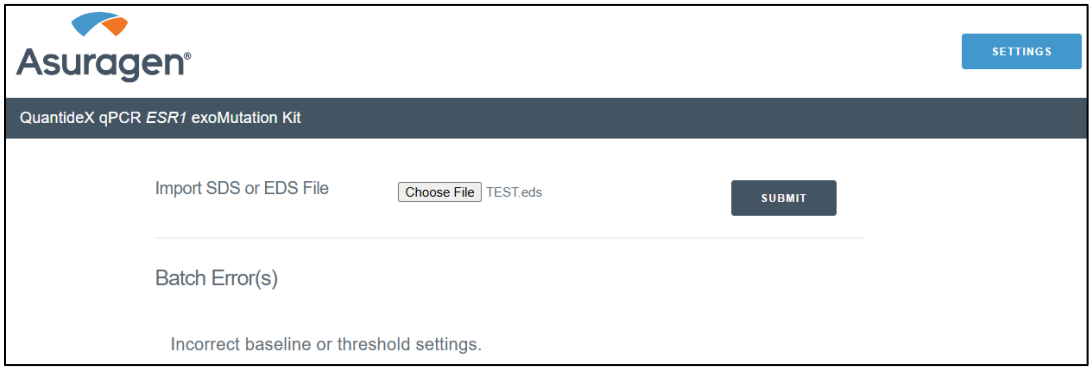
1. Click the **Settings** button located in the upper right corner.



2. Select **Real-Time PCR platform** from the drop-down menu.
    a. **Note:** QS5 Dx and QS7 Pro Dx are grouped together as QuantStudio Real-Time PCR.
3. Toggle the **Auto Baseline** and/or **Auto Threshold** settings if you want these values to be determined automatically instead of using the custom threshold and baseline settings in the following text boxes.
4. If auto threshold is not selected, customize the FAM, VIC, and CY5 Thresholds, either by typing numbers manually into each respective text box or by using the arrows located on the right side.
5. If auto baseline is not selected, customize the baseline start and end values, either by typing numbers manually into each respective text box or by using the arrows located on the right side.
6. Click **Save** to save the custom settings.

Note: The customized baseline and threshold settings of the Software are not saved when the software is uninstalled. New installation will revert to default settings.

Note: The Software cannot adjust settings utilized in SDS Software v1.4/v1.5, Design & Analysis 2, or Diomni Enterprise Software. If the settings entered in the Software do not match what was utilized within the Thermo Fisher provided software, a warning message stating, "Incorrect baseline or threshold settings." will be displayed and analysis in the Software will be blocked from proceeding. An example is shown below:



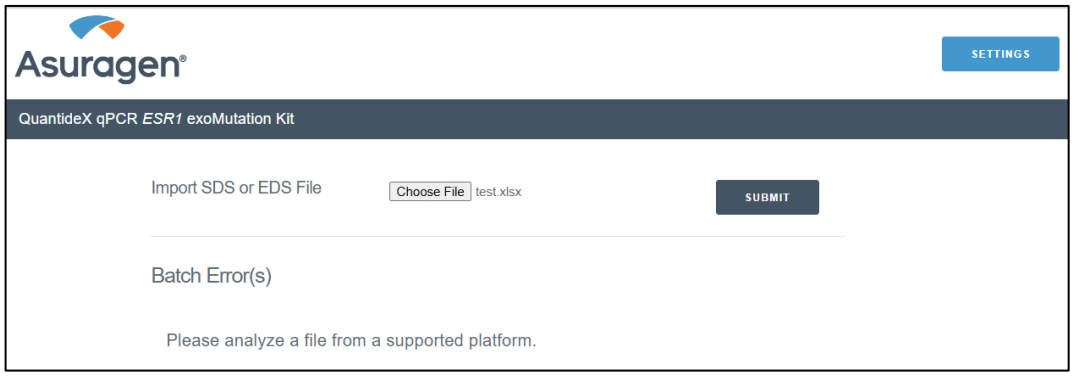
Batch Report

The Software generates a batch report summarizing detected variants and Cq values for all controls and samples for every primer mix in a batch file.

