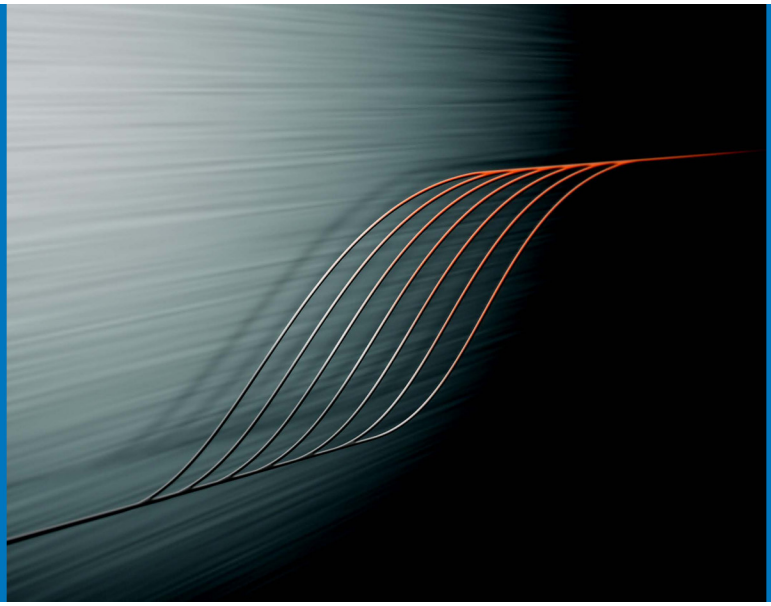
Applied Biosystems

HID Real-Time PCR Analysis Software

Version 1.0

For 7500 Real-Time PCR System



Applied Biosystems

HID Real-Time PCR Analysis Software

Version 1.0

For 7500 Real-Time PCR System

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Preface

How to use this guide

Product overview

The 7500 Real-Time PCR System and HID Real-Time PCR Analysis Software v1.0 detects and quantifies human and/or male DNA in samples.

Purpose of this guide

This guide is intended to help you quickly learn how to use the HID Real-Time PCR Analysis Software v1.0 to perform analysis of samples prepared with the:

- Quantifiler® Human DNA Quantification Kit
- Quantifiler® Y Human Male DNA Quantification Kit
- Quantifiler® Duo DNA Quantification Kit

Use this guide after your plate is prepared and loaded in the 7500 Real-Time PCR System. For instructions on preparing a plate, refer to the *Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual* or the *Quantifiler® Duo DNA Quantification Kit User's Manual*.

Custom experiment option

You can also use the HID Real-Time PCR Analysis Software v1.0 for more complex experiments by selecting the Custom Assay option on the Home screen. If you use the Custom Assay option, refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments* for instructions.

Assumptions

This guide assumes that:

- You are familiar with the Microsoft Windows® operating system, the Internet, and Internet browsers.
- You know how to handle DNA samples and prepare them for PCR.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands that you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

Safety alert words

Safety alert words also appear in user documentation. For general safety information, see Appendix A, "Safety Information," on page 59.

How to obtain more information

Portable document format (PDF) versions of this guide are available at:

www.appliedbiosystems.com.

For additional documentation, see "How to obtain support" on page ix.

To access the HID Real-Time PCR Analysis Software v1.0 Help system, do one of the following:

- Press **F1**
- Click in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen
- Select **Help ▸ Contents and Index**

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating to documentation only. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to obtain support" below).

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**. At the support page you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

For HID Real-Time PCR Analysis Software v1.0 support, you can email **HIDTechSupport@appliedbiosystems.com**.

In North America, you can also call technical support at 1-888-821-4HID (4443), 5:30 a.m. to 5:00 p.m. PST, Monday through Friday.

