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

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:**

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.0</td>
<td>4 November 2020</td>
<td>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.</td>
</tr>
<tr>
<td>B.0</td>
<td>30 April 2020</td>
<td>Changes for version 2.0: Removed send to the instrument run queue; updated analysis setting and presence/absence calls.</td>
</tr>
<tr>
<td>A.0</td>
<td>26 August 2019</td>
<td>New document.</td>
</tr>
</tbody>
</table>

**NOTICE TO PURCHASER: DISCLAIMER OF LICENSE:** Purchase of this software product alone does not imply any license under any process, instrument or other apparatus, system, composition, reagent or kit rights under patent claims owned or otherwise controlled by Thermo Fisher Scientific, either expressly, or by estoppel.

**Trademarks:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

©2020 Thermo Fisher Scientific Inc. All rights reserved.
Contents

- CHAPTER 1  About the Presence Absence Analysis Module ...................... 5
  Compatible data files ................................................................. 5
- CHAPTER 2  Workflow: Presence/absence analysis .............................. 7
- CHAPTER 3  Set up a plate file for presence/absence analysis ................. 8
  Select a system template or existing plate file to set up a new plate file .......... 8
  Confirm or edit the run method for presence absence analysis .................. 9
  Confirm or edit the plate setup for presence/absence analysis .................. 10
  Add samples and assign to wells .................................................. 10
  Add targets and assign to wells ................................................. 10
  Edit reagent information ......................................................... 11
  Select a passive reference ...................................................... 11
  Review and save the plate file .................................................. 12
- CHAPTER 4  Perform presence/absence analysis .................................... 13
  Select the Presence Absence Analysis Module .................................... 13
  Review results in the Amplification Plot ....................................... 13
  Edit Presence Absence Analysis Setting ....................................... 14
    Edit Target Call Rules ............................................................. 14
    Edit Tests settings ....................................................................... 15
    Edit Sample Call Rules ............................................................ 15
    Edit Control Rules ................................................................. 16
  Review presence/absence calls .................................................... 16
  Omit outliers from presence/absence analysis .................................... 17
  (Optional) Add a sample comment ............................................... 17
  (Optional) Review dye signal profile in the Multicomponent Plot .............. 18
  (Optional) Review signal profile in the Raw Data Plot ........................... 18
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”.

Compatible data files

The software is compatible with data files for the following real-time PCR instruments:

• QuantStudio™ 7 Pro Real-Time PCR System (including TaqMan™ Array Card format)
• QuantStudio™ 6 Pro Real-Time PCR System
• QuantStudio™ 5 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)
• QuantStudio™ 3 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)
• QuantStudio™ 1 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)

Data files for the following instruments are a legacy file format that can be opened in the software, but can only be saved as the updated file format:

• QuantStudio™ 5 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
• QuantStudio™ 3 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
• QuantStudio™ 1 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
• QuantStudio™ 6 Flex Real-Time PCR System
• QuantStudio™ 7 Flex Real-Time PCR System (including TaqMan™ Array Card format)
• QuantStudio™ 12K Flex Real-Time PCR System (except OpenArray™ format)
• StepOnePlus™ Real-Time PCR System
• ViiA™ 7 Real-Time PCR System
• 7500/7500 Fast Real-Time PCR System
• 7900HT Real-Time PCR System
Note: To convert a legacy data file into the updated file format, open the data file, then click Actions > Save As.
Workflow: Presence/absence analysis

- Set up a plate file for presence/absence analysis (page 8)
- Select a system template or existing plate file to set up a new plate file (page 8)
- Confirm or edit the run method for presence absence analysis (page 9)
- Confirm or edit the plate setup for presence/absence analysis (page 10)
- Review and save the plate file (page 12)
- Perform presence/absence analysis (page 13)
  - Select the Presence Absence Analysis Module (page 13)
  - Review results in the Amplification Plot (page 13)
  - Edit Presence Absence Analysis Setting (page 14)
  - Review presence/absence calls (page 16)
  - Omit outliers from presence/absence analysis (page 17)
  - (Optional) Add a sample comment (page 17)
  - (Optional) Review dye signal profile in the Multicomponent Plot (page 18)
  - (Optional) Review signal profile in the Raw Data Plot (page 18)
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 13).
3. Navigate to, then select a system template or plate file.