Follow the steps below to generate a report using the Software.

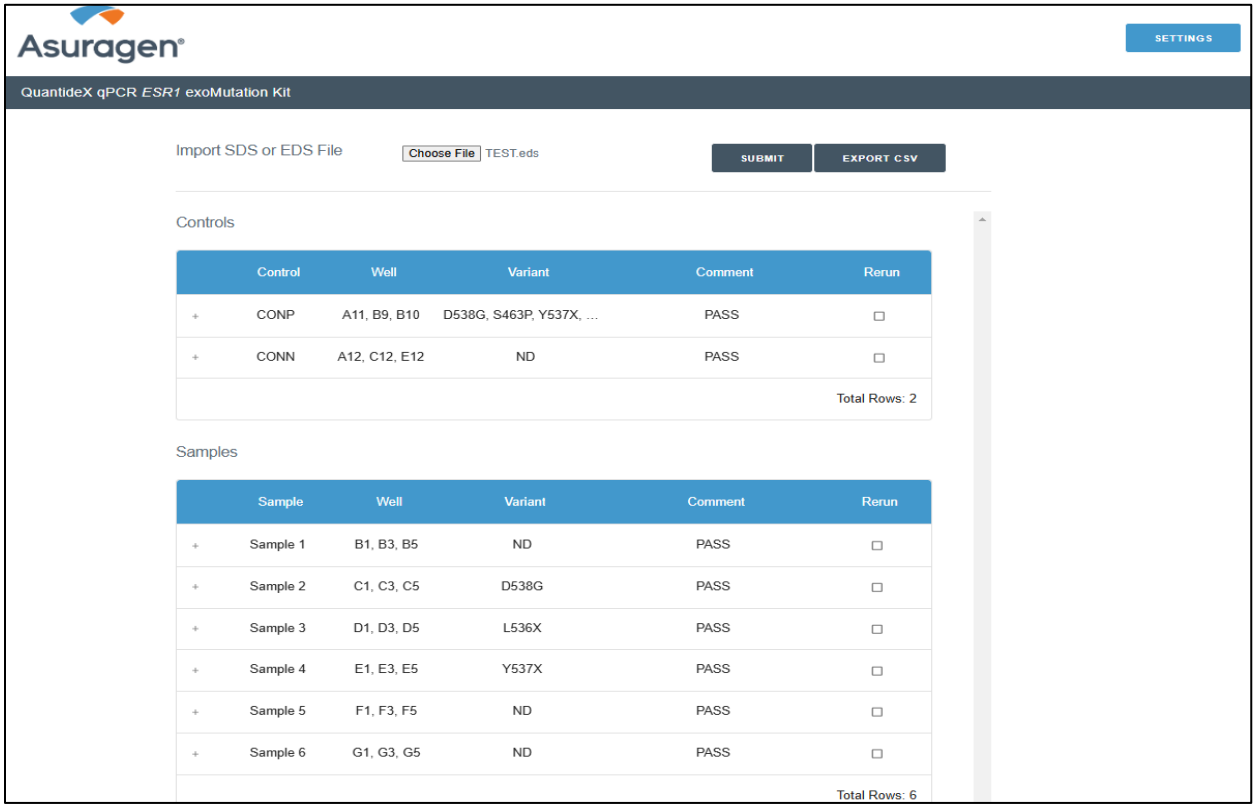
1. Click **Choose File** and select a file from your computer using the File Explorer dialog window.
2. Click **Submit** to generate a batch report for controls and samples in a table on the screen.

Note: If you choose a file not supported by the Software, a warning message stating "Please analyze a file from a supported platform." will be displayed and analysis in the Software will be blocked from proceeding; example shown below:



Note: The recommended number of cycles for the qPCR step is 40. If > or < 40 qPCR cycles were run, a pop-up warning message stating, "Incorrect number of PCR cycles completed." will be displayed. Analysis in the Software will not be blocked from proceeding once the user clicks "OK" on the popup window to accept the results.

