

Presence Absence Analysis Module

USER GUIDE

for use with:

QuantStudio™ Design and Analysis Software v2

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Revision	Date	Description
E.0	15 December 2023	<p>The document was updated for QuantStudio™ Design and Analysis Software v2.8 and the Presence Absence Analysis Module v2.4.</p> <ul style="list-style-type: none">• Permissions were added for the following tasks, when the security settings are enabled:<ul style="list-style-type: none">– Selecting the location when a file is saved (“Select a system template or existing plate file to set up a new plate file” on page 7).– Selecting a passive reference (“Select a passive reference” on page 11).• Instructions were added to export the results (“Export the results” on page 21).
D.0	5 July 2023	<p>The document was updated for the presence absence analysis module of the QuantStudio™ Design and Analysis Software v2.7 and the Presence Absence Analysis Module v2.3.</p> <ul style="list-style-type: none">• The list of compatible data files was removed. See the documentation for the main software for this information.• Information was added about the samples for the OpenArray™ Plate format.• The instructions to edit the target call rules were updated to include the following features:<ul style="list-style-type: none">– An inconclusive call that can be based on a second C_q cutoff value, a minimum C_q confidence value, or a minimum amplification score.– The option to select the method to display a target call for replicates, including based on the positive priority, negative priority, a majority, or concordance.• A tool was added to automatically generate the call rules.• The option was added to display only the targets defined in the call rules.• The instructions to review the presence absence calls were updated to include the consolidated target call.• The option to select how the sample call is made was added.• The option to color by call in the plate layout pane was added.
C.0	4 November 2020	<p>Changes for version 2.1: Added sample comment; added sample name in target call rules and invalidation assessment in control rules; added export/import/scan in reagent information.</p>
B.0	30 April 2020	<p>Changes for version 2.0: Removed send to the instrument run queue; updated analysis setting and presence/absence calls.</p>
A.0	26 August 2019	<p>New document.</p>

The information in this guide is subject to change without notice.

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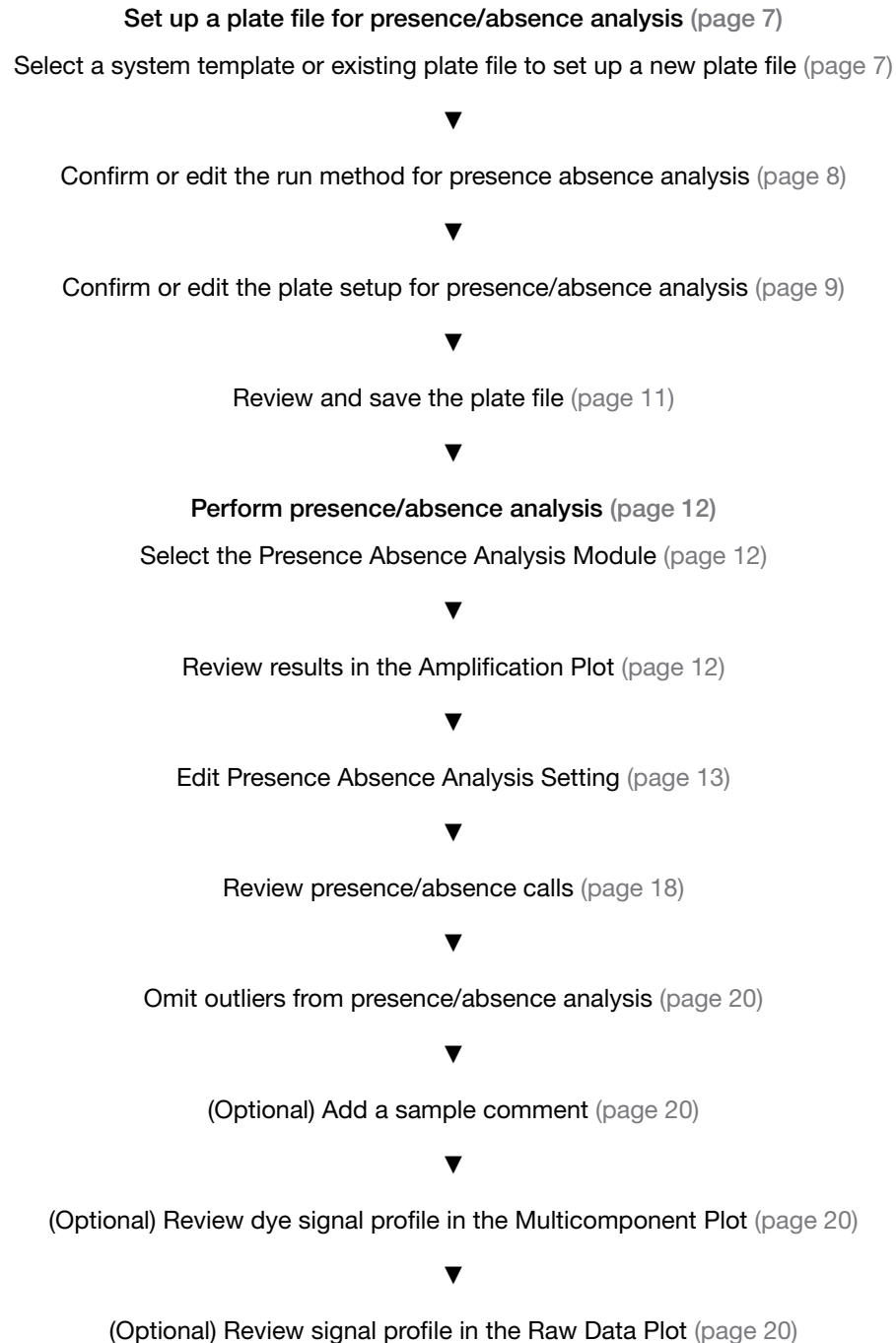
About the Presence Absence Analysis Module

