

QuantStudio™ Design and Analysis Software v2 Command-Line Application

USER GUIDE

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About this guide

Overview

This guide provides technical information for integration of the instrument into a laboratory information system (LIS).

The guide describes the following:

- Command-line interface, which can be used to automate the creation of new experiment template files (EDT files) and the export of data from existing results files (EDS files).
- Specifications of importable and exportable file formats, which are compatible with and generated by the QuantStudio™ Design and Analysis Software v2.

Assumptions for use of this guide

- All software elements (QuantStudio™ Design and Analysis Software v2 and other applications) reside and execute on the same local area network (LAN) or on the same physical computer.
- The QuantStudio™ Design and Analysis Software v2 has been correctly installed.



Command-line application

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About the command-line application

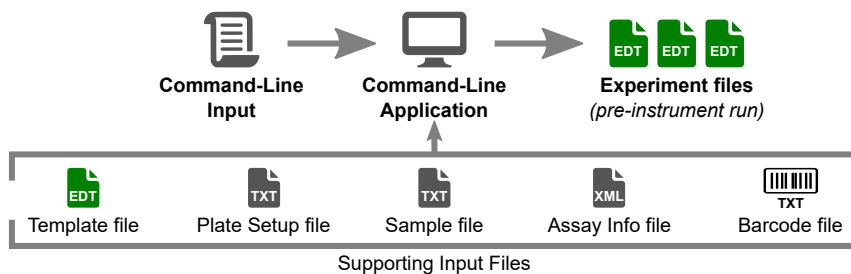
The QuantStudio™ Design and Analysis Software v2 includes a command-line application that allows you to generate template files (EDT files) and export result files (EDS files) from an MS DOS prompt or a batch file. The application is intended for advanced users who choose to create or export experiments using a scripting language.

IMPORTANT! After you use the command-line application to generate experiment files, validate the contents of the files by opening them in the QuantStudio™ Design and Analysis Software v2.

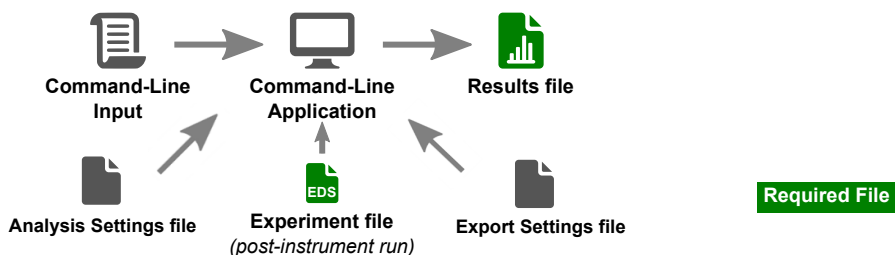
Command-line application workflows

The command-line application supports the workflows in the following figure. For each workflow, the figure shows both the required and optional supporting files.

Experiment file creation workflow



Export workflow



Supporting files

File	Description
assay information file (AIF or AIX)	A tab-delimited or XML data file that is provided by Thermo Fisher Scientific with each assay. The file, which contains data describing the assay, can be imported into the QuantStudio™ Design and Analysis Software v2 for use in related experiments. See “Assay information file” on page 29.
barcode file (TXT)	A user-created, line-separated text file that contains the barcode of each consumable for which you want to create an experiment file. See “Barcode file format” on page 29.
experiment document single file (EDS)	A QuantStudio™ Design and Analysis Software v2 file that contains all information about a particular plate or array card consumable, including metadata (name, barcode, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.

(continued)

File	Description
experiment document template file (EDT)	A QuantStudio™ Design and Analysis Software v2 file used as a template to create experiment files. The file can contain plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, and other plate-specific data.
plate setup file (TXT)	A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the instrument. The file defines the arrangement of assays and samples on the consumable. See “Plate setup files” on page 19.
sample file (TXT)	A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ Design and Analysis Software v2 for use in related experiments. See “Sample files” on page 28.
export settings file (JSON)	A QuantStudio™ Design and Analysis Software v2 file that defines the result column names and the arrangement for the exported results files.
analysis settings file	A QuantStudio™ Design and Analysis Software v2 file used as a template to contain primary analysis and secondary analysis settings. This option can be used for the analysis of legacy EDS or SDS files when a specific set of primary and secondary analysis settings are needed. The primary and secondary analysis settings will be used when the analysis (-a) option is used.

Precedence rules to generate experiment files

When generating experiment template files (EDT files), the QuantStudio™ Design and Analysis Software v2 command-line interface relies on a set of precedence rules (see the following table) to resolve conflicts that arise from the data supplied by the input files.

Many input files can contain data used to populate the same fields of new experiment files. For example, both template files and plate setup files can contain well assignments for samples and assays.

Files used to generate EDS file	Precedence rule
<ul style="list-style-type: none"> Template file (EDT) 	<p>The values in the template file take precedence except for:</p> <ul style="list-style-type: none"> Experiment name—Determined by the File Name Convention preference. Barcode—Determined by the barcode, if present. Otherwise, the value is null. Experiment file name—Determined by the File Name Convention preference.

(continued)

Files used to generate EDS file	Precedence rule
<ul style="list-style-type: none"> • Template file (EDT) • Assay information file (AIF or AIX) 	<p>All values in the template file take precedence, except for:</p> <ul style="list-style-type: none"> • Target / SNP assay definition • Passive reference <p>If any conflicts exist between the assay information file and the template file for the attributes above, then the assay information file values always take precedence.</p>
<ul style="list-style-type: none"> • Template file (EDT) • Plate setup file (TXT) 	<p>All values in the template file take precedence, except for:</p> <ul style="list-style-type: none"> • Target / SNP assay well assignment • Sample color • Sample well assignment • Biological group color • Task well assignment • Target / SNP assay definition • Biological group well assignment • Target / SNP assay color • Well quantity to well assignment • Passive reference
<ul style="list-style-type: none"> • Template file (EDT) • Plate setup file (TXT) • Assay information file (AIF or AIX) • Sample file (TXT) 	<p>All values in the template file take precedence, except for the following.</p> <p>Assay information file values that take precedence over plate setup file and template file values:</p> <ul style="list-style-type: none"> • Target/Assay/Detector/Marker definition • Passive reference <p>Sample file values that take precedence over plate setup file and template file values:</p> <ul style="list-style-type: none"> • Sample name • Sample well assignment <p>Plate setup file values that take precedence over template file values:</p> <ul style="list-style-type: none"> • Block type • Well quantity well assignment • Target/Assay/Marker well assignment • Sample color • Sample well assignment • Biological group color • Task well assignment • Target/SNP assay color • Biological group well assignment

Run the command-line application from a command prompt

Set up the application (Windows™ operating system)

If the software was installed for a single user, the command-line interface is installed at `C:\Users\
<user name>\AppData\Local\Programs\Design and Analysis\resources\bin`.

If the software was installed for all Windows™ operating system users, the command-line interface is installed at `C:\Program Files\Design and Analysis\resources\bin`.

To access the command-line interface without specifying the full path above, you can add the path to your `Path` environmental variable as follows.

1. In the Windows™ task bar, search *environment*.
2. Select the **Edit the system environment variables** search result, then click **Environment Variables**.
3. Select the **Path** variable in the table, then click **Edit...**
4. Click **New**, and add the path to the command-line interface to the end of the list.
5. If you currently have a command prompt window open, close and reopen the window.
6. In the command prompt window, enter `da-cli -h` to open the help contents and verify the command-line interface is working.

Set up the application (Macintosh™ operating system)

The command-line interface is installed at `/Applications/Design\ and\
Analysis.app/Contents/Resources/bin/da-cli.sh`.

To access the command-line interface without specifying the full path above, you can update the `.bash_profile` as follows.

1. Grant execute permission to the `da-cli.sh`.
 - a. Open the terminal and navigate to `/Applications/Design\ and\
Analysis.app/Contents/Resources/bin/`.
 - b. Enter `chmod +x da-cli.sh`.
2. Make the `da-cli.sh` script available from the terminal.
 - a. Open the terminal, and enter `cd ~` to navigate to user home.
 - b. Edit the `.bash_profile` using an available editor, such as `vi`, and set the `PATH`:
 1. `export DA_HOME=/Applications/Design\ and\ Analysis.app/Contents/Resources`
 2. `export PATH=$PATH:$DA_HOME/bin:`

- c. Open a new terminal, and enter `da-cli.sh -h` to open the help contents and verify the command-line interface is working.

Run the application (Windows™ operating system)

1. On your computer, select **Start ▶ Run**.
2. In the **Run** dialog box, enter `cmd` in the **Open** field, then click **OK**.
3. Given the application has been set up in a Windows™ operating system:
 - a. Enter `da-cli`, followed by `expgen` or `export`, then all applicable parameters and arguments. See “Command syntax and arguments” on page 12 for a complete list of command-line parameters.

Run the application (Macintosh™ operating system)

1. On your computer, open the terminal.
2. Given the application has been set up in a Macintosh™ operating system:
 - a. Enter `da-cli.sh`, followed by `expgen` or `export`, then all applicable parameters and arguments. See “Command syntax and arguments” on page 12 for a complete list of command-line parameters.

View the command-line application Help

The command-line application includes a help function that provides the information in this chapter.

- To view help for the entire application:

- ```
da-cli -help
```

## Command syntax and arguments

### Syntax and arguments used for batch file creation

The command used to create batches of template files (EDT files) uses the following syntax:

```
da-cli expgen [parameters]
```

The following is a list of the acceptable parameters that can be included in any order. See “Example commands to create templates” on page 15 for examples of the experiment creation command.