1 Get Started

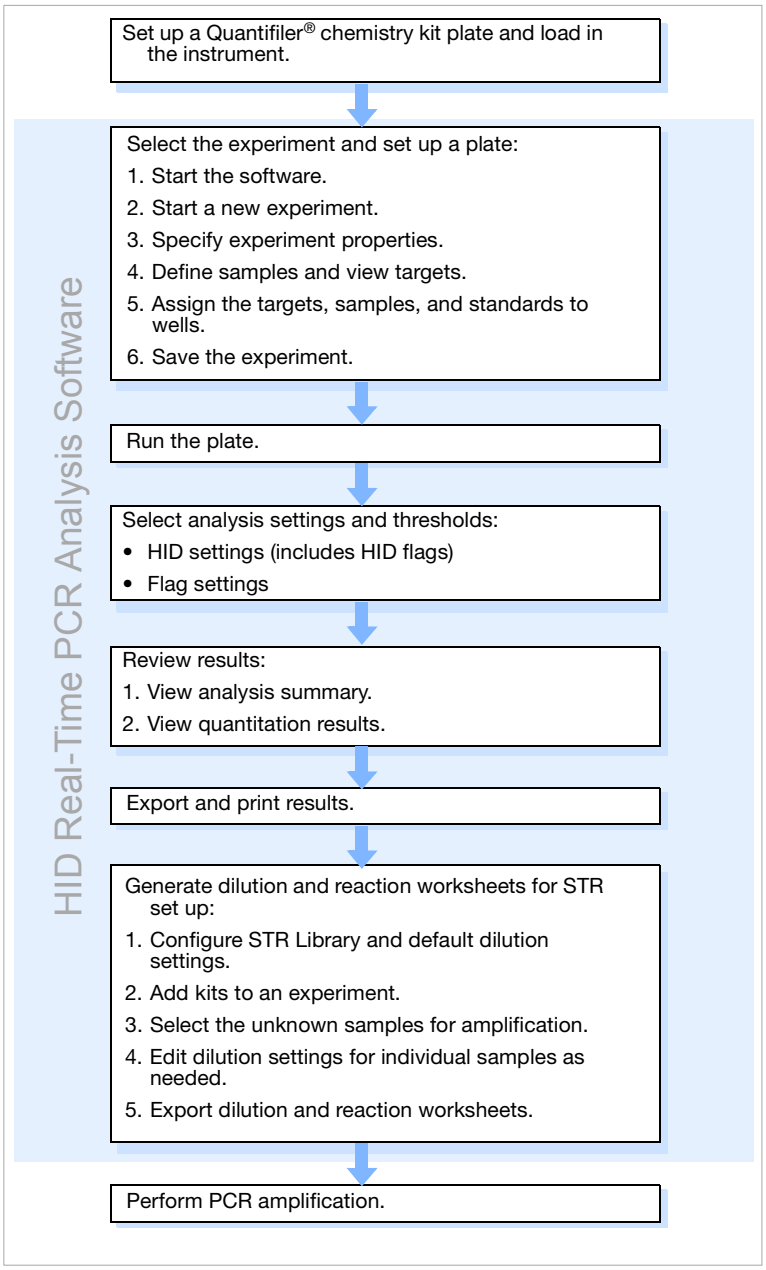
This chapter covers:

Software overview

The HID Real-Time PCR Analysis Software v1.0 is designed specifically to assist human identification laboratories performing DNA quantitation, by simplifying assay setup and streamlining data review and dilution and reaction setup for downstream STR analysis. For example, the software automatically selects the appropriate Quantifiler® target, reporter, quencher, and thermal profile. After a run, the HID Real-Time PCR Analysis Software v1.0 provides an analysis of each well and an analysis summary of all results, STR kit setup instructions, and sample dilutions calculations.

Note: The HID Real-Time PCR Analysis Software v1.0 is for use with the 7500 Real-Time PCR System only.

HID Real-Time PCR Analysis Software v1.0 workflow



Notes

How to use your documentation

HID Real-Time PCR Analysis Software v1.0 users

Refer to the following documents for more information about using HID Real-Time PCR Analysis Software v1.0:

Title	Purpose	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide	Provides information on instrument maintenance.	4412844
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments	Provides further information on system operation and data analysis.	4387779
Quantifiler® Human DNA Quantification Kit and *Quantifiler® Y Human Male DNA Quantification Kit User's Manual*	Provides further information on DNA quantification of samples containing human/male DNA.	4344790
Quantifiler® Duo DNA Quantification Kit User's Manual	Provides further information on DNA quantification of samples containing mixed human and male DNA.	4391294

Documents for custom experiments

Refer to the following documents for information on performing custom experiments instead of using HID Real-Time PCR Analysis Software v1.0:

Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for:	PN
Genotyping Experiments	4387784
Presence/Absence Experiments	4387785
Relative Standard Curve and Comparative C_T Experiments	4387783
Standard Curve Experiments	4387779

Portable document format (PDF) versions of the above documents and this guide are available at **www.appliedbiosystems.com**.

Notes

Before you start

Installation

If the HID Real-Time PCR Analysis Software v1.0 is installed with a new 7500 Real-Time PCR System, both must be installed by an Applied Biosystems technical representative.

Laboratories already using the 7500 Real-Time PCR System can install the HID Real-Time PCR Analysis Software v1.0 by following the instructions provided with the software CD.

Refer to the*Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide* (PN 4412843) for system layout, electrical, power, safety, and other site requirements.

IMPORTANT! Archive all data from analyses using previous software versions before installing HID Real-Time PCR Analysis Software v1.0. Follow the instructions provided with the software CD.