<table>
<thead>
<tr>
<th>Tab</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>System Templates</td>
<td>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.</td>
</tr>
<tr>
<td>My Plate Files</td>
<td>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.</td>
</tr>
<tr>
<td>Recents</td>
<td>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.</td>
</tr>
</tbody>
</table>

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

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. ΔRn 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.
Chapter 3 Set up a plate file for presence/absence analysis

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

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

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Sample name</td>
</tr>
<tr>
<td>Color</td>
<td>Sample color</td>
</tr>
<tr>
<td>Type[1]</td>
<td>Presence absence analysis uses the following sample types.</td>
</tr>
<tr>
<td></td>
<td>• Unknown</td>
</tr>
<tr>
<td></td>
<td>• Positive Control</td>
</tr>
<tr>
<td></td>
<td>• Negative Control</td>
</tr>
</tbody>
</table>

[1] For more information, see “Sample types for presence/absence analysis” on page 19.

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.

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.

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Target name</td>
</tr>
<tr>
<td>Color</td>
<td>Target color</td>
</tr>
</tbody>
</table>
(continued)

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task[1]</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>• Unknown</td>
</tr>
<tr>
<td></td>
<td>• Positive Control</td>
</tr>
<tr>
<td></td>
<td>• Negative Control</td>
</tr>
<tr>
<td></td>
<td>• IPC (Internal Positive Control)[2]</td>
</tr>
<tr>
<td></td>
<td>• Blocked IPC[2]</td>
</tr>
</tbody>
</table>

[1] For more information, see “Sample types for presence/absence analysis” on page 19.
[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

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 reagent **Name**, **Type**, **Barcode**, **Part Number**, **Lot Number**, and **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

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**.

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.
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**

<table>
<thead>
<tr>
<th>Control</th>
<th>Target</th>
<th>Expected Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No template control</td>
<td>IPC</td>
<td>Amplification</td>
</tr>
<tr>
<td></td>
<td>Target</td>
<td>No amplification</td>
</tr>
<tr>
<td>No amplification control (NAC; blocked IPC)</td>
<td>IPC</td>
<td>No amplification</td>
</tr>
<tr>
<td></td>
<td>Target</td>
<td>No amplification</td>
</tr>
<tr>
<td>Positive control</td>
<td>IPC</td>
<td>Amplification</td>
</tr>
<tr>
<td></td>
<td>Target</td>
<td>Amplification</td>
</tr>
</tbody>
</table>

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 Target Call Rules

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

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-PCR Read</td>
<td>ΔRn = Rn_{(post-PCR read)}</td>
</tr>
<tr>
<td>Pre-PCR Read and Post-PCR Read</td>
<td>ΔRn = Rn_{(post-PCR read)} − Rn_{(pre-PCR read)}</td>
</tr>
<tr>
<td>Real-time Rn Data</td>
<td>ΔRn = Rn_{(last PCR cycle)} − Rn_{(first PCR cycle)}</td>
</tr>
</tbody>
</table>

   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 9).

2. In the Target Call Rules tab, in the table:
   - Click ➔ (Add) to add a new target call rule.
   - Click in a cell to edit the following settings if needed.
     - Sample Name
     - Target
     - \( C_q \) Cutoff
     - ΔRn Threshold
   - Click ☺ (Remove) to delete a target call rule.

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

   The data is 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 (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.

Edit Sample Call Rules

For detailed information about sample call rules and example sample call rule settings, see “About sample call rules” on page 21.

1. In the Presence Absence Analysis Setting window, in the Sample Call Rules tab, click (Add) to add a new sample call rule.

2. In the table, click in a cell to edit the following settings if needed.
   - Sample Name
   - Presence Targets
   - Absence Targets
   - Test Code (see “Edit Tests settings” on page 15 for more information)
   - Call
   - Assessment

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

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

The data is reanalyzed using the updated analysis settings.
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

To enable well calls, you must define sample call rules in the Presence Absence Analysis Settings (see “Edit Presence Absence Analysis Setting” on page 14).