---

**IMPORTANT!** Enclose file paths in double quotes to allow spaces in the string.

---

- **-a** *<filepath>*  
(Optional) Specifies the path and name (*<filepath>*) of the assay information file (AIF or AIX) that the software uses to create new experiment files (EDS files).  
Example: -a "C:\assayfiles\assayfile.aif"
- **-b** *<filepath>*  
(Optional) Specifies the path and name (*<filepath>*) of the barcode file that the software uses to create new files. If the **-b** parameter is not used, then the software creates the number of experiments specified by the **-n** parameter.  
Example: -b "C:\barcodefiles\barcodefile.txt"
- **-c** *<string>*  
(Optional) When the **-c** parameter is included, specifies the alphanumeric string that the software includes in the file names of the new experiments. If no value is supplied, "custom" is used as the default value.  
Example: -c "Batch001\_"
- **-f** *<option>*  
(Optional) Specifies the convention that the software uses to name the new files. The convention can consist of all or some of the following interchangeable arguments, in any order:
  - Custom Name Field—The alphanumeric string specified by the **-c** parameter.
  - ID—The barcode of the plate specified in the barcode file specified by the **-b** parameter.
 Example: -f "Custom Name Field\_ID"  
If the **-f** parameter is used without arguments, then the software names files according to the following convention: "Custom Name Field\_ID".
- **-l** *<dirpath>*  
(Required) Specifies the path of the directory (*<dirpath>*) to which the software saves the new files.  
Example: -l "C:\Applied Biosystems\QuantStudio Design and Analysis Software v2\User Files\experiments"  
Before creating experiment template files (EDT files), the software confirms whether the destination directory exists and aborts if the location does not exist.
- **-m** *<filepath>*  
(Optional) Specifies the path (*<path>*) of a sample file or a directory containing sample files that the software uses during the analysis and export of results. The sample name will be updated in the save as file if the **-o** option is used. The **-b** option to include the barcode will be ignored if the

specified `-m` option is a directory, in which case the file name containing the samples is assumed as the barcode.

Examples:

```
-m "C:\setupfiles\samplefilesdirectory"
-m "C:\setupfiles\sample.txt"
```

- **-n** *<integer>*  
(Optional) If the `-b` parameter is not included, specifies number of experiments (*<integer>*) that the software will create. If no value is supplied, the software creates 25 experiments by default.  
Example: `-n 31`
- **-s** *<filepath>*  
(Optional) Specifies the path and name (*<filepath>*) of the plate setup file that the software uses to create new files.  
Example: `-s "C:\setupfiles\setupfile.txt"`
- **-t** *<filepath>*  
(Required) Specifies the path and name (*<filepath>*) of the QuantStudio™ Design and Analysis Software v2 template file that the software uses to create new files.  
Example: `-t "C:\Applied Biosystems\QuantStudio Design and Analysis Software v2\User Files\experiments\templates\standard_curve.edt"`
- **-v**  
(Optional) Configures the software to operate in verbose mode, where the software displays each operation as it is performed.

## Syntax and arguments used for results export

The command used to export the results from experiment files (EDS files) uses the following syntax:

```
da-cli export [parameters]
```

The following table lists the acceptable parameters that can be included in any order. See “Example commands for results export” on page 16 for examples of the export command.

---

**IMPORTANT!** Enclose file paths in double quotes to allow spaces in the string.

---

- **-e** *<dirpath>*  
(Required) Specifies the path (*<filepath>*) of a specific file or the path of the directory (*<dirpath>*) that contains the experiment files (EDS files) for which the software exports data.  
Example: `-e "C:\Applied Biosystems\QuantStudio Design and Analysis Software v2\User Files\experiments"`
- **-f** *<option>*  
(Required) Specifies the format of the exported data (see “About the exportable files” on page 30 for the export file specifications):
  - **QuantStudio**—Exports data in a native format compatible with the QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems, QuantStudio™ 3 and 5 Real-Time PCR Systems, and QuantStudio™ 6 and 7 Flex Real-Time PCR Systems.
  - **RDML**—Exports data in the real-time data markup language (RDML) format. Example: `-f "RDML"`

- **-l** *<path>*  
(Optional) Specifies the path (*<path>*) of the directory to which the software saves the exported files.  
Example: `-l "C:\exports\"`
- **-m** *<filepath>*  
(Optional) Specifies the path (*<path>*) of the sample file that the software uses during the analysis and export of results. The sample name will be updated in the save as file if the `-o` option is used.  
Example: `-m "C:\setupfiles\samples.txt"`
- **-n** *<filepath>*  
(Optional) Specifies the path (*<path>*) of the analysis settings file that the software uses to obtain the primary and secondary analysis settings. The primary and secondary analysis settings will be used when the analysis (`-a`) option is used.  
Example: `-n "C:\setupfiles\sds7500AnalysisSettingFile.edt"`
- **-o** *<filepath>*  
(Optional) Specifies the path (*<path>*) of the save as file that the software uses to save as the experiment file. The save as file will be saved in a native format compatible only with the QuantStudio™ Design and Analysis Software v2.  
Example: `-o "C:\export\saveAsSds7500.eds"`
- **-s** *<option>*  
(Optional) Specifies the data spanning option (*<option>*) that determines how the software exports data from multiple experiments:
  - **single**—Exports data for all experiments into one single XLS or XLSX file.
  - **multiple**—Exports data for each experiment to a separate XLS or XLSX file.Example: `-s "multiple"`
- **-t** *<filepath>*  
(Optional) Specifies the path (*<filepath>*) of the export settings file which is exported from the QuantStudio™ Design and Analysis Software v2.  
Example: `-t "C:\export\export-settings.json"`
- **-x** *<filepath>*  
(Required) Specifies the file format of the exported file:
  - QuantStudio export format—TXT, CSV, XLS, or XLSX
  - RDML export format—RDMLExample: `-x "rdml"`

## Example commands to create templates

### Create EDT files with sample assignments from a directory

This example requires preparing one or more sample files in a directory with each sample file name representing the plate barcode and using the `expgen` command. The generated EDT files will contain sample assignments.

---

**Note:** In this example, the sample file names are assumed as the plate barcode and the `-b` option is ignored.

---

1. Prepare the template file containing the target assignments and run method (without sample assignments and barcode).
2. Prepare the sample file containing sample assignments.
3. Name the sample files with the barcode of the corresponding plate.
4. Place all the sample files under a single directory.
5. Run the command-line application.

```
expgen -t "/Users/user1/cli/create/template-file/qs7pro-9602.edt" -l "/Users/user1/cli/create/destination" -m "/Users/user1/cli/create/sample-file-directory"
```

Assuming the sample file directory folder contains two files (`barcode-1.txt` containing the sample assignments for the plate `barcode-1` and `barcode-2.txt` containing the sample assignments for the plate `barcode-2`), the example above will generate the following two EDT files.

- `barcode-1.edt` with sample assignments from `barcode-1.txt` and barcode `barcode-1`
- `barcode-2.edt` with sample assignments from `barcode-2.txt` and barcode `barcode-2`

### Create EDT files with sample assignments from a file

This example requires preparing a sample file and a barcode file for the plate and using the `expgen` command. The generated EDT file will contain sample assignments.

1. Prepare the template file containing the target assignments and run method (without sample assignments and barcode).
2. Prepare the sample file containing sample assignments.
3. Prepare the barcode file containing one barcode.
4. Run the command-line application.

```
expgen -t "/Users/user1/cli/create/template-file/qs7pro-9602.edt" -l "/Users/user1/cli/create/destination" -m "/Users/user1/cli/create/sample-file.txt" -b "/Users/user1/cli/create/barcode-file/barcode-file.txt" -f "ID"
```

Assuming the `barcode-file1.txt` contains `barcode-1`, the example above will generate the following EDT file.

- `barcode-1.edt` with sample assignments from `sample-file1.txt` and barcode `barcode-1`

## Create EDT files without sample assignments

This example requires preparing only one barcode file (with multiple barcodes) and using the `expgen` command only once to generate multiple EDT files. The generated EDT files will have no sample assignments. The sample assignments can be performed after the run using the `export` command (see “Example commands for results export” on page 16).

1. Prepare the template file containing the target assignments and run method (without sample assignments and barcode).
2. Prepare the barcode file with multiple barcodes.
3. Run the command-line application.

```
expgen -t "/Users/user1/cli/create/template-file/qs7pro-9602.edt" -l "/Users/user1/cli/create/destination" -b "/Users/user1/cli/create/barcode-file/barcode-file.txt" -f "ID"
```

Assuming the `barcode-file.txt` contains two lines of barcodes (`barcode-1` and `barcode-2`), the example above will generate the following two EDT files and both will have no sample assignments.

- `barcode-1.edt` with barcode `barcode-1`
- `barcode-2.edt` with barcode `barcode-2`

## Example commands for results export

### Export multiple EDS/SDS files

If all of the EDS/SDS files contain sample assignments, you can export the results for all of them by using the command-line application once.

1. Place all of the EDS/SDS files to export in the same folder.
2. Prepare the export settings file (`export-settings.json` in the example below) using the QuantStudio™ Design and Analysis Software v2.
3. If the analysis settings need to be updated, prepare an EDT file (`7500-setting-template.edt` in the example below) with the desired analysis settings using the QuantStudio™ Design and Analysis Software v2. The EDT file should use the same run method as your EDS/SDS file, with expected targets and expected samples (unknown samples that change from plate to plate should not be included).
4. Run the command-line application.