Systems purchased before February 2008

Tower and laptop computers of Applied Biosystems 7500/7500 Fast Real-Time PCR Systems purchased before February 2008 require a memory upgrade before the computers can install the HID Real-Time PCR Analysis Software v1.0. Refer to the Applied Biosystems 7500/7500 Fast Real-Time PCR Systems User Bulletin *Memory Upgrade Requirements for 7500 Software v2.0* (PN 4379705) for information.

Applicable HID kits

You can use the HID Real-Time PCR Analysis Software v1.0 with the following kits:

- Quantifiler® Human DNA Quantification Kit
- Quantifiler® Y Human Male DNA Quantification Kit
- Quantifiler® Duo DNA Quantification Kit

For more information

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screens, or by selecting **Help ▸ Contents and Index**.

Notes

2 Select the Experiment and Set Up a Plate

This chapter describes how to:

This chapter assumes that you have prepared a plate according to the instructions in the *Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual* or the *Quantifiler® Duo DNA Quantification Kit User's Manual.*

Start the software

1. On your desktop, double-click **HID Real-Time PCR Analysis Software v1.0** or select **Start ▸ All Programs ▸ Applied Biosystems ▸ HID Real-Time PCR Analysis Software v1.0**. To open the Login Screen.

2. In the User Name field, select your user name from the drop-down list. Click **OK** to open the Home screen (Figure 1).

Figure 1 Home screen

Notes

Start a new experiment

1. In the Home screen:
   - If the drop-down list of HID experiments is not visible on the Home screen Figure 1), click the down arrow below the Quantifiler® Duo button to open the list (Figure 2).

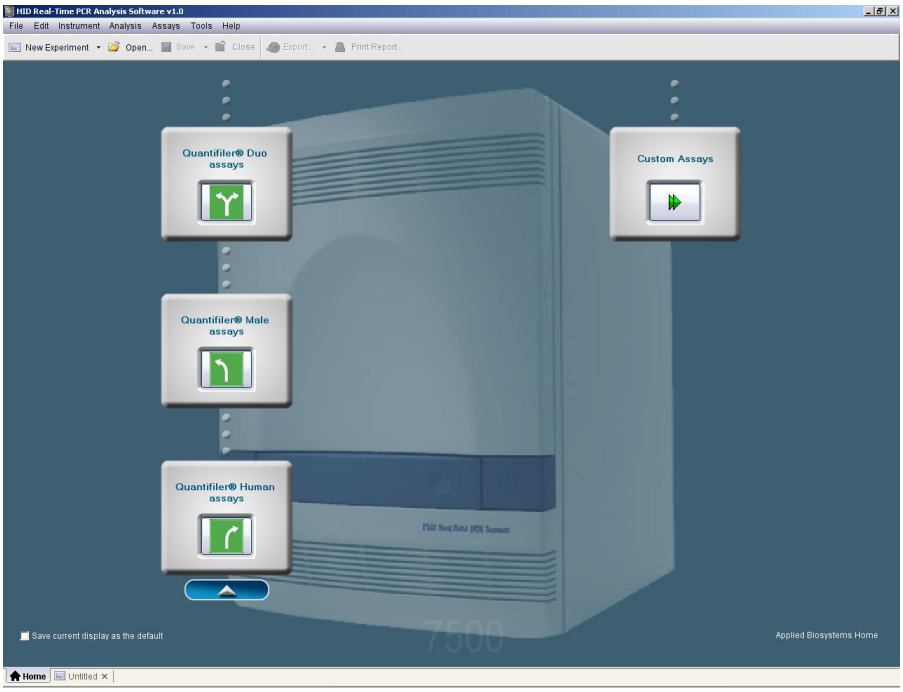


Figure 2 HID Real-Time PCR Analysis Software v1.0 Home Screen

or

   - In the toolbar, click **New Experiment** (Figure 3).

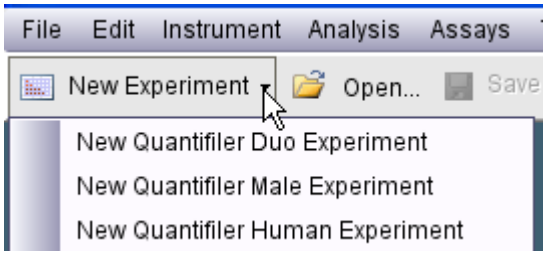


Figure 3 New Experiment

2. Select the type of experiment that you want to perform:
   - **Quantifiler® Duo**
   - **Quantifiler® Male**
   - **Quantifiler® Human**

Note: To use a (hybrid) Quantifiler® Human and Male plate, select either **Quantifiler® Human** or **Quantifiler® Male**.