Once a file is analyzed, two main tables are shown: Controls and Samples. An example is shown below.

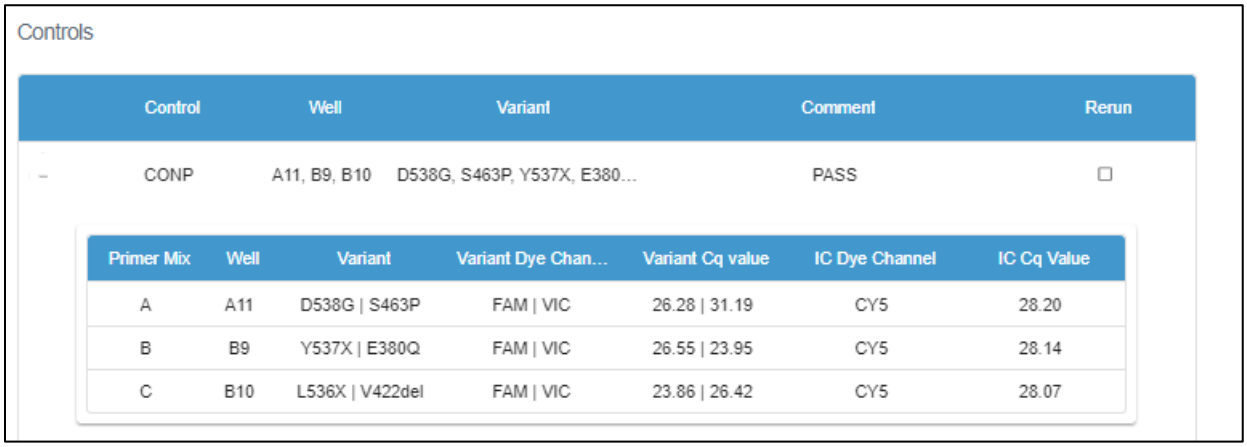


Understanding Results

The following information is displayed in the main table for each control (CONP and CONN) and sample.

Field	How to Interpret
Control/Sample	The name of the sample or control.
Well	A comma separated list of well names for the respective sample. Samples are run across three wells representing each of the primer mixes.
Variant	A comma separated list of detected variants for the respective sample.
Comment	A comma separated list of status messages for the respective sample.
Rerun	A checkbox that is automatically set during analysis if a QC flag was triggered with the control and/or sample. Users may also click and unclick the checkbox to manually change rerun status, which will be tracked within the Comment field.

Click the plus (+) icon on the left of a control or sample name to open up a sub-table with additional information. An example is shown below for Control CONP.



Field	How to Interpret
Primer Mix	The name of the primer mix of the respective control/sample (A, B, or C).
Well	The name of the well on the plate where this primer mix was used.
Variant	A list of variants (if identified) separated by "|" for each primer mix. Valid variants for primer mixes are • **A**: D538G, S463P • **B**: Y537X, E380Q • **C**: L536X, V422del If no variant was detected, ND will be placed in this field.
Variant Dye Channel	A list of dye channels separated by "|" for variants (if identified) for each primer mix (FAM or VIC). If no variant was detected, ND will be placed in this field.

Field	How to Interpret
Variant Cq value	A list of Cq values separated by "|" for each primer mix containing variants. If no variant was detected, ND will be placed in this field.
IC Dye Channel	The CY5 dye channel for the primer mix containing the Internal Control (IC).
IC Cq Value	The Cq value of the IC for the primer mix containing the Internal Control (IC).

Data Export

1. Click **Export CSV** button in the upper right to generate a comma-separated values (CSV) file of the results.
2. Refer to the QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for additional information regarding the interpretation of results.

An example CSV file is shown below.