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:

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
</table>
| Plate layout | 1. In the plate layout pane, in the Color By dropdown list, select Sample.  
              2. Hover over a well to see the individual calls for each target in the well.                                                       |
| Target Call  | In the table pane, click Target Call, then review the call, $C_q$, and $\Delta Rn$ for each target in a well.                                      |
| Table        | For more information, see “About call types” on page 20.                                                                                    |
In the Presence Absence tab, use one of the following options to review well calls:

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
</table>
| Plate layout         | 1. In the plate layout pane, in the Color By dropdown list, select Sample.  
                     | 2. Review the call for each well sample, as indicated by the icon displayed in the middle of the well:  
                     |   - ➕ (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  

Note:  
- 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.

Well Call Table  
In the table pane, click Well Call, then review the calls.  
For more information, see “About call types” on page 20.

Sample Call Table  
In the table pane, click Sample Call, then review the calls.  
For more information, see “About call types” on page 20.

Control Status Table  
In the table pane, click Control Status, then review the calls.


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.

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omit wells in the Plate Layout</td>
<td>Select outlier wells, then click ⋯ (Actions) &gt; Omit Wells.</td>
</tr>
<tr>
<td>Omit wells in the Target Call Table</td>
<td>Select Omit in the row of the outlier well.</td>
</tr>
</tbody>
</table>

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.
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 Rn.
- If endpoint experiments include pre-PCR data points, the software calculates the ΔRn value according to the following formula:
  \[ \Delta Rn = Rn_{(\text{post-PCR read})} - Rn_{(\text{pre-PCR read})} \]
  where \( Rn \) = 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.

<table>
<thead>
<tr>
<th>Sample type (Type column in Samples table)</th>
<th>Sample description</th>
<th>Target task assignment(^{(1)}) (Task column in Targets table)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>- Test sample</td>
<td>- Unknown</td>
</tr>
<tr>
<td></td>
<td>- IPC template</td>
<td>- IPC(^{(2)})</td>
</tr>
<tr>
<td>Negative control</td>
<td>No template control(^{(3)})</td>
<td>- Negative control</td>
</tr>
<tr>
<td></td>
<td>- Water or buffer</td>
<td>- IPC(^{(1)})</td>
</tr>
<tr>
<td></td>
<td>- IPC template</td>
<td></td>
</tr>
</tbody>
</table>
(continued)

<table>
<thead>
<tr>
<th>Sample type (Type column in Samples table)</th>
<th>Sample description</th>
<th>Target task assignment (Task column in Targets table)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>No amplification control (NAC; blocked IPC)</td>
<td>Negative control</td>
</tr>
<tr>
<td></td>
<td>- Water or buffer plus a blocking agent</td>
<td>Blocked IPC [1]</td>
</tr>
<tr>
<td></td>
<td>- IPC template; amplification prevented by blocking agent</td>
<td></td>
</tr>
</tbody>
</table>

[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.  

- Singleplex presence/absence experiments without IPC

<table>
<thead>
<tr>
<th>Sample type (Type column in Samples table)</th>
<th>Sample description</th>
<th>Target task assignment (Task column in Targets table)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>Test sample</td>
<td>Unknown</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Water or buffer</td>
<td>Negative Control</td>
</tr>
</tbody>
</table>

[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.

<table>
<thead>
<tr>
<th>Call type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Call</td>
<td>- Each target call is for one particular target in a particular well.</td>
</tr>
<tr>
<td></td>
<td>- A target call can be presence or absence, based on the target call rules.</td>
</tr>
<tr>
<td>Well Call</td>
<td>- Each well call is for one particular test in a particular well.</td>
</tr>
<tr>
<td></td>
<td>- 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.</td>
</tr>
<tr>
<td></td>
<td>- For multiple tests in the same well, the Well Call Table has multiple rows for the same well, with one row for each test.</td>
</tr>
</tbody>
</table>
(continued)

<table>
<thead>
<tr>
<th>Call type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Call</td>
<td>• Each sample call is for one particular test of one particular sample.</td>
</tr>
<tr>
<td></td>
<td>• 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.</td>
</tr>
<tr>
<td></td>
<td>• If the same sample has different calls in different wells for the same test, the sample call becomes inconclusive for that particular test.</td>
</tr>
</tbody>
</table>