Notes

For custom experiments

To perform a non-HID experiment, or a modified experiment, click:

- **Custom Assay** in the Home screen.

  or

- **Assays** in the toolbar, then select **Custom Assays** in the drop-down list,

For information on running custom experiments, refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

Navigate the software

Each HID Real-Time PCR Analysis Software v1.0 experiment screen displays instructions for a step in the experiment. Use the Experiment Menu (Figure 4) at the left of any screen to navigate the software.

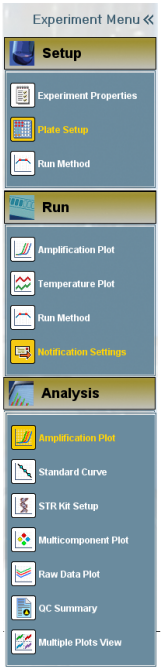


Figure 4 Experiment Menu (Showing the fully expanded menu.)

Click >> (Expand) to expand the Experiment Menu.

Click << (Collapse) to collapse the Experiment Menu.

Click **Setup**, **Run**, or **Analysis**, to display screens used in the corresponding process.

You can access HID Real-Time PCR Analysis Software v1.0 screens in any sequence.

To return to the Home screen at any time, click (Home) at the bottom left of any screen.

Notes

Specify experiment properties

1. In the Experiment Menu, select **Setup ▸ Experiment Properties** (Figure 5).

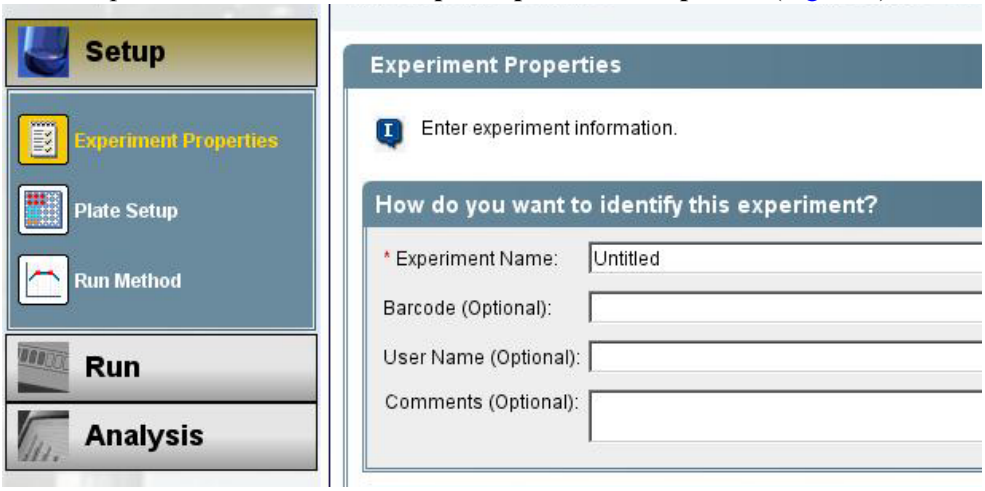


Figure 5 Experiment Properties

2. In the "How do you want to identify this experiment?" section, enter in the Experiment Name field the *name of the plate* or experiment information. Entries in the other fields are optional.

Note: The name you enter in the Experiment Name field appears on the data report and on *.xls spreadsheets of data that you export. If you do not enter a name, "Untitled" appears on the report and spreadsheet that correspond to the experiment.

The following parameters are automatically set:

- Instrument: 7500 (96 wells)
- Experiment: Quantitation-Standard Curve (HID Quantitation)
- Reagents: Taqman® Reagents
- Ramp Speed: Standard (~2 hours to complete a run)

Define samples and view targets

Note: Targets and an NTC sample are automatically listed and named. Standards dilutions 1 to 8 are listed by default for each Quantifiler® Kit. For information about the standard included in the Quantifiler® kit, refer to your Quantifiler® kit user's manual (see "How to use your documentation" on page 3).

View targets

1. In the Experiment Menu, select **Setup ▸ Plate Setup**.

Notes

2. Select the **Define Targets and Samples** tab to open the Defined Targets area. View the targets list (Figure 6) to verify that you selected the correct experiment in step 2 on page 7.