---

**Note:** `-a` is optional when `-m` or `-n` is used.

---

```
export -a -e "/Users/user1/cli/export/qs7pro-eds" -n "/Users/user1/cli/export/7500-setting-template.edt" -l "/Users/user1/cli/export/destination" -f "QuantStudio" -t "/Users/user1/cli/export/export-settings.json" -x "csv"
```



The above example generates multiple CSV files. The file names are prefixed with the corresponding EDS/SDS file name and they are located in the destination folder according to the `export-settings.json`.

## Export single EDS/SDS files

If your EDS/SDS files do not contain sample assignments, you can perform the sample assignments and export by using the command-line application once per EDS/SDS file.

1. Prepare the export settings file (`export-settings.json` in the example below) using the QuantStudio™ Design and Analysis Software v2.
2. If the analysis settings need to be updated, prepare an EDT file (`7500-setting-template.edt` in the example below) with the desired analysis setting using the QuantStudio™ Design and Analysis Software v2. The EDT file should use the same run method as your EDS/SDS file, with expected targets and expected samples (unknown samples that change from plate to plate should not be included).
3. Prepare the sample file (`samples.txt` in the example below).
4. Run the command-line application.

---

**Note:** `-a` is optional when `-m` or `-n` is used.

---

```
export -a -e "/Users/user1/cli/export/sds7500.sds" -l "/Users/
user1/cli/export/destination" -f "QuantStudio" -m "/Users/user1/cli/export/
samples.txt" -n "/Users/user1/cli/export/7500-setting-template.edt" -t "/
Users/user1/cli/export/export-settings.json" -x "csv"
```

The above example generates multiple CSV files. The file names are prefixed with the EDS/SDS file name and they are located in the destination folder according to the `export-settings.json`.



# Import file formats and specifications

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## About the importable files

The QuantStudio™ Design and Analysis Software v2 supports several import file formats that can be used to automate creating experiments and importing assay and sample data. The files can be used with the command-line application (see Chapter 2, “Command-line application”) to integrate the instrument into a laboratory information system (LIS).

---

**Note:** The file specifications listed in this appendix are subject to change. For updated information, review the release notes for the QuantStudio™ Design and Analysis Software v2.

---

## About the import file formats

| File format                         | Description                                                                                                                                                                                                                              | See     |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Plate setup file (TXT)              | A user-created, tab-delimited text file that describes the layout of a reaction consumable for an experiment to be run on the instrument. The file defines the arrangement of assays and samples on the reaction consumable.             | page 19 |
| Sample file (TXT)                   | A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ Design and Analysis Software v2 for use in related experiments.                                                                | page 28 |
| Assay information file (AIF or AIX) | A tab-delimited or XML data file that is provided by Thermo Fisher Scientific. The file, which contains data describing the assay, can be imported into the QuantStudio™ Design and Analysis Software v2 for use in related experiments. | page 29 |
| Barcode file (TXT)                  | A user-created, text file containing the barcodes of consumables for which you want to create experiment files (EDS files) using the command-line application.                                                                           | page 29 |

## Conventions

The following conventions are used in the remainder of this section:

- `normal`  
Normal text must be entered exactly as it appears.
- `< text >`  
Italicized text between brackets must be substituted with custom values.
- `[ required text ]`  
Text appearing between brackets is required information. All information inside the brackets must be present for the QuantStudio™ Design and Analysis Software v2 to import it.
- `{ optional text }`  
Text appearing between braces is optional.
- Unless noted otherwise, separate all fields in a row using a tab character (U+0009).
- Unless noted otherwise, end all rows using a carriage-return character (U+000D).

## Plate setup files

You can use plate setup files to automatically populate setup information into an open experiment in the QuantStudio™ Design and Analysis Software v2 or into new experiments generated by the command-line application (see page 6).

A plate setup file is a tab-delimited ASCII text file (TXT file) that contains data that describes the arrangement of assays and samples on the reaction consumable. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**Note:** To ensure successful import of the plate setup file into an experiment, the file must contain all the elements described in the following sections and in the order that they appear.

---

## File structure

The plate setup file consists of a header, which specifies the instrument model for which the experiment is designed, and a sample setup section.

| Section                                        | Description                                                                                                  | See     |
|------------------------------------------------|--------------------------------------------------------------------------------------------------------------|---------|
| Header of plate setup file                     | Defines the instrument model for which the experiment is designed and the dye used as the passive reference. | page 20 |
| Body of plate setup file body (sample section) | Defines the contents of an instrument consumable, including target, SNP assay, sample, and task assignments. | page 21 |

## Plate setup file header

The plate setup file begins with a header that consists of two lines. Each line starts with an asterisk (\*) and ends with a carriage return in the following pattern:

```
* <field name> = <field value>
```

The header must contain the lines shown in the following table.

| Field             | Description                                                   | Valid Values                                                                                                                                                                                                                             |
|-------------------|---------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument Type   | The model of instrument for which the experiment is designed. | <ul style="list-style-type: none"> <li>QuantStudio 6 Pro or QuantStudio 7 Pro</li> </ul>                                                                                                                                                 |
| Passive Reference | The dye that the experiment will use as a passive reference.  | <ul style="list-style-type: none"> <li>The name of a dye in the Dye Library of the QuantStudio™ Design and Analysis Software v2<sup>[1]</sup></li> <li>Blank if the reaction consumable does not contain a passive reference.</li> </ul> |

<sup>[1]</sup> Custom dyes are allowed if they are in the Dye Library.

---

**Note:** The software automatically removes any leading and trailing white space around the field name and field value.

---

### Example (reaction consumable with a passive reference)

```
* Instrument Type = QuantStudio 6 Pro
* Passive Reference = ROX
```

### Example (reaction consumable with no passive reference)

```
* Instrument Type = QuantStudio 6 Pro
* Passive Reference =
```

## Plate setup file body

The body of a plate setup file contains either:

- Target information—Can be imported into all experiments except genotyping
- SNP assay information—Can be imported into genotyping experiments only

The body (sample setup) consists of three required elements (the header, the column header, and the body) that describe the contents of an instrument consumable. The sample setup column header and body can appear in any order.

---

**IMPORTANT!** Guidelines when creating a plate setup file:

- Do not insert blank lines between the sample setup header and the column header.
  - Do not use illegal characters, including backslash (\), tab, asterisk (\*), hard return, soft return, brackets([ or ]), or comma (,).
- 

### Sample setup header

The header contains the label that defines the beginning of the sample setup data.

**Example:**

```
[Sample Setup]
```

### Sample setup column headers

The column headers contain the headings that define the positions of the data columns in the sample setup body. The headings are separated by tab characters.

See “Plate setup file data columns” on page 22 for a list of the data column headers.

**Example:**

```
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name...
```

### Sample setup body

The body contains the sample setup data where each row defines the contents of a single well on the consumable, including:

- Well contents (sample, target, or SNP assay added to the well)
- Task assignments
- Comments

---

**Note:** If a well contains multiple assays (multiplex PCR), the data for the additional assays are defined on separate lines by repeating the well designation.

---

See “Plate setup file data columns” on page 22 for a list of the data column headers.

---

**Note:** The sample setup data rows can occur in any order.

---

**Example:**

| Well | Sample Name | Sample Color    | Biogroup Name | Biogroup Color | Target Name... |
|------|-------------|-----------------|---------------|----------------|----------------|
| 1    | Liver cDNA  | "RGB (25,0,0) " |               |                | CCKAR          |
| 2    | Liver cDNA  | "RGB (25,0,0) " |               |                | CCKAR          |
| 3    | Liver cDNA  | "RGB (25,0,0) " |               |                | CCKAR          |
| 4    | Heart cDNA  | "RGB (0,25,0) " |               |                | CCKAR          |
| 5    | Heart cDNA  | "RGB (0,25,0) " |               |                | CCKAR          |
| ...  |             |                 |               |                |                |

## Plate setup file data columns

The following table lists the headings and columns that are present in the body of plate setup files for experiments. Note the differences between genotyping and non-genotyping experiments.

| Column name                                   | Description                                                                                                                                                                       | Valid values                                             |
|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|
| <b>All experiments (including genotyping)</b> |                                                                                                                                                                                   |                                                          |
| Well                                          | The number of the well on the consumable <sup>[1]</sup>                                                                                                                           | <Positive integer (1 to 96/384)> <sup>[2]</sup>          |
| Sample Name                                   | The name of the sample in the associated well.                                                                                                                                    | <up to 100-character string>                             |
| Sample Color                                  | (Optional) The RGB color of the associated sample.                                                                                                                                | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                     |
| Biogroup Name                                 | (Optional) The name of the associated biological group.                                                                                                                           | <up to 100-character string>                             |
| Biogroup Color                                | (Optional) The RGB color of the biological group.                                                                                                                                 | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                     |
| Comments                                      | (Optional) Additional text that describes the well.                                                                                                                               | <up to 1024-character string>                            |
| <b>All experiments (except genotyping)</b>    |                                                                                                                                                                                   |                                                          |
| Target Name                                   | The name of the target detected or amplified by the assay in the associated well.                                                                                                 | <up to 100-character string> <sup>[4]</sup>              |
| Target Color                                  | (Optional) The RGB color of the target.                                                                                                                                           | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                     |
| Task                                          | The task assignment of the target assay in the well. <sup>[5]</sup>                                                                                                               | UNKNOWN   STANDARD   NTC   ENDOGENOUS   IPC   BlockedIPC |
| Reporter                                      | The reporter dye used by the associated target assay.                                                                                                                             | <dye name> <sup>[4,6]</sup>                              |
| Quencher                                      | The quencher dye used by the associated target assay.                                                                                                                             | <dye name> <sup>[4]</sup>                                |
| Quantity                                      | (Optional) The quantity of standard present in the given well expressed as a float or integer. If the associated well is not assigned the STANDARD task, then the field is blank. | <float or integer>                                       |