The Presence Absence Analysis Module for QuantStudio™ Design and Analysis Software v2 is used to determine the presence or absence of a target nucleic acid sequence in a sample.

For more information about presence/absence analysis, see Chapter 5, “About presence/absence analysis”.



Workflow: Presence/absence analysis





Set up a plate file for presence/absence analysis

For detailed instructions about setting up a plate file, see [? Help ▶ Help Contents](#).


Select a system template or existing plate file to set up a new plate file

A plate file contains the information that is necessary to perform an instrument run, including instrument setup, run method, plate setup, and analysis setting.

A system template is a non-editable plate file that is included with the software.

A new plate file must be created from a system template or a previously created plate file.

For detailed information about system templates and plate files, see [? Help ▶ Help Contents](#).

1. In the home screen, click  **Set Up Plate**.
The **Plate Gallery** opens to the **System Templates** tab.

2. **IMPORTANT!** Select a system template or a plate file that corresponds to your instrument, block, and run mode. These properties are not editable after the plate file has been created.

In the left pane, select the appropriate options to filter the system template and plate file lists.

- **Instrument**
- **Block**
- **Run Mode**
- **Analysis**

Note: Thermal protocol, plate setup, and post-run analysis options are independent of analysis module selection. Analysis module selection can be changed at any point during plate file set or post-run analysis (see “Select the Presence Absence Analysis Module” on page 12).

3. Navigate to, then select a system template or plate file.

Tab	Description
System Templates	Contains system templates, non-editable plate files that are included with the software. Select a system template to automatically generate a new plate file that can be edited, then saved.
My Plate Files	Contains plate files that were previously saved to My Plate Files . plate files that are included with the software. Select an existing plate file to edit, then save, or to save as a new plate file.
Recents	Contains plate files that were recently opened. Recently opened plate files from System Templates and My Plate Files do not populate this tab. Select an existing plate file to edit, then save, or to save as a new plate file.

Note: To view all options for opening the plate file, hover over the plate file, then click ... **(Actions)**.

Selecting the location where the file is saved is a controlled function. If the **Browse** button in the **Save As** dialog box is inactive, the security has been enabled in the software. The user role does not have the permission to select the location where the file is saved. The user role must have the permission of **Edit Save As Destination**.

The plate file opens in the **Run Method** tab.

Confirm or edit the run method for presence absence analysis

For most analysis, the default run method is appropriate. The following options are compatible with presence absence analysis.

- PCR
- 1-step RT-PCR
- 2-step RT-PCR
- In a plate file, in the **Run Method** tab, adjust the run method elements as needed.
For detailed instructions about editing the run method, see [?](#) **Help** ▶ **Help Contents**.
- *(Optional)* Confirm that data collection is turned on in the **Pre Read** stage.
 ΔR_n calculation requires pre-PCR read data.
- *(Recommended)* Confirm that data collection is turned on in the **PCR** stage.
We recommend collecting real-time amplification data during the PCR stage, for troubleshooting purposes.