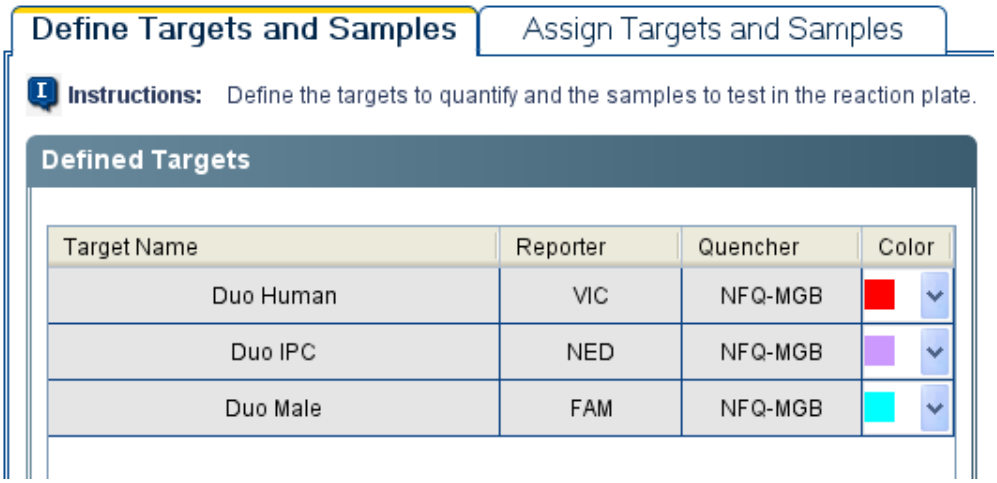


Figure 6 Defined Targets area (example shows Quantifiler® Duo experiment targets.)

The quencher for all Quantifiler® experiments is NFQ-MGB. The reporter dyes for the Quantifiler® Duo and Quantifiler® Male or Human kits are different:

- Quantifiler® Duo – Human: VIC®, Male: FAM™, IPC: NED™
- Quantifiler® Male or Human – Human and Male: FAM™, IPC: VIC®

Change color designation

To change the color that represents a target in the data analysis:

1. Click (down arrow) in the Color column.
2. Select a color in the drop-down list.

Notes

Define samples

1. In the Define Samples area of the Define Targets and Samples tab, specify sample names (Figure 7):

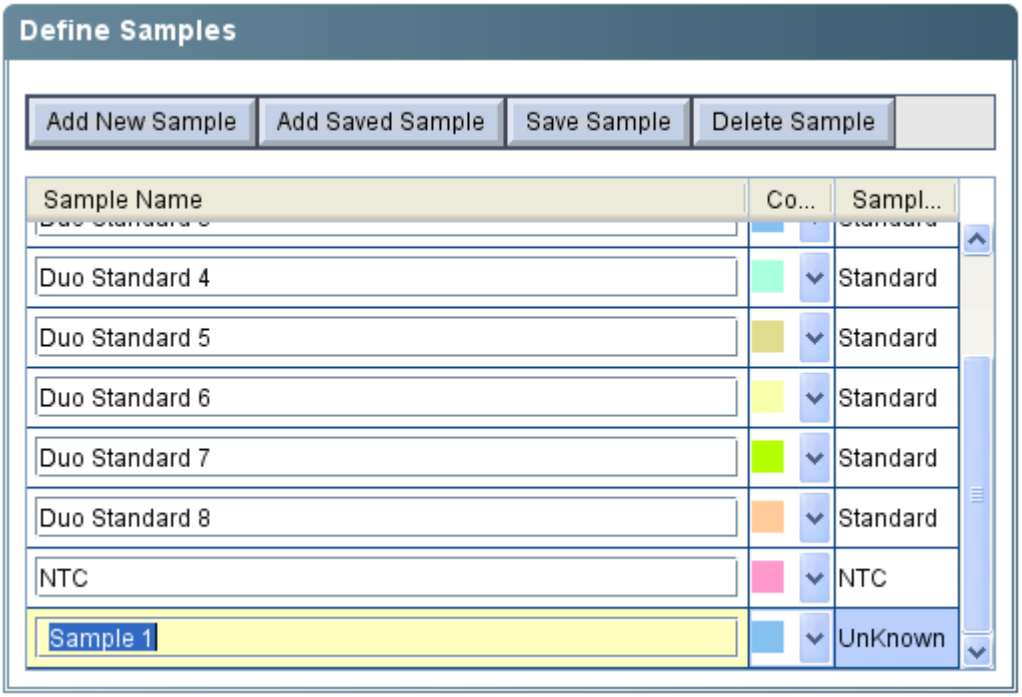


Figure 7 Define samples area (New sample added)

- To define a new sample:
  – Click **Add New Sample**. A new line appears in the Sample Name field,
  or
  – In the toolbar, select **Sample Library** to open the sample library screen, then click **New**.

  The default name for the new sample is Sample X (where X=1 or the highest listed Sample # + 1).You can enter a new name for the sample. To save the name of the sample for future experiments, click **Save Sample.**
- To use a sample from your sample library:
  a. In the Define Samples pane, select **Add Saved Sample**.
  b. Select the sample(s) to use.

2. Select the sample type: Standard, NTC, or Unknown.

Note: Unknown is the default sample type for new samples.

When you assign the sample type, the software automatically assigns the appropriate task to each target.

Notes

3. Repeat step 1and step 2 for each sample.

IMPORTANT! List each sample individually. For replicates (identical samples), add the sample name only once. To assign the replicate to a well in the plate, in step 3 on page 13, select the well, then select the check box next to the sample name.