(continued)

| Column name                        | Description                                                                          | Valid values                                               |
|------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------|
| <b>Genotyping experiments only</b> |                                                                                      |                                                            |
| SNP Assay Name                     | The name of the SNP assay detected or amplified by the assay in the associated well. | <up to 100-character string> <sup>[4]</sup>                |
| SNP Assay Color                    | (Optional) SNP assay color in RGB                                                    | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                       |
| Task                               | The task assignment of the SNP assay in the well. <sup>[5]</sup>                     | UNKNOWN   NTC   PC_ALLELE_1   PC_ALLELE_2   PC_ALLELE_BOTH |
| Allele1 Name                       | The name of the first allele detected by the SNP assay.                              | <up to 100-character string> <sup>[4]</sup>                |
| Allele1 Color                      | The RGB color used to represent data for the first allele.                           | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                       |
| Allele1 Reporter                   | The reporter dye used to label the probe for the first allele.                       | <dye name> <sup>[4,6]</sup>                                |
| Allele1 Quencher                   | The quencher dye used to label the probe for the first allele.                       | <dye name> <sup>[6]</sup>                                  |
| Allele2 Name                       | The name of the second allele detected by the SNP assay.                             | <up to 100-character string> <sup>[4]</sup>                |
| Allele2 Color                      | The RGB color used to represent data for the second allele.                          | "RGB (<r>, <g>, <b>)" <sup>[3]</sup>                       |
| Allele2 Reporter                   | The reporter dye used to label the probe for the second allele.                      | <dye name> <sup>[4,6]</sup>                                |
| Allele2 Quencher                   | The quencher dye used to label the probe for the second allele.                      | <dye name> <sup>[6]</sup>                                  |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

<sup>[2]</sup> Cannot be blank.

<sup>[3]</sup> Contains (r)ed, (b)lue, and (g)reen color values between 0 to 255. The field must be set within double quotes with no spaces between the values.

<sup>[4]</sup> Can be empty if the Task field is empty. Otherwise, the field must contain a value.

<sup>[5]</sup> To determine the tasks applicable to your experiment, see the *QuantStudio™ Design and Analysis Software v2 User Guide* (Pub. No. MAN0018200).

<sup>[6]</sup> The dye must already exist in the Dye Library. The dye name must be 100 characters or less.

## Examples

### Quantitative PCR experiments

The following example shows a plate setup file created for a quantitative PCR experiment to be run on a QuantStudio™ 6 Pro Real-Time PCR System or a QuantStudio™ 7 Pro Real-Time PCR System.

The experiment evaluates the expression of two targets (CCKAR and GH1) in three samples (cDNA from the liver, heart, and brain). For both assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB). Biological groups are not used in this experiment.

```
* Instrument Type = QuantStudio 6 Pro or QuantStudio 7 Pro
* Passive Reference = ROX
[Sample Setup]
```

| Well | Sample Name | Sample Color  | Biogroup Name | Biogroup Color | Target Name | Target Color   | Task       | Reporter | Quencher | Quantity | Comment |
|------|-------------|---------------|---------------|----------------|-------------|----------------|------------|----------|----------|----------|---------|
| 1    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 2    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 3    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 4    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 5    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 6    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 7    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 8    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| 9    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |         |
| ...  |             |               |               |                |             |                |            |          |          |          |         |
| 376  | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 377  | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 378  | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 379  | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 380  | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 381  | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 382  | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 383  | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |
| 384  | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |         |

The following example shows a plate setup file for a multiplex version of the experiment above, where the assays for the two targets (CCKAR and GH1 targets) are added to the same well. For both assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB).



\* Instrument Type = QuantStudio 6 Pro or QuantStudio 7 Pro  
 \* Passive Reference = ROX

[Sample Setup]

| Well | Sample Name | Sample Color  | Biogroup Name | Biogroup Color | Target Name | Target Color   | Task       | Reporter | Quencher | Quantity | Comments |
|------|-------------|---------------|---------------|----------------|-------------|----------------|------------|----------|----------|----------|----------|
| 1    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 1    | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 2    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 2    | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 3    | Liver cDNA  | "RGB(25,0,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 3    | Liver cDNA  | "RGB(25,0,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 4    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 4    | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 5    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 5    | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 6    | Heart cDNA  | "RGB(0,25,0)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 6    | Heart cDNA  | "RGB(0,25,0)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 7    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 7    | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 8    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 8    | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |
| 9    | Brain cDNA  | "RGB(0,0,25)" |               |                | CCKAR       | "RGB(98,25,0)" | ENDOGENOUS | FAM      | NFQ-MGB  |          |          |
| 9    | Brain cDNA  | "RGB(0,0,25)" |               |                | GH1         | "RGB(0,0,105)" | UNKNOWN    | FAM      | NFQ-MGB  |          |          |

## Presence/absence experiments

The following example shows a plate setup file created for a presence/absence experiment to be run on a QuantStudio™ 6 Pro Real-Time PCR System or a QuantStudio™ 7 Pro Real-Time PCR System.

The experiment screens samples for the presence of a pathogen (*E. coli* 0157:H7). The detection assay uses FAM™ and VIC™ dye-labeled probes to amplify a unique genomic sequence and an internal positive control (IPC).

```
* Instrument Type = QuantStudio 6 Pro or QuantStudio 7 Pro
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name Target Color Task Reporter Quencher Quantity Comments
1 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
1 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
2 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
2 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
3 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
3 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
4 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
4 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
5 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
5 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
6 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
6 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
7 Blocked IPC "RGB(0,0,25)" E.coli "RGB(98,25,0)" BlockedIPC FAM NFQ-MGB
7 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
10 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
10 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
11 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
11 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
..
```

## Genotyping experiments

The following example shows a plate setup file created for a genotyping experiment to be run on a QuantStudio™ 6 Pro Real-Time PCR System or a QuantStudio™ 7 Pro Real-Time PCR System.

The experiment screens samples for one SNP targets (rs15934), using a set of allele-specific probes labeled with the FAM™ and VIC™ reporter dyes and the non-fluorescent quencher (NFQ-MGB).

```
* Instrument Type = QuantStudio 6 Pro or QuantStudio 7 Pro
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color SNP Assay Name SNP Assay Color Task Allele1 Name Allele1 Color Allele1 Reporter Allele1 Quencher Allele2 Name Allele2 Color Allele2 Reporter Allele2 Quencher Comments
1 Neg Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* NTC G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
2 Neg Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* NTC G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
3 Neg Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* NTC G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
4 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_1 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
5 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_1 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
6 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_1 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
7 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_2 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
8 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_2 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
9 All Control *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* PC_ALLELE_2 G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
10 Sample01 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
11 Sample01 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
12 Sample01 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
13 Sample02 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
14 Sample02 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
15 Sample02 *RGB(25,0,0)* SNP rs15934 *RGB(0,75,0)* UNKNOWN G *RGB(0,0,50)* VIC NFQ-MGB A *RGB(0,50,0)* FAM NFQ-MGB
..
```

## Sample files

You can use sample files to automatically populate sample information into new experiments generated by the command-line application (see page 6).

A sample file is a tab-delimited ASCII text file (TXT) that contains sample/well designations and custom sample properties. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**IMPORTANT!** The sample file must contain all the elements described in the following section and in the order that they appear.

The *sample file* used by the command-line application differs from the *sample definition file* used for direct import into open experiment files in the QuantStudio™ Design and Analysis Software v2. The sample file used by the command-line application requires well assignment data.

---

**Note:** When used concurrently with the command-line application, sample file values take precedence over plate setup file values, including sample well assignments.

---

## File structure

### Sample file header row

The sample file begins with an optional header row that contains column headers for well number (“Well”), sample name (“Sample Name”), and optional custom sample attributes names. The order of the columns is important and cannot be changed.

### Sample file body

The body is a set of rows that follows the optional header row, with each row containing the sample data. Each body row defines the sample information for a single well on the consumable, including: well number, sample name, and any applicable custom fields. The body can contain data for a subset of wells on the consumable, so the rows for empty wells can be omitted from the file. The sample body rows can occur in any order.

## Sample file data columns

| Column name            | Description                                                       | Valid values                     |
|------------------------|-------------------------------------------------------------------|----------------------------------|
| Well                   | The number of the well on the consumable <sup>[1]</sup>           | <Positive integer (1 to 96/384)> |
| Sample Name            | The name of the sample contained by the associated well.          | <up to 100-character string>     |
| Custom1...-<br>Custom6 | (Optional) Additional text that describes the sample in the well. | <up to 1024-character string>    |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Sample file example

| Well | Sample Name | Sex    | Age | HairColor | Dosage1 |
|------|-------------|--------|-----|-----------|---------|
| 21   | Sample 1    | Female | 25  | black     | Yes     |
| 22   | Sample 2    | Male   | 26  | brown     | No      |
| 23   | Sample 3    | Female | 27  | black     | Yes     |
| 1    | Sample 4    | Female | 28  | blonde    | No      |
| 2    | Sample 5    | Male   | 26  | red       | No      |
| 3    | Sample 6    | Female | 22  | brown     | No      |
| ...  |             |        |     |           |         |

## Barcode file format

The command-line application can import barcode files to populate experiment files (EDS files) it generates with barcode information. A barcode file is a tab-delimited ASCII text file (TXT file) that contains a list of barcodes. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**IMPORTANT!** To guarantee successful import, the file must contain all the elements described in the following section and in the order that they appear.