**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 14).

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

**Table 2 Sample call rule table settings**

<table>
<thead>
<tr>
<th>Sample call rule setting</th>
<th>Description</th>
</tr>
</thead>
</table>
| Sample Name              | 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. The sample name can contain wildcard characters, such as * for any number of characters and ? for exactly one character. For example:  
  • "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                | *(Optional)* 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. |
Table 2  Sample call rule table settings  (continued)

<table>
<thead>
<tr>
<th>Sample call rule setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Call</td>
<td>Select one of the following call options:</td>
</tr>
<tr>
<td></td>
<td>• Presence—The target nucleic acid sequence is present in the sample</td>
</tr>
<tr>
<td></td>
<td>• Absence—The target nucleic acid sequence is absent in the sample</td>
</tr>
<tr>
<td></td>
<td>• Warning—The sample data needs review for possible errors</td>
</tr>
<tr>
<td></td>
<td>• Inconclusive—A well call cannot be made</td>
</tr>
<tr>
<td></td>
<td>• Invalid—The IPC failed</td>
</tr>
<tr>
<td>Assessment</td>
<td>(Optional) Enter an assessment or recommended action for the sample call.</td>
</tr>
</tbody>
</table>

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 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>Target 1</td>
<td>—</td>
<td>Invalid</td>
<td>Repeat test</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Target 2</td>
<td>—</td>
<td>Absence</td>
<td>Report results</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Target 3</td>
<td>—</td>
<td>Inconclusive[4]</td>
<td>Repeat test</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Target 1</td>
<td>—</td>
<td>Inconclusive[4]</td>
<td>Repeat test</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Target 2</td>
<td>—</td>
<td>Inconclusive[4]</td>
<td>Repeat test</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Target 3</td>
<td>—</td>
<td>Inconclusive[4]</td>
<td>Repeat test</td>
</tr>
</tbody>
</table>
Table 3  Example sample call rule settings  *(continued)*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target 1</td>
<td>Target 3</td>
<td></td>
<td>Presence[4]</td>
<td>Report results</td>
</tr>
<tr>
<td></td>
<td>Target 2</td>
<td>Target 1</td>
<td></td>
<td>Presence[4]</td>
<td>Report results</td>
</tr>
<tr>
<td></td>
<td>Target 3</td>
<td>Target 2</td>
<td></td>
<td>Presence[4]</td>
<td>Report results</td>
</tr>
<tr>
<td></td>
<td>Target 1</td>
<td>Target 3</td>
<td></td>
<td>Presence[4]</td>
<td>Report results</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Target 1</td>
<td>—</td>
<td></td>
<td>Presence</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Target 2</td>
<td>—</td>
<td></td>
<td>Presence</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Target 3</td>
<td>IPC</td>
<td></td>
<td>Absence</td>
<td>—</td>
</tr>
</tbody>
</table>

[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.


[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

<table>
<thead>
<tr>
<th>Document</th>
<th>Publication number</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantStudio™ Design and Analysis Software v2 User Guide</td>
<td>MAN0018200</td>
</tr>
<tr>
<td>QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro</td>
<td>MAN0018045</td>
</tr>
<tr>
<td>Real-Time PCR System User Guide</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro</td>
<td>MAN0017992</td>
</tr>
<tr>
<td>Real-Time PCR System Site Preparation Guide</td>
<td></td>
</tr>
</tbody>
</table>

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.
A
Amplification Plot, review 13
analysis module, select 13
analysis settings
  edit 14
Tests 15

C
Calls, review 16, 20
compatible data file types 5
control rules, edit 16

L
limited product warranty 24

M
MultiComponent Plot, review 18

O
omit wells, Presence Absence 17

P
passive reference, select 11
plate file
  analysis type 8
  create 8
  save 12
  set up 8, 9
plate setup
  samples 10
  targets 10
presence absence analysis, samples 10

presence absence analysis module, about 5
presence/absence analysis
  IPC 19
  sample types 19
  workflow 7

R
Raw Data Plot, review 18
reagents, information 11
related documentation 24
run method 9

S
sample call rules, edit 15
Sample Call Table, review calls 16
sample comment, Presence Absence 17
sample types, presence/absence analysis 19
samples 10
standard curve analysis, targets 10
support, customer and technical 24

T
target 10
target call rules, edit 14
Target Call Table, review calls 16
terms and conditions 24
Tests, analysis settings 15

W
warranty 24
Well Call Table, review calls 16
workflow 7