- Confirm that data collection is turned on in the **Post Read** stage.
Post-PCR read data is used to determine presence or absence calls.
- Click ... **(Actions)** ▶ **Filter Settings** to confirm or edit filter settings.

Confirm or edit the plate setup for presence/absence analysis

For detailed instructions about plate setup, or to download example plate setup files, see [? Help ▶ Help Contents](#).

Add samples and assign to wells

For detailed instructions about plate setup, see [? Help ▶ Help Contents](#).

1. In the **Plate Setup** tab, add samples and assign to wells using the following options.
 - Import a plate setup file
 - Manually add samples to the **Samples** table
 - Manually add samples to wells in the plate layout

For the OpenArray™ Plate format, a sample layout can be imported. Samples cannot be added manually. The sample names and target names can be edited.

The other formats allow a plate setup file to be imported, including targets and assays.

2. Confirm or edit sample information in the **Samples** table.

Column	Description
Name	Sample name
Color	Sample color
Type ^[1]	Presence absence analysis uses the following sample types. <ul style="list-style-type: none">• Unknown• Positive Control• Negative Control

^[1] For more information, see “Sample types for presence/absence analysis” on page 23.

3. Confirm or edit sample well assignments in the plate layout.

Add targets and assign to wells

For detailed instructions about plate setup, see [?](#) **Help** ▶ **Help Contents**.

Targets or SNP assays cannot be imported or assigned for the OpenArray™ Plate format. The names can be edited.

1. In the **Plate Setup** tab, add targets and assign to wells using the following options.

- Import an AIF file
- Import a plate setup file
- Manually add targets to the **Targets** table
- Manually add targets to wells in the plate layout
- Import TaqMan™ assay plate and card files

2. Confirm or edit the target information in the **Target** table.

Column	Description
Name	Target name
Color	Target color
Task ^[1]	The software automatically assigns a task to the target in a well based on the sample type in that well. The following tasks are used for presence absence analysis. <ul style="list-style-type: none"> • Unknown • Positive Control • Negative Control • IPC (Internal Positive Control or Internal Process Control)^[2] • Blocked IPC^[2]

^[1] For more information, see “Sample types for presence/absence analysis” on page 23.

^[2] To assign this task type, select the task from the drop-down list.

3. Confirm or edit the target well assignments in the plate layout.

Edit reagent information

Reagents can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.
2. In the **Reagents** table, click **+** (**Add**).

Note:

- Click **⋮ (Actions)** ▶ **Export Reagents** to export reagents.
 - Click **⋮ (Actions)** ▶ **Import Reagents** to import reagents.
 - Click **⋮ (Actions)** ▶ **Scan Reagents** to scan reagents.
-

3. Enter the following information for each reagent.

- Name
- Type
- Barcode
- Part Number
- Lot Number
- Expiration Date

Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

4. (Optional) Click **✕ (Remove)** in the row of a reagent to delete it from the table.

Select a passive reference

Editing the passive reference is a controlled function. If security has been enabled in the software, the user role must have the permission of **Edit Passive Reference**.

1. In the upper-left corner of the **Plate Setup** tab, select a passive reference from the dropdown list.
2. (Optional) Save the plate file or data file.

Review and save the plate file

1. In the **Run Summary** tab, review the run method selections, then edit if needed.
2. Review the plate setup, then edit if needed.
3. (Optional) Click the barcode field, then scan the plate barcode.
4. (Optional) Select **Add to My Plates**.
This option allows you to create new plate files using the current plate file as a template.
5. Select an instrument from the list.
If the instrument does not appear on the list, click **⚙ System ▶ Instruments** to add a new instrument.
6. Save the plate file.

Start the run on an instrument. For specifics on starting an instrument run, see the instrument documentation.

4

Perform presence/absence analysis

Select the Presence Absence Analysis Module

1. In an open data file, click **Actions** ▶ **Analysis Modules**.
2. In the **Analysis Modules** window, select **Presence Absence**, then click **Ok**.
The Presence Absence Analysis Module opens.

Click **Analyze**, then review the results in the **Presence Absence** tab.

Review results in the Amplification Plot

For detailed instructions about reviewing results in the **Amplification Plot** in the **Quality Control** tab, see [?](#) **Help** ▶ **Help Contents**.

If no data are displayed in the **Presence Absence** tab, or if reanalysis is required, click **Analyze**.

1. In the **Presence Absence** tab, in the plot pane, review the overall shape of the curves in the **Amplification Plot**.
2. Review the amplification status for each well.