---

### File structure

The barcode file contains a list of barcodes, where each line defines a single barcode terminated by a carriage return. The barcodes can occur in any order and cannot contain starting or trailing white space.

---

**Note:** The command-line application does not validate the barcodes.

---

### Barcode file example

```
HA996346102
IB894812348
DD834814679
EK209825848
AF092387348
FF225676243
```

## Assay information file

The command-line application can import data for Thermo Fisher Scientific assays from assay information files (AIF). AIF are provided with each assay order and contain technical details about all assays in the shipment. It includes information about:

- Assay concentrations
- Reporter and quencher dyes for the assays
- Part numbers and lot numbers
- Assay, vial, and/or plate ID numbers

The file name includes the number from the barcode on the plate (if applicable).

# 4

## Export file formats and specifications

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- RDML export format ..... 49

### About the exportable files

This chapter describes the export formats supported by the QuantStudio™ Design and Analysis Software v2. The information provided is intended for users who want to integrate the software with third-party applications, including downstream analysis software and laboratory information system (LIS) tools.

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**Note:** The file specifications listed in this appendix are subject to change. For updated information, review the release notes for the QuantStudio™ Design and Analysis Software v2.

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### Export formats

The QuantStudio™ Design and Analysis Software v2 can export setup and results data from experiment files (EDS files) in several file formats that allow further downstream analysis. The export formats feature standardized data structures and markup to maximize accessibility by downstream applications.

The software supports the following export formats:

| File format             | Description                                                                                                                                                                                                                                | See     |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| QuantStudio export file | A QuantStudio-formatted text file that contains setup and/or results data exported from an experiment file (EDS file).                                                                                                                     | page 31 |
| RDML export file        | A compressed XML file that contains setup and/or results data exported from an experiment file (EDS file) and parsed in Real-time PCR Data Markup Language (RDML). The file is stored as a compressed file using the PKZIP archive format. | page 49 |

## QuantStudio export files

The software can export setup and results data from experiment files (EDS file) to tab-delimited text files (TXT file) in a native QuantStudio export format.

Data exported in the QuantStudio export format can be opened by common spreadsheet applications or imported by laboratory information management system (LIS) applications or databases that have been configured to parse the file format.

### QuantStudio export file: Structure

The following table shows the data structure common to data exported in the QuantStudio export format, regardless of experiment type. Each row represents one or more lines of data in the exported file corresponding to a common functional group.

The software export format allows the user to customize and/or omit columns. The columns and orders described below are the default configuration (all columns in their natural order). Actual files may contain fewer columns if the user modified the configuration.

| Section             | Description                                                                                                                                                                                                                                                                              | See     |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| File header         | Describes the qualities of the instrument used to run the experiment and several general experiment properties, such as the date and time of the run and the dye used as the passive reference.                                                                                          | page 32 |
| Sample setup data   | Describes the configuration of samples on the reaction consumable, including sample location, target or SNP assay properties, and task assignments.                                                                                                                                      | page 34 |
| Raw data            | Contains the raw data collected by the instrument during the experiment run.                                                                                                                                                                                                             | page 36 |
| Amplification data  | Contains the normalized data collected during the cycling stage of PCR amplification, which the software uses to generate the amplification plot.<br><b>Note:</b> Not applicable for presence/absence, genotyping, or melt curve experiments that are run without a PCR (cycling) stage. | page 37 |
| Multicomponent data | Contains the spectral data used by the software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.                                                                                                                         | page 38 |
| Results data        | Contains the normalized, processed, and analyzed data generated by the software.                                                                                                                                                                                                         | page 39 |

## QuantStudio export file: Header

The plate setup file begins with a header that describes the instrument used to run the experiment and several other general experiment properties.

Each line starts with an asterisk (#) and ends with a carriage return:

```
<field name>: <field value>
```

**Note:** The software automatically removes any leading and trailing white space around the field name and field value.

**Table 1** QuantStudio export file: Header

| Field                      | Description                                                                          | Output                                    |
|----------------------------|--------------------------------------------------------------------------------------|-------------------------------------------|
| Plate File Name            | The path to the experiment file (EDS file) on the local computer hard drive.         | <i>&lt;filepath&gt;</i>                   |
| Description                | The description entered into the Description field.                                  | <i>&lt;up to 100-character string&gt;</i> |
| User Name                  | The name of the user entered into the User Name field.                               | <i>&lt;up to 100-character string&gt;</i> |
| Last Modified Date/Time    | The last modified date and time.                                                     | <i>&lt;date and time&gt;</i>              |
| Date Created               | The date and time that the instrument started exporting the experiment.              | <i>&lt;date and time&gt;</i>              |
| Barcode                    | The barcode of the PCR reaction plate entered into the optional Plate Barcode field. | <i>&lt;up to 100-character string&gt;</i> |
| Instrument Type            | The model of the instrument for the run.                                             | QuantStudio                               |
| Block Type                 | The model of the sample block installed to the instrument at the time of the run.    | 96-well   384-well   array card           |
| Instrument Name            | The name of the instrument that ran the experiment.                                  | <i>&lt;up to 100-character string&gt;</i> |
| Instrument Serial Number   | The serial number of the instrument for the run.                                     | <i>&lt;up to 100-character string&gt;</i> |
| Heated Cover Serial Number | The serial number of the heated cover for the run.                                   | <i>&lt;up to 100-character string&gt;</i> |
| Block Serial Number        | The serial number of the block for the run.                                          | <i>&lt;up to 100-character string&gt;</i> |
| Plate Run Start Date/Time  | The date and time that the instrument started the run.                               | <i>&lt;date and time&gt;</i>              |
| Plate Run End Date/Time    | The date and time that the instrument finished the run.                              | <i>&lt;date and time&gt;</i>              |



Table 1 QuantStudio export file: Header (continued)

| Field                                         | Description                                                                                       | Output                                                   |
|-----------------------------------------------|---------------------------------------------------------------------------------------------------|----------------------------------------------------------|
| Run Duration                                  | The duration of the run.                                                                          | <code>&lt;time&gt;</code>                                |
| Sample Volume                                 | The sample volume ( $\mu\text{L}$ ) specified in the run method.                                  | <code>&lt;Float&gt;</code>                               |
| Cover Temperature                             | The cover temperature ( $^{\circ}\text{C}$ ) specified in the run method.                         | <code>&lt;Float&gt;</code>                               |
| PCR Stage / Cycle where Analysis is performed | The stage and cycle during the thermal cycling protocol when the instrument collected data.       | <code>Stage &lt;integer&gt;, Step &lt;integer&gt;</code> |
| Pre-read Stage/Step                           | The pre-read stage and cycle during the thermal cycling protocol.                                 | <code>Stage &lt;integer&gt;, Step &lt;integer&gt;</code> |
| Post-read Stage/Step                          | The post-read stage and cycle during the thermal cycling protocol.                                | <code>Stage &lt;integer&gt;, Step &lt;integer&gt;</code> |
| Passive Reference                             | The dye used as a passive reference for the run (or blank if the consumable did not contain one). | <code>&lt;up to 100-character string&gt;</code>          |
| Quantification Cycle Method                   | The method of quantification for the associated experiment.                                       | <code>&lt;up to 100-character string&gt;</code>          |

## QuantStudio export file: Sample setup data

When selected as an export option, the software exports sample setup data after the file header. The sample setup data describes the sample configuration on the reaction consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consists of a column header followed by the sample data fields, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the export file lists the data for each additional assay on separate rows, repeating the well number and sample information.

The data included in the sample setup data export varies depending on experiment type.

This section describes the following sample setup data formats:

- Quantification and presence/absence tests (see page 34)
- Genotyping tests (see page 35)

### Quantification and presence/absence tests

The following table describes the sample setup data that can be exported from absolute quantification, relative quantification, or presence/absence tests.

The body can contain all or some of the data columns below depending on the export configuration.

**Table 2 QuantStudio export file (sample setup data): Quantification and presence/absence tests**

| Column       | Description                                                                                                        | Output                                                        |
|--------------|--------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| Well         | The number of the well on the consumable.                                                                          | <i>&lt;Integer (1 to 96/384)&gt;</i> <sup>[1]</sup>           |
| Sample Name  | The name of the sample in the well.                                                                                | <i>&lt;up to 100-character string&gt;</i>                     |
| Sample Color | The RGB color of the associated sample.                                                                            | <i>"RGB (&lt;r&gt;, &lt;g&gt;, &lt;b&gt;)"</i> <sup>[2]</sup> |
| Target Name  | The name of one target in the well, if applicable. If a well contains multiple targets one row is used per target. | <i>&lt;up to 100-character string&gt;</i>                     |
| Target Color | The RGB color of the associated assay.                                                                             | <i>"RGB (&lt;r&gt;, &lt;g&gt;, &lt;b&gt;)"</i> <sup>[2]</sup> |
| Task         | The task assignment of the target in the well.                                                                     | UNKNOWN   STANDARD   IPC   NTC   BlockedIPC                   |
| Reporter     | The reporter dye that labels the probe for the target assay.                                                       | <i>&lt;up to 100-character string&gt;</i>                     |
| Quencher     | The quencher dye that labels the probe for the target assay.                                                       | <i>&lt;up to 100-character string&gt;</i>                     |

**Table 2 QuantStudio export file (sample setup data): Quantification and presence/absence tests** (continued)

| Column   | Description                                                                           | Output                        |
|----------|---------------------------------------------------------------------------------------|-------------------------------|
| Quantity | Standard quantity (if applicable). This column only appears for standard curve tests. | <Float>   <Integer>           |
| Comments | Additional text that describes the well.                                              | <up to 1024-character string> |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

<sup>[2]</sup> Contains (r)ed, (b)lue, and (g)reen color values, each between 0 to 255. The field is enclosed in double quotes with no spaces between the values.