	A	B	C	D	E	F	G	H	I	J	K
1	#File Information:										
2	Run Name	Analysis Version	Regulatory Label	Analyzed Date	PCR End Cycle	Comment					
3	TEST	1.0.3	Research use only. Not for use in diagnostic procedures	11/5/2024	40	PASS					
4											
5	#Threshold/Baseline Information										
6	Target	Threshold	Baseline Start	Baseline End							
7	D538G	0.1	3	15							
8	S463P	0.1	3	15							
9	IC1	0.1	3	15							
10	Y537X	0.1	3	15							
11	E380Q	0.1	3	15							
12	IC2	0.1	3	15							
13	L536X	0.1	3	15							
14	V422del	0.1	3	15							
15	IC3	0.1	3	15							
16											
17											
18	#Controls										
19	Control	Well	Primer Mix	FAM Target	FAM Cq	VIC Target	VIC Cq	CY5 Target	CY5 Cq	Comment	Rerun
20	CONP	A11	A	D538G	26.28	S463P	31.19	IC1	28.2	PASS	No
21	CONP	B9	B	Y537X	26.55	E380Q	23.95	IC2	28.14	PASS	No
22	CONP	B10	C	L536X	23.86	V422del	26.42	IC3	28.07	PASS	No
23	CONN	A12	A	D538G	ND	S463P	ND	IC1	27.14	PASS	No
24	CONN	C12	B	Y537X	ND	E380Q	ND	IC2	27.18	PASS	No
25	CONN	E12	C	L536X	ND	V422del	ND	IC3	27.37	PASS	No
26											
27	#Samples										
28	Sample	Well	Primer Mix	FAM Target	FAM Cq	VIC Target	VIC Cq	CY5 Target	CY5 Cq	Comment	Rerun
29	Sample 1	B1	A	D538G	ND	S463P	ND	IC1	31.51	PASS	No
30	Sample 1	B3	B	Y537X	ND	E380Q	ND	IC2	31.27	PASS	No
31	Sample 1	B5	C	L536X	ND	V422del	ND	IC3	31.52	PASS	No
32	Sample 2	C1	A	D538G	31.22	S463P	ND	IC1	31.27	PASS	No
33	Sample 2	C3	B	Y537X	ND	E380Q	ND	IC2	31.1	PASS	No
34	Sample 2	C5	C	L536X	ND	V422del	ND	IC3	31.12	PASS	No

Uninstallation Instructions

1. Open Programs and Features from the **Start** menu. This can usually be done by clicking **Control Panel** and then **Programs and Features**, but if you do not see the option then use the search bar.
2. Select **QuantideX qPCR ESR1 exoMutation Analysis Module**, and then click **Uninstall**.

Known Limitations

- When the user attempts to install the Software for the first time on a Windows machine, Microsoft® Defender may block the installation and display a notification indicating that the Software is an unrecognized app, warning that running it might pose a risk to the PC. To proceed with the installation, the user should select **More info** and the **Run anyway** option.
- If the storage device (e.g., hard drive) where the Software is installed runs out of space, the Software may slow down or fail to respond, and analyses will not complete. Ensure you have at least 10 GB disk space before you initiate an analysis.

Quality Control Procedures

Positive Control (CONP)

The positive control (CONP) is required in singleton for all 3 qPCR primer mixes in every batch run for data analysis and quality control. The CONP generates a positive signal in all dye channels (FAM, VIC, CY5) across all qPCR primer mixes. Specifically, per qPCR primer mix, the CONP will generate a positive signal for two variants and the internal control (IC).

If an expected variant is not detected then the comment column for CONP will display “Expected Variant(s) not detected” and the rerun box for CONP and all samples will be checked. If the internal controls (IC1, IC2 and/or IC3) are not detected, then the comment column for CONP will display “Internal Control: not detected” and the rerun box for CONP and all samples will be checked. If the expected targets are not identified then the comment column for CONP will display “Invalid number of target(s)” and the rerun box for CONP and all samples will be checked. These QC failures alert the user that the batch run is invalid. For further details, refer to the Troubleshooting section.

A valid CONP will display PASS in the comment column in the results view of the Software and in the control section of the CSV report. In the results view, the CONP dropdown table will display the variants detected, the variant dye channel, and associated Cq values. The internal control (IC) dye channel and IC Cq values will be displayed.