Table 1 Expected amplification status for control reactions

Control	Target	Expected Result
No template control	IPC	Amplification
	Target of interest	No amplification
No amplification control (NAC; blocked IPC)	IPC	No amplification
	Target of interest	No amplification
Positive control	IPC	Amplification
	Target of interest	Amplification

3. Review or edit threshold settings.
4. Review or edit baseline settings.

Edit Presence Absence Analysis Setting

To enable well calls and sample calls, you must set up sample call rules, in addition to target call rules. Open the Presence Absence Analysis Module.

1. Click **Actions** ▶ **Presence Absence Analysis Setting**.

Note:

- Click **Import** to import settings.
- Click **Export** to export settings.

2. Once you are finished editing all of the analysis settings, click **Apply**.

The data is reanalyzed using the updated analysis settings.

Edit the target call rules

1. In the **Presence Absence Analysis Setting** window, in the **Target Call Rules** tab, select an option from the **Analyze Data** dropdown list to determine the method used to calculate ΔRn (Rn = normalized readings).

Option	Description
Post-PCR Read	$\Delta Rn = Rn_{(\text{post-PCR read})}$
Pre-PCR Read and Post-PCR Read	$\Delta Rn = Rn_{(\text{post-PCR read})} - Rn_{(\text{pre-PCR read})}$
Real-time Rn Data	$\Delta Rn = Rn_{(\text{last PCR cycle})} - Rn_{(\text{first PCR cycle})}$

Note: If you did not turn on data collection for a specific stage of the thermal protocol during plate file setup, data analysis for that stage will not be available (see “Confirm or edit the run method for presence absence analysis” on page 8).

2. Toggle the **Use Inconclusive Call** setting on or off. When the **Use Inconclusive Call** setting is on, the following columns in the **Customization** table can be edited:

- **Upper Cq Cutoff** column
- **Min Cq Conf** column
- **Min Amp Score** column

When the **Use Inconclusive Call** setting is off, these columns in the table are not available.

3. Enter the following items in the **Customization** table.
 - Click **+** (**Add**) to add a new target call rule.

- Click in a cell to edit the following settings if needed.
 - **Sample Name** field (optional)
The call rule applies to all of the targets on the plate if the sample name is not defined.
 - **Target** field
 - **C_q Cutoff** field
 - **ΔRn Threshold** field
 - Click **✕ (Remove)** to delete a target call rule.
4. Enter a value in the each of the following fields in the **Customization** table if an inconclusive call is used.
- **Upper C_q Cutoff** field
An inconclusive call uses two C_q cutoff values.
A target is interpreted as inconclusive the both of the following conditions are met:
 - The C_q value is greater than the C_q cutoff value.
 - The C_q value is less than or equal to the upper C_q cutoff value.
 - **Min C_q Conf** field
A target is interpreted as inconclusive the both of the following conditions are met:
 - The C_q value is less than or equal to the C_q cutoff value.
 - The C_q value is less than or equal to the minimum C_q confidence value.
 - **Min Amp Score** field
A target is interpreted as inconclusive the both of the following conditions are met:
 - The C_q value is less than or equal to the C_q cutoff value.
 - The amplification score is less than or equal to the minimum amplification score.
5. Select a value in the **Consolidate by** column of the **Customization** table.
Click the table cell to access the list in order to select the value.
- **Positive Priority**
A single positive call in the replicate group results in a positive target call.
 - **Negative Priority**
A single negative call in the replicate group results in a negative target call.
 - **Majority**
The target call with the majority in the replicate group is displayed.
 - **Concordance**
The target call of the replicate group is used if all of the target calls within the group are concordant. If there is any discrepancy between target calls for the replicate group, the target call is displayed as inconclusive.

The values that are available in the **Consolidate by** column depend on whether the inconclusive call setting is on or off (see step 2). All of the values are available if the inconclusive call setting is on. Positive priority and negative priority are the values that are available if the inconclusive call setting is off.

Positive priority is the default value if the inconclusive call setting is off.

The following table shows the target call based on the consolidation value. It uses four replicates.

Replicate target calls	Replicate target group call			
	By positive priority	By negative priority	By majority	By concordance
4 x				
3 x 1 x				
2 x 2 x				
1 x 3 x				
4 x				
2 x 1 x 1 x				
1 x 1 x 2 x				
2 x 2 x				
1 x 2 x 1 x				
4 x				

6. Click **Apply**.

The data are reanalyzed using the updated analysis settings.