## Genotyping tests

The following table describes the sample setup data that can be exported from a genotyping test.

The body can contain all or some of the data columns below depending on the export configuration.

**Table 3 QuantStudio export file (sample setup data): Genotyping tests**

| Column           | Description                                                                                                                                         | Output                                 |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| Well             | The number of the well on the consumable.                                                                                                           | <Integer (1 to 96/384)> <sup>[1]</sup> |
| Sample Name      | The name of the sample in the well.                                                                                                                 | <up to 100-character string>           |
| Sample Color     | The RGB color of the associated sample.                                                                                                             | "RGB (<r>, <g>, <b>)" <sup>[2]</sup>   |
| SNP Assay Name   | The name of the SNP assay applied to the well. If the well contains multiple assays, the data for each SNP assay are exported in an additional row. | <up to 100-character string>           |
| SNP Assay Color  | The RGB color of the associated SNP assay.                                                                                                          | "RGB (<r>, <g>, <b>)" <sup>[2]</sup>   |
| Task             | The task assignment of the SNP assay in the well.                                                                                                   | UNKNOWN   NTC                          |
| Allele1 Name     | The name of the first allele for the associated SNP assay.                                                                                          | <up to 100-character string>           |
| Allele1 Color    | The RGB color of the first allele for the associated SNP assay.                                                                                     | "RGB (<r>, <g>, <b>)" <sup>[2]</sup>   |
| Allele1 Reporter | The reporter dye that labels the probe for the first allele.                                                                                        | <up to 100-character string>           |
| Allele1 Quencher | The quencher dye that labels the probe for the first allele.                                                                                        | <up to 100-character string>           |
| Allele2 Name     | The name of the second allele for the associated SNP assay.                                                                                         | <up to 100-character string>           |

Table 3 QuantStudio export file (sample setup data): Genotyping tests (continued)

| Column           | Description                                                      | Output                               |
|------------------|------------------------------------------------------------------|--------------------------------------|
| Allele2 Color    | The RGB color of the second allele for the associated SNP assay. | "RGB (<r>, <g>, <b>)" <sup>[2]</sup> |
| Allele2 Reporter | The reporter dye that labels the probe for the second allele.    | <up to 100-character string>         |
| Allele2 Quencher | The quencher dye that labels the probe for the second allele.    | <up to 100-character string>         |
| Comments         | Additional text that describes the well                          | <up to 100-character string>         |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

<sup>[2]</sup> Contains (r)ed, (b)lue, and (g)reen color values, each between 0 to 255. The field is enclosed in double quotes with no spaces between the values.

## QuantStudio export file: Raw data

The software can export the unprocessed raw data (R) collected by the instrument during the run. The raw data consists of fluorescence readings collected by the instrument that have not been normalized to the passive reference.

This section of the export file begins with a column header followed by the raw data, where each row contains the data for a single well separated by tab characters. Each line of raw data consists of readings sorted by filter set, where each filter set represents an excitation/emission filter pair that was selected during setup. The filter sets are named for the corresponding filter combination according to the following convention:

```
<excitation filter name>_<emission filter name>
```

Table 4 QuantStudio export file: Raw data

| Column        | Description                                                                                                         | Output                                            |
|---------------|---------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| Well          | The number of the well on the consumable.                                                                           | <Integer (1 to 96/384)> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable.                                                                         | <A1 to H12 for 96-well or A1 to P24 for 384-well> |
| Cycle Number  | The cycle of the run during which the instrument recorded the fluorescence.                                         | <Integer>                                         |
| <Filter Set>  | The raw fluorescence for the well measured by the instrument for the associated filter set at the designated cycle. | <Float>                                           |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## QuantStudio export file: Melt curve raw data

The following table describes the raw data exported from a melt curve experiment. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

**Table 5 QuantStudio export file: Melt curve raw data**

| Column         | Description                                                            | Output                                                         |
|----------------|------------------------------------------------------------------------|----------------------------------------------------------------|
| Well           | The number of the well on the consumable.                              | <i>&lt;Integer (1 to 96/384)&gt;</i> <sup>[1]</sup>            |
| Well Position  | The position of the well on the consumable.                            | <i>&lt;A1 to H12 for 96-well or A1 to P24 for 384-well&gt;</i> |
| Reading Number | The 1-based index of the reading.                                      | <i>&lt;Integer&gt;</i>                                         |
| Target         | The name of the target in the well.                                    | <i>&lt;up to 100-character string&gt;</i>                      |
| Temperature    | The temperature in °C.                                                 | <i>&lt;Float&gt;</i>                                           |
| Fluorescence   | The fluorescence value.                                                | <i>&lt;Float&gt;</i>                                           |
| Derivative     | The value of the fluorescence curve derivative for this reading point. | <i>&lt;Float&gt;</i>                                           |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## QuantStudio export file: Amplification data

The software can export the amplification data used to generate the amplification plot of a real-time PCR. The amplification data ( $R_n$ ) are the raw fluorescence readings collected by the instrument normalized to the fluorescence from the passive reference. If available, the exported amplification data also exports the baseline-compensated normalized fluorescence data ( $\Delta R_n$ ) calculated by the software.

This section of the export file begins with a column header followed by the amplification data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the export file lists the data for each additional assay on separate rows, repeating the well number and sample information.

**Table 6 QuantStudio export file: Amplification data**

| Column        | Description                                                                          | Output                                                         |
|---------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Well          | The number of the well on the consumable.                                            | <i>&lt;Integer (1 to 96/384)&gt;</i> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable.                                          | <i>&lt;A1 to H12 for 96-well or A1 to P24 for 384-well&gt;</i> |
| Cycle Number  | The cycle of the run during which the instrument recorded the fluorescence.          | <i>&lt;Integer&gt;</i>                                         |
| Target        | <i>(Genotyping tests)</i> The name of the SNP assay in the well and the allele name. | <i>&lt;SNP assay name&gt;-&lt;allele name&gt;</i>              |

Table 6 QuantStudio export file: Amplification data (continued)

| Column | Description                                                                                                                                             | Output                       |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Target | (All other tests) The name of the target in the well.                                                                                                   | <target name>                |
| Rn     | The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye (reporter signal or passive reference signal). | <Float>                      |
| dRn    | The baseline compensated R <sub>n</sub> value for the associated well.                                                                                  | <Float>                      |
| Sample | The name of the sample in the well.                                                                                                                     | <up to 100-character string> |
| Omit   | Whether the well is omitted from analysis.                                                                                                              | TRUE   FALSE                 |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## QuantStudio export file: Multicomponent data

The software can export the data used to generate the multicomponent plot of a real-time PCR. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the run.

This section of the export file begins with a column header followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Table 7 QuantStudio export file: Multicomponent data

| Column        | Description                                                                                             | Output                                            |
|---------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| Well          | The number of the well on the consumable.                                                               | <Integer (1 to 96/384)> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable.                                                             | <A1 to H12 for 96-well or A1 to P24 for 384-well> |
| Cycle Number  | The cycle of the run during which the instrument recorded the fluorescence.                             | <Integer>                                         |
| <Dye Name>    | The raw fluorescence for the designated dye measured by the instrument at the specified well and cycle. | <Float>                                           |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## QuantStudio export file: Results data

The software can export the results data from an analyzed experiment file (EDS file). The format and content of the results data depends on the experiment type and the configured analysis settings.

This section of the export file begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the export file lists the data for each additional assay on separate rows, repeating the well number and sample information.

The following section describes the following results data formats:

- Primary analysis results (see page 39)
- Replicate group results (see page 41)
- Genotyping results (see page 41)
- Melt curve results (see page 42)
- High resolution melt curve results (see page 43)
- Presence/absence results (see page 44)
- Standard curve results (see page 46)
- Relative standard curve results (see page 48)

### Primary analysis results

The following table describes the primary analysis data exported from a test.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

**Table 8 QuantStudio export file (results data): Primary analysis results**

| Column        | Description                                                  | Output                                                             |
|---------------|--------------------------------------------------------------|--------------------------------------------------------------------|
| Well          | The number of the well on the consumable.                    | <Integer (1 to 96/384)> <sup>[1]</sup>                             |
| Well Position | The position of the well on the consumable.                  | <A1 to H12 for 96-well or A1 to P24 for 384-well>                  |
| Omit          | Whether the well is omitted from analysis.                   | TRUE   FALSE                                                       |
| Sample        | The name of the sample in the well.                          | <up to 100-character string>                                       |
| Target        | The name of the target in the well.                          | <up to 100-character string>                                       |
| Task          | The task assigned to the target in the well.                 | UNKNOWN   NTC   BLOCKED_IPC   IPC<br>  POSITIVE_CONTROL   STANDARD |
| Reporter      | The reporter dye that labels the probe for the target assay. | <up to 100-character string>                                       |
| Quencher      | The quencher dye that labels the probe for the target assay. | <up to 100-character string>                                       |
| Amp Status    | The amplification status result.                             | Amp   No Amp   Inconclusive   N/A                                  |