Assign the targets, samples, and standards to wells

1. In the Experiment Menu, select **Setup ▸ Plate Setup**.
2. Select the **Assign Targets and Samples** tab (Figure 8).

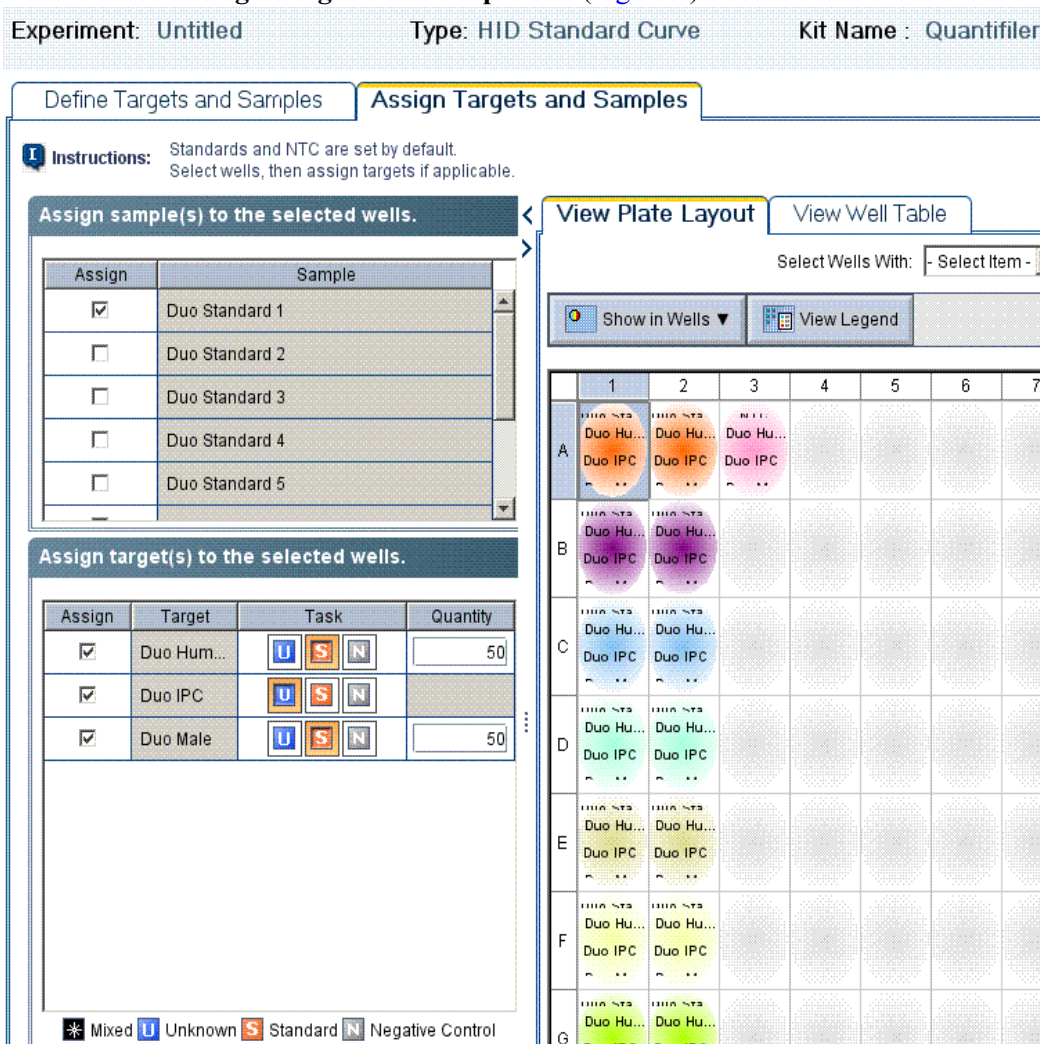


Figure 8 Assign Targets and Samples area

Note: The passive reference is ROX™ dye.

Notes

Assign Using Plate Layout

To assign samples, standards, and NTCs using the View Plate Layout tab:

1. Select the **View Plate Layout** tab in the pane on the right of the screen (Figure 9).

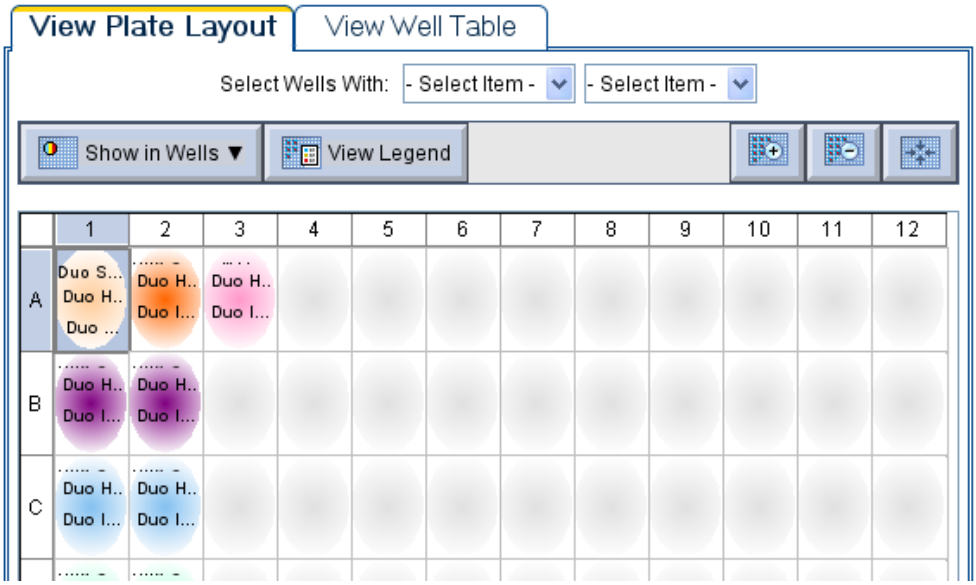


Figure 9 Plate layout area

   To select wells with specific characteristics:

   a. Click the left **Select Wells With** button above the layout diagram.

   b. Select **Sample**, **Target**, or **Task** in the drop-down list.

   c. Click the right **Select Wells With** button.

   d. Select a specific sample, target, or task.

2. Specify the information to display in the wells:

   a. Click **Show in Wells** to open the drop-down list. Items that are marked with a check (☑) are selected for display.

   b. Click an item to select or deselect it for display.

3. Assign standards, NTCs, and unknown samples to well(s):

   a. To select:

   - **Well** – Click the well
   - **Row of wells** – Click a letter on the side of the layout
   - **Column of wells** – Click a number at the top of a column
   - **More than one well, row, or column** – Drag the pointer over the wells, letters, or columns to select.

Notes

b. In the Assign Sample(s) to the Selected Wells section to the left of the plate layout, select the check box in the Assign column corresponding to the unknown, standard, or NTC sample in the well(s). The target for each sample is set by default.

Note: <Sample 1> is automatically assigned to all wells that are not assigned as standard(s) or NTC(s).

4. To change the quantity of standards (optional), enter the quantity in ng/µLin the Quantity field.

5. Repeat step 3 and step 4 until you assign samples, standards, and NTCs to all wells that you use in the experiment. You can delete empty wells after data analysis.

Note: If you delete the samples/standards/NTCs in a well and then restore them, you must reenter the well information.

The task for each target/sample combination is set automatically.

6. Clear all wells not assigned:

a. Select the well(s) to clear.

b. Right-click the well(s).

c. Select **Clear** from the drop-down list.

Assign Using Well Table

To assign samples, standards, and NTCs using the View Well Table tab:

1. Select the **View Well Table** tab (Figure 10).

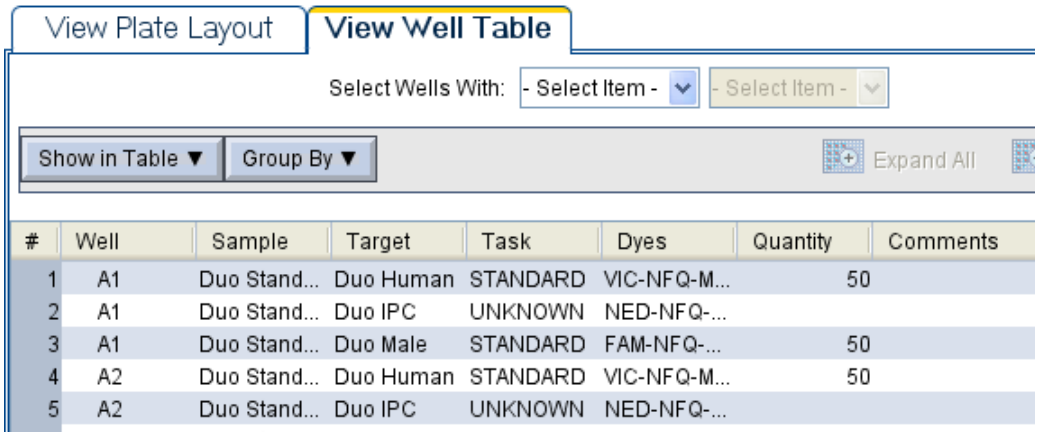


Figure 10 Well table area

Each row in the table represents one well. To group the rows by a characteristic, click the column header. For example, click **Task** to group rows by task.