Edit Tests settings

In **Presence Absence Analysis Setting**, a test refers to a test for a particular pathogen. If you are doing analysis with multiple tests, define the tests to differentiate for the sample call rules and control rules.

1. In the **Presence Absence Analysis Setting** window, in the **Tests** tab, click **+ (Add)** to add a new test.
2. Click in a cell to edit the following settings if needed.
 - **Test Code**
 - **Description**

3. (Optional) Click **X (Remove)** to delete the settings.
A test code should not be deleted if it is used in sample call rules or control rules.
4. Once you are finished editing all analysis settings, click **Apply**.

The data is reanalyzed using the updated analysis settings.

Edit Sample Call Rules

For detailed information about sample call rules and example sample call rule settings, see “About sample call rules” on page 24.

1. In the **Sample Call Using** dropdown list, select one of the following options.
 - **Well Call**
 - **Consolidated Target Call**
 - **Hybrid (Well Call or Consolidated Target Call)**

The **Hybrid (Well Call or Consolidated Target Call)** is selected as the default.

2. Toggle the **Sample call result table only displays targets defined in the Call Rules** on or off.
When this setting is off, all targets assigned with the sample in the well (for the **Well Call** table) or on the plate (for the **Sample Call** table) are displayed in the **Presence Targets** column, the **Absence Targets** column, and the **Inconclusive Targets** column. This is regardless of whether the target name is part of the sample call rule that leads to the call.
When this setting is on, the target names that are not part of the sample call rule are not displayed.
3. In the **Presence Absence Analysis Setting** window, in the **Sample Call Rules** tab, click **+ (Add)** to add a new sample call rule.
4. (Optional) Click **Auto Generate Rules**.
5. In the **Auto Generate Rules** dialog box, enter or select the following information.
 - Select the internal positive controls from the **IPC Target** dropdown list.
One internal positive control or multiple internal positive controls can be selected.
 - (Optional) Enter the name of the no template control sample in the **NTC Sample Name** field.
 - (Optional) Enter the name of the negative extraction control sample in the **NEC Sample Name** field.

The name of the internal positive control target can be typed if there are many targets. Typing filters the list of targets available in the dropdown list.

You can use the * wildcard character for the no template control or negative extraction control. These samples can be set up with a prefix to allow the * wildcard character instead of entering each value.

You can use the ? wildcard character for the no template control or negative extraction control. This character matches a character in a specific position.

For more information about the tool, see “Overview of the tool to automatically generate the rules” on page 17.

IMPORTANT! If the rules are automatically generated with the tool, the settings overwrite any previous test codes, sample call rules, and control rules.

6. In the table, click in a cell to edit the following settings if needed.

- **Sample Name**
- **Presence Targets**
- **Absence Targets**
- **Inconclusive Targets**

The **Inconclusive Targets** column of the table is available if the inconclusive call was enabled when the target call rules were set up (see “Edit the target call rules” on page 13).

- **Test Code** (see “Edit Tests settings” on page 15 for more information)
- **Call**
- **Assessment**

7. (Optional) In the table, click **✕ (Remove)** to delete a sample call rule.

8. Once you are finished editing all analysis settings, click **Apply**.

The data is reanalyzed using the updated analysis settings.

Overview of the tool to automatically generate the rules

A tool is available to set up the tests and sample call rules. It is available to simplify the procedure to set up the call rules when there is a large number of targets. It sets up the call rules instead of requiring you to set them up manually.

When you select the IPC target or multiple IPC targets, the tool creates the test codes and sample call rules.

A no template control and an negative extraction control can also be defined.

IMPORTANT! If the rules are automatically generated with the tool, the settings overwrite any previous test codes, sample call rules, and control rules.

The tool is available in the **Presence Absence Analysis Setting** dialog box.

The should not be used when a positive call is based on the presence of multiple target combinations. It is applicable to assays in which a positive call is based on a single non-IPC target mapped to a test code.

Edit Control Rules

1. In the **Presence Absence Analysis Setting** window, in the **Control Rules** tab, click **+ (Add)** to add a new control rule.
2. In the table, click in a cell to edit the following settings if needed.
 - **Sample Name**
 - **Test Code** (see “Edit Tests settings” on page 15 for more information)
 - **Expected Call**
 - **Invalidate Calls**
 - **Invalidation Assessment**

Note: If you select **Invalidate Calls**, the software will automatically set well calls and sample calls for all other samples to invalid if the control result does not match the **Expected Call**. The well call and sample call for the control will still display as either Presence or Absence to indicate why the control failed. Only well calls and sample calls for the indicated **Test Code** will be affected.