Table 8 QuantStudio export file (results data): Primary analysis results (continued)

| Column                | Description                                                                                                                 | Output                                                                             |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Amp Score             | The calculated amplification score result.                                                                                  | <Float>                                                                            |
| Curve Quality         | Whether the curve has quality issues.                                                                                       | Warning                                                                            |
| Result Quality Issues | The list of possible quality issues separated by commas.                                                                    | <i>DRN, AMP_STATUS, AMP_SCORE, CQ_STATUS, CQ, CQ_CONF, CQ_SD, OUTLIER, MTP, TM</i> |
| Cq                    | The calculated threshold cycle ( $C_t$ ) or the relative threshold cycle ( $C_{rt}$ ) for the target in the specified well. | <Float>                                                                            |
| Cq Confidence         | The calculated $C_q$ confidence score result.                                                                               | <Float>                                                                            |
| Cq Mean               | The average $C_q$ of the replicates.                                                                                        | <Float>                                                                            |
| Cq SD                 | The standard deviation of the $C_q$ of the replicates.                                                                      | <Float>                                                                            |
| Auto Threshold        | Whether the auto threshold setting is used.                                                                                 | TRUE   FALSE                                                                       |
| Threshold             | The threshold cycle ( $C_t$ ) for the sample in the well.                                                                   | <Float>                                                                            |
| Auto Baseline         | Whether the baseline was determined automatically (true) or manually (false).                                               | TRUE   FALSE                                                                       |
| Baseline Start        | The first cycle used to calculate the baseline.                                                                             | <Integer>                                                                          |
| Baseline End          | The last cycle used to calculate the baseline.                                                                              | <Integer>                                                                          |
| Tm1...Tm4             | The first, second, third, and fourth melting temperatures calculated in °C.                                                 | <Float>                                                                            |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.



## Replicate group results

The following table describes the replicate group data exported from a test.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

**Table 9 QuantStudio export file (results data): Replicate group results**

| Column               | Description                                                           | Output                       |
|----------------------|-----------------------------------------------------------------------|------------------------------|
| Sample               | The name of the sample in the well.                                   | <up to 100-character string> |
| Target               | The name of the target in the well.                                   | <up to 100-character string> |
| Number of Replicates | The number of technical replicates.                                   | <Integer>                    |
| Quantity             | The quantity of the sample at the well.                               | <Float>                      |
| Number of Cqs        | The number of C <sub>q</sub> values used in the computation.          | <Integer>                    |
| Cq Mean              | The average C <sub>q</sub> of the replicates.                         | <Float>                      |
| Cq SD                | The standard deviation of the C <sub>q</sub> of the replicates.       | <Float>                      |
| Cq SE                | The standard deviation error of the C <sub>q</sub> of the replicates. | <Float>                      |
| Biogroup             | The name of the biological group.                                     | <up to 100-character string> |

## Genotyping results

The following table describes the results data exported from genotyping tests.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

**Table 10 QuantStudio export file (results data): Genotyping results**

| Column        | Description                                 | Output                                            |
|---------------|---------------------------------------------|---------------------------------------------------|
| Well          | The number of the well on the consumable.   | <Integer (1 to 96/384)> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable. | <A1 to H12 for 96-well or A1 to P24 for 384-well> |
| Sample        | The name of the sample in the well.         | <up to 100-character string>                      |
| Sample Type   | The sample type associated with the sample. | Unknown   Negative Control                        |
| SNP Assay     | The name of the SNP assay in the well.      | <up to 100-character string>                      |

Table 10 QuantStudio export file (results data): Genotyping results (continued)

| Column       | Description                                                                                                                                       | Output                                                                              |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Assay ID     | The ID of the SNP assay in the well.                                                                                                              | <up to 100-character string>                                                        |
| Task         | The task assigned to the target in the well.                                                                                                      | Unknown   Negative Control                                                          |
| Confidence   | The confidence of the automatic allele call.                                                                                                      | <Float>                                                                             |
| Allele 1     | The raw fluorescence associated with the allele 1 probe of the SNP assay in the well normalized to the fluorescence of the passive reference dye. | <Float>                                                                             |
| Allele 2     | The raw fluorescence associated with the allele 2 probe of the SNP assay in the well normalized to the fluorescence of the passive reference dye. | <Float>                                                                             |
| Call         | The allele call assigned to the sample in the specified well.                                                                                     | Allele 1/Allele 1   Allele 2/<br>Allele 2   Allele 1/Allele 2   No<br>Amplification |
| Manual Call  | Whether the manual method is used to call alleles.                                                                                                | TRUE   FALSE                                                                        |
| Cycle Number | The cycle of the run during which the instrument recorded the fluorescence.                                                                       | <Integer>                                                                           |
| Omit         | Whether the well is omitted from analysis.                                                                                                        | TRUE   FALSE                                                                        |

[1] Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Melt curve results

The following table describes the results data exported from melt curve tests.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

Table 11 QuantStudio export file (results data): Melt curve results

| Column        | Description                                 | Output                                               |
|---------------|---------------------------------------------|------------------------------------------------------|
| Well          | The number of the well on the consumable.   | <Integer (1 to 96/384)>[1]                           |
| Well Position | The position of the well on the consumable. | <A1 to H12 for 96-well or A1 to<br>P24 for 384-well> |
| Sample        | The name of the sample in the well.         | <up to 100-character string>                         |
| Target        | The name of the target in the well.         | <up to 100-character string>                         |

Table 11 QuantStudio export file (results data): Melt curve results (continued)

| Column           | Description                                                             | Output  |
|------------------|-------------------------------------------------------------------------|---------|
| Tm               | The melting temperature calculated in °C.                               | <Float> |
| Melt Peak Height | The raw fluorescence for the designated dye at the melting temperature. | <Float> |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## High resolution melt curve results

**Note:** High resolution melt curve experiments and data are available only through the purchase and installation of the High Resolution Melt Analysis Module for the QuantStudio™ Design and Analysis Software v2.

The following table describes the results data exported from high resolution melt curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

Table 12 QuantStudio export file (results data): High resolution melt curve results

| Column                   | Description                                                                            | Output                                            |
|--------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------|
| Well                     | The number of the well on the consumable.                                              | <Integer (1 to 96/384)> <sup>[1]</sup>            |
| Well Position            | The position of the well on the consumable.                                            | <A1 to H12 for 96-well or A1 to P24 for 384-well> |
| Sample                   | The name of the sample in the well.                                                    | <up to 100-character string>                      |
| Sample Type              | The sample type associated with the sample.                                            | POSITIVE_CONTROL   UNKNOWN   NTC                  |
| Target                   | The name of the target in the well.                                                    | <up to 100-character string>                      |
| Task                     | The task assigned to the target in the well.                                           | POSITIVE_CONTROL   UNKNOWN   NTC                  |
| Variant Call             | The variant call assigned to the sample in the specified well.                         | AA   GG   AG                                      |
| Silhouette Score         | The calculated silhouette score.                                                       | <Float>                                           |
| Method                   | The method used to call the variant.                                                   | AUTO   MANUAL                                     |
| Calibrated Tm1...<br>Tm4 | The first, second, third, and fourth calibrated melting temperatures calculated in °C. | <Float>                                           |
| Omit                     | Whether the well is omitted from analysis.                                             | TRUE   FALSE                                      |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Presence/absence results

The following tables describe the results data exported from presence/absence tests.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

**Table 13 QuantStudio export file (results data): Control status**

| Column    | Description                                                            | Output                                    |
|-----------|------------------------------------------------------------------------|-------------------------------------------|
| Sample    | The name of the sample in the well.                                    | <i>&lt;up to 100-character string&gt;</i> |
| Test Code | The associated test code.                                              | <i>&lt;up to 100-character string&gt;</i> |
| Wells     | The list of well positions assigned to the sample separated by commas. | <i>&lt;well position,...&gt;</i>          |
| Call      | The calculated presence/absence call.                                  | NEGATIVE   POSITIVE                       |
| Status    | The status result.                                                     | FAILED   PASSED                           |

**Table 14 QuantStudio export file (results data): Target call**

| Column        | Description                                                                                                                 | Output                                                         |
|---------------|-----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Well          | The number of the well on the consumable.                                                                                   | <i>&lt;Integer (1 to 96/384)&gt;</i> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable.                                                                                 | <i>&lt;A1 to H12 for 96-well or A1 to P24 for 384-well&gt;</i> |
| Sample        | The name of the sample in the well.                                                                                         | <i>&lt;up to 100-character string&gt;</i>                      |
| Target        | The name of the target in the well.                                                                                         | <i>&lt;up to 100-character string&gt;</i>                      |
| Task          | The task assigned to the target in the well.                                                                                | UNKNOWN   NTC   Blocked_IPC   IPC                              |
| Reporter      | The reporter dye that labels the probe for the target assay.                                                                | <i>&lt;up to 100-character string&gt;</i>                      |
| Quencher      | The quencher dye that labels the probe for the target assay.                                                                | <i>&lt;up to 100-character string&gt;</i>                      |
| Amp Status    | The amplification status result.                                                                                            | Amp   No Amp   Inconclusive   N/A                              |
| Cq            | The calculated threshold cycle ( $C_t$ ) or the relative threshold cycle ( $C_{rt}$ ) for the target in the specified well. | <i>&lt;Float&gt;</i>                                           |
| Cq Mean       | The average $C_q$ of the replicates.                                                                                        | <i>&lt;Float&gt;</i>                                           |
| Cq Confidence | The calculated $C_q$ confidence score result.                                                                               | <i>&lt;Float&gt;</i>                                           |
| Cq SD         | The standard deviation of the $C_q$ of the replicates.                                                                      | <i>&lt;Float&gt;</i>                                           |