Negative Control (CONN)

The negative control (CONN) is required in singleton in every batch run for data analysis and quality control. The CONN generates a signal in only the CY5 dye channel for the internal control (IC) across all qPCR primer mixes.

If a variant is detected then the comment column for CONN will display “Variant(s) detected” and the rerun box for CONN and all samples will be checked. If the internal controls (IC1, IC2 and/or IC3) are not detected, then the comment column for CONN will display “Internal Control: not detected” and the rerun box for CONN and all samples will be checked. If the expected targets are not identified then the comment column for CONN will display “Invalid number of target(s)” and the rerun box for CONN and all samples will be checked. These QC failures alert the user that the batch run is invalid. For further details, refer to the Troubleshooting section.

A valid CONN will display PASS in the comment column in the results view of the Software and in the control section of the CSV report. In the results view, the CONN dropdown table will display ND (Not Detected) in the variant, the variant dye channel, and variant Cq value columns. The internal control (IC) dye channel and IC Cq value will be displayed.

Samples

Each sample is only required to be tested in singleton. It is expected for each sample to generate a signal in the CY5 dye channel for the internal control (IC) across all qPCR primer mixes. A valid sample will display PASS in the comment column in the results view of the Software and in the sample section of the generated CSV report.

If no variants are detected, the results view of the Software sample dropdown table will display ND (Not Detected) in the variant, variant dye channel, and variant Cq value columns. The internal control (IC) dye channel and Cq value will be displayed.

If variants are detected, the results view of the Software sample dropdown table will display the variants detected, the variant dye channel, and associated Cq values. ND (Not Detected) will be displayed for variants not present in the sample. The internal control (IC) dye channel and IC Cq values will be displayed.

Troubleshooting

Observation	Potential Cause	Action
Incorrect baseline or threshold settings	Analysis settings for the baseline and/or threshold do not match expected settings	Open the .sds or .eds file within the Thermo Fisher provided software and review the baseline and threshold settings. See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for baseline and threshold settings appropriate per qPCR instrument. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module.
Please analyze a file from a supported platform	Non-supported instrument file submitted for analysis	Confirm the correct file was submitted for analysis. See §**Overview** for a list of supported qPCR instruments.
Invalid number of target(s)	Incorrect target name setup	See the QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for appropriate target name. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module.
	Incorrect assignment of target(s) to plate layout	Ensure qPCR setup naming of all controls and samples contain a single assignment of each target. A valid sample or control contains 9 targets. See QuantideX qPCR *ESR1* exoMutation Kit protocol guide for appropriate target name.
	Incorrect control and/or sample naming	Ensure CONN and CONP are labeled as identified in the QuantideX qPCR *ESR1* exoMutation Kit protocol guide. Incorrectly named controls lead to incorrect assignment in the QuantideX qPCR *ESR1* exoMutation Analysis Module. The affected control(s) will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun, including samples that were named correctly. Misnaming of sample replicates results in the sample flagged for a rerun. Ensure sample name is consistent across all wells for the sample as identified in QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide.
	Variant observed in CONN	Verify no contamination has occurred. Verify qPCR master mixes were properly formulated. Confirm CONN was added to the appropriate well. If CONN has a variant detected, CONN will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun.