3. Click **× (Remove)** to delete the settings.
4. Once you are finished editing all analysis settings, click **Apply**.

The data is reanalyzed using the updated analysis settings.

Review presence/absence calls

Note: If there is no information that is displayed in a table, the settings that were defined are incorrect. For example, for an assay that uses multiple wells to make a call, there is no data displayed in the **Well Call** table. The settings must be adjusted. The recommendation is to use the hybrid sample call (see “Edit Sample Call Rules” on page 16).

In order to enable well calls, the following conditions must be met:

- The sample call rules are defined (see “Edit Presence Absence Analysis Setting” on page 13)
- Each well contains more than one target
- Each well contains the targets that are defined in the sample call rules

If no data are displayed in the **Presence Absence** tab, or if reanalysis is required, click **Analyze**.

- In the **Presence Absence** tab, use one of the following options to review target calls:

Option	Description
Plate layout	<p>a. In the plate layout pane, in the Color By dropdown list, select Sample or Target.</p> <p>b. Hover over a well to see the individual calls for each target in the well.</p>
Target Call Table	<p>In the table pane, click Target Call, then review the call, the consolidated target call, the C_q value, C_q confidence, amplification score, and ΔR_n for each target in a well.</p> <p>For more information about the consolidated target call, see “Edit the target call rules” on page 13.</p> <p>For more information, see “About call types” on page 23.</p>

- In the **Presence Absence** tab, use one of the following options to review well calls:

Option	Description ^[1]
Plate layout	<p>a. In the plate layout pane, in the Color By dropdown list, select Sample or Call.</p> <p>b. Review the call for each well sample, as indicated by the icon displayed in the middle of the well:</p> <ul style="list-style-type: none"> - + (Presence)—The target nucleic acid sequence is present in the sample - — (Absence)—The target nucleic acid sequence is absent in the sample - ! (Warning)—The sample data needs review for possible errors - ? (Inconclusive)—A well call cannot be made - × (Invalid)—The IPC failed <p>Note:</p> <ul style="list-style-type: none"> • You can also hover over a well to see the call for each well sample. • If there is no icon displayed in the well, the well call is undetermined. • The icons that are displayed in the plate layout are different than the icons that are displayed in the table pane. If there is a single test result in a well, then the icons are the same. If there are multiple test results in a well, then the well might have different calls for each test. The symbol that is displayed in the plate layout is based on the severity of the results. For example, a positive result is considered more severe than a negative result.
Well Call Table	<p>In the table pane, click Well Call, then review the calls.</p> <p>For more information, see “About call types” on page 23.</p>
Sample Call Table	<p>In the table pane, click Sample Call, then review the calls.</p> <p>For more information, see “About call types” on page 23.</p>
Control Status Table	<p>In the table pane, click Control Status, then review the calls.</p>

^[1] For more information about sample call rules, see “About sample call rules” on page 24.

Omit outliers from presence/absence analysis

Outlier wells have C_q values that differ significantly from the average for the associated replicate wells. To ensure C_q precision, consider omitting the outliers from analysis.

1. In the **Presence Absence** tab, select an option to omit wells from analysis.

Option	Description
Omit wells in the Plate Layout	Select outlier wells, then click ⋮ (Actions) ▶ Omit Wells .
Omit wells in the Target Call Table	Select Omit in the row of the outlier well.

2. Click **Analyze** to reanalyze the data with any outliers removed.

(Optional) Add a sample comment

1. In the **Presence Absence** tab, in the table pane, click **Sample Call**.
2. Click a cell in the **Comment** column to enter a sample comment.
3. Click **Actions ▶ Save** or **Actions ▶ Save As** to save the sample comment.

(Optional) Review dye signal profile in the Multicomponent Plot

For more information about the **Multicomponent Plot**, see [? Help ▶ Help Contents](#).

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Review the signal profiles for the passive reference dye, reporter dye, and negative control wells.
3. Review the plot to ensure that there are no irregularities in the dye signals.

(Optional) Review signal profile in the Raw Data Plot

For detailed instructions about reviewing results in the **Raw Data Plot**, see [? Help ▶ Help Contents](#).

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.
2. Click-drag the **Cycle Number** slider through all of the cycles, then confirm that each filter displays the characteristic signal increase.