Table 14 QuantStudio export file (results data): Target call (continued)

| Column         | Description                                                                   | Output                             |
|----------------|-------------------------------------------------------------------------------|------------------------------------|
| Auto Threshold | Whether the auto threshold setting is used.                                   | TRUE   FALSE                       |
| Threshold      | The threshold cycle ( $C_t$ ) for the sample in the well.                     | <Float>                            |
| Auto Baseline  | Whether the baseline was determined automatically (true) or manually (false). | TRUE   FALSE                       |
| Baseline Start | The first cycle used to calculate the baseline.                               | <Integer>                          |
| Baseline End   | The last cycle used to calculate the baseline.                                | <Integer>                          |
| Delta Rn       | The calculated normalized delta reporter signal.                              | <Float>                            |
| Call           | The calculated target call.                                                   | NEGATIVE   POSITIVE   UNDETERMINED |
| Omit           | Whether the well is omitted from analysis.                                    | TRUE   FALSE                       |

<sup>[1]</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Table 15 QuantStudio export file (results data): Sample call

| Column           | Description                                                            | Output                                                                      |
|------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Sample           | The name of the sample in the well.                                    | <up to 100-character string>                                                |
| Test Code        | The associated test code.                                              | <up to 100-character string>                                                |
| Wells            | The list of well positions assigned to the sample separated by commas. | <well position,...>                                                         |
| Presence Targets | The list of presence targets separated by commas.                      | <target name,...>                                                           |
| Absence Targets  | The list of absence targets separated by commas.                       | <target name,...>                                                           |
| Call             | The calculated well call.                                              | NEGATIVE   POSITIVE   WARNING<br>  INCONCLUSIVE   UNDETERMINED  <br>INVALID |
| Assessment       | The description of the call assessment.                                | <up to 100-character string>                                                |

Table 16 QuantStudio export file (results data): Well call

| Column           | Description                                               | Output                                                                      |
|------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------|
| Well Position    | The position of the well on the consumable.               | <A1 to H12 for 96-well or A1 to P24 for 384-well>                           |
| Sample           | The name of the sample in the well.                       | <up to 100-character string>                                                |
| Test Code        | The associated test code.                                 | <up to 100-character string>                                                |
| Presence Targets | The list of presence targets separated by commas.         | <target name,...>                                                           |
| Absence Targets  | The list of absence targets separated by commas.          | <target name,...>                                                           |
| Call             | The sample call made within the context of a single well. | NEGATIVE   POSITIVE   WARNING<br>  INCONCLUSIVE   UNDETERMINED  <br>INVALID |
| Assessment       | The description of the call assessment.                   | <up to 100-character string>                                                |

### Standard curve results

The following table describes the results data exported from standard curve tests.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

Table 17 QuantStudio export file (results data): Standard curve results

| Column        | Description                                                                                                                                                                                        | Output                                            |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|
| Well          | The number of the well on the consumable.                                                                                                                                                          | <Integer (1 to 96/384)> <sup>[1]</sup>            |
| Well Position | The position of the well on the consumable.                                                                                                                                                        | <A1 to H12 for 96-well or A1 to P24 for 384-well> |
| Sample        | The name of the sample in the well.                                                                                                                                                                | <up to 100-character string>                      |
| Quantity      | <ul style="list-style-type: none"> <li>Unknown wells — The calculated quantity for the sample at the well.</li> <li>Standard wells — The quantity assigned to the standard at the well.</li> </ul> | <Float>                                           |
| Target        | The name of the target in the well.                                                                                                                                                                | <up to 100-character string>                      |
| Dye           | The reporter dye that labels the probe for the target assay.                                                                                                                                       | <up to 100-character string>                      |
| Task          | The task assigned to the target in the well.                                                                                                                                                       | UNKNOWN   NTC   STANDARD                          |
| Reporter      | The reporter dye that labels the probe for the target assay.                                                                                                                                       | <up to 100-character string>                      |

Table 17 QuantStudio export file (results data): Standard curve results (continued)

| Column             | Description                                                                                                                 | Output                            |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| Quencher           | The quencher dye that labels the probe for the target assay.                                                                | <up to 100-character string>      |
| Amp Status         | The amplification status result.                                                                                            | Amp   No Amp   Inconclusive   N/A |
| Cq                 | The calculated threshold cycle ( $C_t$ ) or the relative threshold cycle ( $C_{rt}$ ) for the target in the specified well. | <Float>                           |
| Cq Mean            | The average $C_q$ of the replicates.                                                                                        | <Float>                           |
| Cq Confidence      | The calculated $C_q$ confidence score result.                                                                               | <Float>                           |
| Cq SD              | The standard deviation of the $C_q$ of the replicates.                                                                      | <Float>                           |
| Auto Threshold     | Whether the auto threshold setting is used.                                                                                 | TRUE   FALSE                      |
| Threshold          | The threshold cycle ( $C_t$ ) for the sample in the well.                                                                   | <Float>                           |
| Auto Baseline      | Whether the baseline was determined automatically (true) or manually (false).                                               | TRUE   FALSE                      |
| Baseline Start     | The first cycle used to calculate the baseline.                                                                             | <Integer>                         |
| Baseline End       | The last cycle used to calculate the baseline.                                                                              | <Integer>                         |
| Tm1...Tm4          | The first, second, third and fourth melting temperatures calculated in °C.                                                  | <Float>                           |
| Y-Intercept        | The Y-intercept value of the standard curve regression line.                                                                | <Float>                           |
| R2                 | The $R^2$ value of the standard curve regression line.                                                                      | <Float>                           |
| Slope              | The slope value of the standard curve regression line.                                                                      | <Float>                           |
| Efficiency         | The efficiency value of the standard curve regression line.                                                                 | <Float>                           |
| Standard Deviation | The standard deviation value of the standard curve regression line.                                                         | <Float>                           |
| Standard Error     | The standard error value of the standard curve regression line.                                                             | <Float>                           |
| Omit               | Whether the well is omitted from analysis.                                                                                  | TRUE   FALSE                      |

[1] Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Relative standard curve results

The following tables describe the results data exported from relative standard curve tests.

Because columns can be omitted from the results, the exported file may contain a subset of the following data columns.

**Table 18 QuantStudio export file (results data): RQ replicate group results**

| Column             | Description                                                                                                                                    | Output                       |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Sample             | The name of the sample in the well.                                                                                                            | <up to 100-character string> |
| Target             | The name of the target in the well.                                                                                                            | <up to 100-character string> |
| EqCq Mean          | The EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                           | <Float>                      |
| Adjusted EqCq Mean | The adjusted EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                  | <Float>                      |
| Delta EqCq Mean    | The delta EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                     | <Float>                      |
| Delta EqCq SD      | The delta EqC <sub>q</sub> standard deviation value calculated for the replicate wells of the target/sample combination.                       | <Float>                      |
| Delta EqCq SE      | The delta EqC <sub>q</sub> standard error value calculated for the replicate wells of the target/sample combination.                           | <Float>                      |
| Delta Delta EqCq   | The delta delta EqC <sub>q</sub> value calculated for the replicate wells of the target/sample combination.                                    | <Float>                      |
| Rq                 | The relative quantity calculated for the replicate wells of the target/sample combination.                                                     | <Float>                      |
| Rq Min             | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval. | <Float>                      |
| Rq Max             | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval. | <Float>                      |
| Omit               | Whether the well is omitted from analysis.                                                                                                     | TRUE   FALSE                 |



Table 19 QuantStudio export file (results data): RQ biological group results

| Column             | Description                                                                                                                                    | Output                       |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Biological Group   | The name of the biological group in the well.                                                                                                  | <up to 100-character string> |
| Target             | The name of the target in the well.                                                                                                            | <up to 100-character string> |
| EqCq Mean          | The EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                           | <Float>                      |
| Adjusted EqCq Mean | The adjusted EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                  | <Float>                      |
| Delta EqCq Mean    | The delta EqC <sub>q</sub> mean value calculated for the replicate wells of the target/sample combination.                                     | <Float>                      |
| Delta EqCq SD      | The delta EqC <sub>q</sub> standard deviation value calculated for the replicate wells of the target/sample combination.                       | <Float>                      |
| Delta EqCq SE      | The delta EqC <sub>q</sub> standard error value calculated for the replicate wells of the target/sample combination.                           | <Float>                      |
| Delta Delta EqCq   | The delta delta EqC <sub>q</sub> value calculated for the replicate wells of the target/sample combination.                                    | <Float>                      |
| Rq                 | The relative quantity calculated for the replicate wells of the target/sample combination.                                                     | <Float>                      |
| Rq Min             | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval. | <Float>                      |
| Rq Max             | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval. | <Float>                      |

## RDML export format

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**IMPORTANT!** The RDML export format is available only for standard curve, gene expression, and relative standard curve tests.

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The software can export data from real-time quantitative PCR tests as well-formed Real-time PCR Data Markup Language (RDML), a structured extensible markup language (XML) standard for quantitative PCR (qPCR) data. In combination with the Minimal Information (MIQPCR) guidelines, the RDML element structure describes all aspects of a qPCR test, including setup, analysis, and data interpretation. The exported RDML data is saved as a flat text file that can be used to transfer qPCR data between the software and third-party applications.

The RDML standard is maintained by the RDML consortium, an organization that consists of key developer groups and a member community. For more information on the RDML format, visit the RDM organization website ([rdml.org](http://rdml.org)). The website features free data management tools, including an on-line RDML file generator and RDML software libraries.



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