Observation	Potential Cause	Action
	Absence of a positive signal in all channels for CONP	Verify qPCR master mixes were properly formulated. Verify CONP was added to the appropriate well. If CONP has a variant detected, CONP will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun.
Invalid reporter(s) for target(s)	Incorrect target name setup	See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for proper target name and reporter association. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module. All affected controls and samples in the batch will be marked for rerun.
CONP not found	Incorrect control naming	Ensure CONP is labeled as identified in QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide. Incorrectly named CONP leads to incorrect assignment in the QuantideX qPCR *ESR1* exoMutation Analysis Module. CONP will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun, including samples that were named correctly.
CONN not found	Incorrect control naming	Ensure CONN is labeled as identified in QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide. Incorrectly named CONN leads to incorrect assignment in the QuantideX qPCR *ESR1* exoMutation Analysis Module. CONN will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun, including samples that were named correctly.
PCR Cycle QC	Incorrect number of PCR cycles completed	The recommended number of cycles for the qPCR step is 40. If > or < 40 qPCR cycles were intentionally run, click “OK” on the popup window to accept the results. See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for recommended cycling conditions.
Internal Control: not detected	No signal detected for IC1, IC2, and/or IC3	Ensure correct volume of template was utilized in all steps. Verify qPCR master mixes were properly formulated. If CONN or CONP have internal control not detected (ND) for IC1, IC2, and/or IC3, affected control(s) and all samples will be flagged for rerun.

Observation	Potential Cause	Action
	Incorrect target name setup	See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for appropriate target name. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module.
	Incorrect assignment of target(s) to plate layout	Ensure qPCR setup naming of all controls and samples contain a single assignment of each target. A valid sample or control contains 9 targets. See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for appropriate target name.
	Incorrect internal control naming	Ensure IC1, IC2 and IC3 are labeled as identified in QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide. Incorrectly named IC1, IC2 and IC3 lead to incorrect assignment in the QuantideX qPCR *ESR1* exoMutation Analysis Module. The affected control(s) will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun, including samples that were named correctly.
	Variant observed in CONN	Verify no contamination has occurred. Verify qPCR master mixes were properly formulated. Confirm CONN was added to the appropriate well. If CONN has a variant detected, CONN will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun.
	Absence of a positive signal in all channels for CONP positive control	Verify qPCR master mixes were properly formulated. Verify CONP was added to the appropriate well. If CONP has a variant detected, CONP will be flagged for rerun. Additionally, all samples in the batch will be marked for rerun.
Expected Variant(s) not detected	One or more expected variants are not detected (ND) in CONP	Ensure correct control was used for CONP. Verify qPCR master mixes were properly formulated. CONP and all samples will be flagged for rerun.
	Incorrect target name setup	See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for appropriate target name. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module.

Observation	Potential Cause	Action
Variant(s) detected	Unexpected variant(s) have been detected in CONN	Ensure correct control was used for CONN. Verify no contamination has occurred. CONN and all samples will be flagged for rerun.
	Incorrect target name setup	See QuantideX qPCR *ESR1* exoMutation Kit Protocol Guide for appropriate target name. Reanalyze the run and submit for analysis in QuantideX qPCR *ESR1* exoMutation Analysis Module.

Third Party Applications and Software Licenses

This product incorporates third-party software libraries and components. Below is a list of these components and their respective licenses.

Software	Version	License	Description / Purpose
.NET	8.0	MIT License	Core framework for server logic. Primary uses include ASP.NET Core for business logic and Kestrel as a lightweight web server for managing local web traffic.
React	18.2.0	MIT License	Core framework for the web browser-based user interface.
MUI X DataGrid Pro	7.1.1	Commercial License	User interface framework used primarily to display results data using DataGrid tables.
CsvHelper	32.0.1	MIT License	Used to facilitate the handling of CSV data.
Emotion	11.11.1	MIT License	A CSS-in-JS library for styling React components, allowing for dynamic, scoped styles with high performance.
Milligram	1.4.1	MIT License	A minimal CSS framework providing a clean and simple base for styling web applications.

Disclaimers

1. This Product is intended to be Research Use Only. Not for use in diagnostic procedures.
2. This Product may not be resold, modified for resale, or used to manufacture commercial products without the written approval of Asuragen®.
3. Trademarks are the property of their respective owners.
4. All instrumentation must be maintained and operated according to manufacturer's instructions.
5. TO THE EXTENT PERMITTED BY APPLICABLE LAW, IN NO EVENT SHALL ASURAGEN BE LIABLE IN ANY WAY (WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE) FOR ANY CLAIM ARISING IN CONNECTION WITH OR FROM THE USE OF THIS PRODUCT.



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