Export the results

1. In the table pane, click the table associated with the results to export.
 - **Target Call**
 - **Well Call**
 - **Sample Call**
 - **Control Status**
2. Click ... **(Actions)** ▶ **Export**.
3. *(Optional)* In the **Export CSV** dialog box, edit the file name in the **File Name** field.
The **File Name** field is populated with a default file name.
4. Click **Browse** to select a location to save the file.
Selecting the location where the file is saved is a controlled function. If the **Browse** button in the **Export CSV** dialog box is inactive, the security has been enabled in the software. The user role does not have the permission to select the location where the file is saved. The user role must have the permission of **Edit Export Destination**.
5. Click **Export**.



About presence/absence analysis

Overview of presence/absence analysis

Use presence/absence analysis to determine the presence or absence of a target nucleic acid sequence in a sample. The software calls the target present or absent based on an algorithmically determined call threshold. (The call threshold is different from the C_q threshold; the C_q threshold is not used to make calls.)

Presence/absence calls are based on real-time PCR data, or endpoint data (data collected after the PCR stage).

- The data that is collected is the normalized intensity of the reporter dye, or R_n .
- If endpoint experiments include pre-PCR data points, the software calculates the ΔR_n value according to the following formula:

$$\Delta R_n = R_n_{(\text{post-PCR read})} - R_n_{(\text{pre-PCR read})}, \text{ where } R_n = \text{normalized readings.}$$

We recommend collecting real-time amplification data during the PCR stage, for troubleshooting purposes.

Sample types for presence/absence analysis

Presence/absence sample types depend on whether the experiment is set up with or without an internal positive control (IPC).

- **Multiplex presence/absence experiments using IPC (recommended)**—multiplex assays for the target of interest and the IPC target. The IPC is used to confirm that a negative result for the target of interest is not caused by a failed PCR.

Sample type (Type column in Samples table)	Sample description	Target task assignment ^[1] (Task column in Targets table)
Unknown	<ul style="list-style-type: none"> – Test sample – IPC template 	<ul style="list-style-type: none"> – Unknown – IPC^[2]
Negative control	No template control ^[3] <ul style="list-style-type: none"> – Water or buffer – IPC template 	<ul style="list-style-type: none"> – Negative control – IPC^[1]
	No amplification control or negative extraction control (NAC, NEC, or blocked IPC) ^[3] <ul style="list-style-type: none"> – Water or buffer plus a blocking agent – IPC template; amplification prevented by blocking agent 	<ul style="list-style-type: none"> – Negative control – Blocked IPC^[1]

^[1] The software automatically assigns a task to the target in a well based on the sample type in that well.

^[2] To edit the automatic target task assignment, select an option from the dropdown list.

^[3] Minimum of two replicates is required for this control.

- **Singleplex presence/absence experiments without IPC**

Sample type (Type column in Samples table)	Sample description	Target task assignment ^[1] (Task column in Targets table)
Unknown	Test sample	Unknown
Negative Control	Water or buffer	Negative Control

^[1] The software automatically assigns a task to the target in a well based on the sample type in that well.

The software makes calls for individual wells. Running three or more replicates of each reaction can help identify outlier wells that may be present.

About call types

Presence/absence analysis uses the following call types.

Call type	Description
Target Call	<ul style="list-style-type: none"> • Each target call is for one particular target in a particular well. • A target call can be presence or absence, based on the target call rules.

(continued)

Call type	Description
Well Call	<ul style="list-style-type: none"> Each well call is for one particular test in a particular well. A well call can be derived from multiple target calls from the same well. For example, a well call for one test can be derived from four target calls from the same well. For multiple tests in the same well, the Well Call Table has multiple rows for the same well, with one row for each test.
Sample Call	<ul style="list-style-type: none"> Each sample call is for one particular test of one particular sample. A sample call is derived from well calls. Since the same sample can be run on multiple wells, either as replicates or for different tests, the Sample Call Table can be very different from the Well Call Table. If the same sample has different calls in different wells for the same test, the sample call becomes inconclusive for that particular test.

About sample call rules

To enable well calls, you must define sample call rules in the **Presence Absence Analysis Setting** (see “Edit Presence Absence Analysis Setting” on page 13).

The sample call can be made with one of the following options:

- Well call—The software makes sample calls using well call results. If there is no meaningful well call, a sample call is not made.
- Consolidated target call—The software makes a sample call using the consolidated target call results. The software disregards the well call result table, if present.
- Hybrid (well call or consolidated target call)—The software attempts to make sample calls using well call results first. If no well call result is available for the sample, the software changes to using consolidated target calls.

The hybrid option is selected by default.

Option	Well call available	Description
Hybrid (well call or consolidated target call)	Yes	Well call results are used
	No	Consolidated target call results are used
Well call result	Yes	Well call results are used
	No	No call is made
Consolidated target call result	—	Consolidated target call results are used

An example of a well call not being available is an assay where only the unknown target is being tested in the well. There is no internal positive control associated with the well, therefore a well call cannot be made. If the hybrid option is selected, the software determines whether a well call can be made. If it cannot be made, the software identifies the internal positive control associated with the sample and uses the consolidated target call results.

For each sample type, create a unique sample call rule for all applicable presence/absence target combinations.

Table 2 Sample call rule table settings

Sample call rule setting	Description
Sample Name	<p>Enter a sample name. Call rules that include a sample name are given priority over call rules that do not include a sample name. For example, if multiple call rules match the results for a given sample, the call rule that matches the sample name will be used.</p> <p>The sample name can contain wildcard characters, such as * for any number of characters and ? for exactly one character. For example:</p> <ul style="list-style-type: none"> • "PositiveControl*" will match "PositiveControl", "PositiveControl1" and "PositiveControl-A" • "PositiveControl?" will match "PositiveControl1", but not "PositiveControl" or "PositiveControl-A"
Presence Targets	Select all of the targets that are present for a particular call.
Absence Targets	Select all of the targets that are absent for a particular call.
Test Code	<i>(Optional)</i> If there are multiple tests in one well, enter or select a test from the dropdown list. To edit test settings, see “Edit Tests settings” on page 15.
Call	<p>Select one of the following call options:</p> <ul style="list-style-type: none"> • Presence—The target nucleic acid sequence is present in the sample • Absence—The target nucleic acid sequence is absent in the sample • Warning—The sample data needs review for possible errors • Inconclusive—A well call cannot be made • Invalid—The IPC failed
Assessment	<i>(Optional)</i> Enter an assessment or recommended action for the sample call.

Two sample call rules are considered conflicting if both can be applied to a sample (the sample name, presence targets and absence targets match) but each give a different call.

If a sample call rule does not exist for a particular sample name/target combination, the software cannot make a call for that well, and the result will be undetermined.

The target call rules can be set up to define an inconclusive call.

The undetermined call is not included in the Sample Call Table or Well Call Table, only in the Target Call Table.

Example sample call rule settings

In the following example, there is one test that includes four targets per well: Target 1, Target 2, Target 3, and IPC.

Table 3 Example sample call rule settings

Sample Name ^[1]	Presence Targets	Absence Targets	Test Code ^[2]	Call	Assessment ^[3]
—	—	Target 1 Target 2 Target 3 IPC	—	Invalid	Repeat test
—	IPC	Target 1 Target 2 Target 3	—	Absence	Report results
—	Target 1	Target 2 Target 3	—	Inconclusive ^[4]	Repeat test
—	Target 2	Target 1 Target 3	—	Inconclusive ^[4]	Repeat test
—	Target 3	Target 1 Target 2	—	Inconclusive ^[4]	Repeat test
—	Target 1 Target 2	Target 3	—	Presence ^[4]	Report results
—	Target 2 Target 3	Target 1	—	Presence ^[4]	Report results
—	Target 1 Target 3	Target 2	—	Presence ^[4]	Report results
—	Target 1 Target 2 Target 3	—	—	Presence ^[4]	Report results

Table 3 Example sample call rule settings (continued)

Sample Name ^[1]	Presence Targets	Absence Targets	Test Code ^[2]	Call	Assessment ^[3]
Positive Control	Target 1 Target 2 Target 3 IPC	—	—	Presence	—
Negative Control	IPC	Target 1 Target 2 Target 3	—	Absence	—

^[1] Call rules that include a sample name are given priority over call rules that do not include a sample name. For example, if multiple call rules match the results for a given sample, the call rule that matches the sample name will be used.

^[2] The **Test Code** is not defined because there is only one test.

^[3] (Optional) Enter an assessment for a sample call rule.

^[4] For this sample call, the IPC target call can be Presence or Absence. Therefore, the IPC is not included in the sample call rule.



Documentation and support

Related documentation

Document	Publication number
<i>QuantStudio™ Design and Analysis Software v2 User Guide</i>	MAN0018200
<i>QuantStudio™ Design and Analysis v2 User Guide (Thermo Fisher™ Connect Platform)</i>	MAN0018202

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