To select wells with specific characteristics:

Notes

a. Click the left **Select Wells With** button above the layout diagram.

b. Select **Sample**, **Target**, or **Task** in the drop-down list.

c. Click the right **Select Wells With** button.

d. Select a specific sample, target, or task.

2. Specify the information to display in the table:

a. Click **Show in table** to open the drop-down list. Items that are checked in the check box (☑) are selected for display.

b. Click an item to select or deselect it for display.

3. Assign samples, standards, and NTCs to well(s):

a. Select the well(s). To select:

- **Well** – Click under one of the column headings in the row next to the well location (for example, to select well A6, click in row A6 under Sample).
- **More than one well** – Drag the pointer over the wells that you want to select, or **Contr+click** the wells that you want to select.

b. In the Assign Sample(s) to the Selected Wells section, select the check box in the Assign column corresponding to the unknown, standards, or NTC sample in the well(s). The target for each sample is set by default.

Note: <Sample 1> is automatically assigned to all wells that are not assigned as standard(s) or NTC(s).

4. To change the quantity of standards (optional), enter the quantity in ng/µLin the Quantity field. The quantity of samples is set by default.

5. Repeat step 3 and step 4 until you assign samples, standards, and NTCs to all wells that you use in the experiment. You can delete empty wells after data analysis.

Note: If you delete the samples, standards, or NTCs in a well and then restore them, you must reenter the well information.

The task for each target/sample combination is set automatically.

6. Clear all wells not assigned:

a. Click the left Select Wells With button at the top of the table.

b. Select **Sample** from the drop-down list.

c. In the well table, select the sample name(s) of the well(s) to clear.

d. In the Assign samples to the selected wells area, deselect the checkbox in the Assign column beside the sample name.

Notes

Save plate layout as *.eds or template

IMPORTANT! Do not save the experiment to the network folder until the plate run is completed.

1. To save your plate layout, in the toolbar, click the down arrow next to Save, then in the drop-down list, select:
   - **Save** – to save the plate layout as an Experiment Document Single (*.eds) file
   - **Save as** – to save the plate layout as a *.eds file with a different name

     or
   - **Save as Template** – to save the experiment file as a template for future experiments.

   IMPORTANT! Be sure to close any open template (*.edt) file before you save your (*.eds) file as a template. If a *.edt file is open and you attempt to overwrite it, the *.edt file will be corrupted.

2. If you want to save the file with a different name, enter the new name in the File Name field.
3. Click **Save**.
4. Before you start the run, verify that the plate is loaded in the instrument, as described in the Quantifiler® kit user manual:
   - *Applied Biosystems Quantifiler® Kits Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual*
   - *Applied Biosystems Quantifiler® Duo DNA Quantification Kit User's Manual*

Link your template to a Home screen button

You can link your template to the Quantifiler® Duo assays, Quantifiler® Male assays, or Quantifiler® Human assays button on the HID Real-Time PCR Analysis Software v1.0 Home screen. The software will automatically use the template as the default experiment when you click the corresponding button. You will still be able to use a different template by opening a different experiment.

IMPORTANT! Be sure to close any open template (*.edt) file before you save your (*.eds) file as a template. If an *.edt file is open and you attempt to overwrite it, the *.edt file might be corrupted.

1. Before you link your template file to a button on the Home screen, save a copy of the original template:

Notes

a. Navigate to *C:\Applied Biosystems\7500\config\templates*.

b. Select **Edit ▸ Copy** to copy the folder *C:\Applied Biosystems\7500\config\templates*.

c. Navigate to a safe location on your computer.

d. Select **Edit ▸ Paste** to insert a copy of the templates folder in the location you select.

2. Link your template to a button on the Home screen:

a. In the toolbar, from the file that you want to link, click the down arrow next to **Save**.

b. In the drop-down menu, select **Save as Template**.

c. Navigate to *C:\Applied Biosystems\7500\config\templates*.

d. Select the file corresponding to the Quantifiler® Duo assays, Quantifiler® Male assays, or Quantifiler® Human assays button that you want to replace *QuantifilerDuo.edt*, *QuantifilerMale.edt*, or *QuantifilerHuman.edt*.

IMPORTANT! Be sure to give the file exactly the same name as the file corresponding to the button that you want to replace: *QuantifilerDuo.edt*, *QuantifilerMale.edt*, or *QuantifilerHuman.edt*.

e. Click **Save**.

For more information

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

Notes

Notes

3 Run the Plate

This chapter describes how to:

View the run method

1. In the Experiment Menu, select **Setup ▸ Run Method** to open the Run Method screen.
2. Select the **Graphical View** tab to open the thermal profile for Quantifiler® Duo (Figure 11) or Quantifiler® Human and Male (Figure 12).

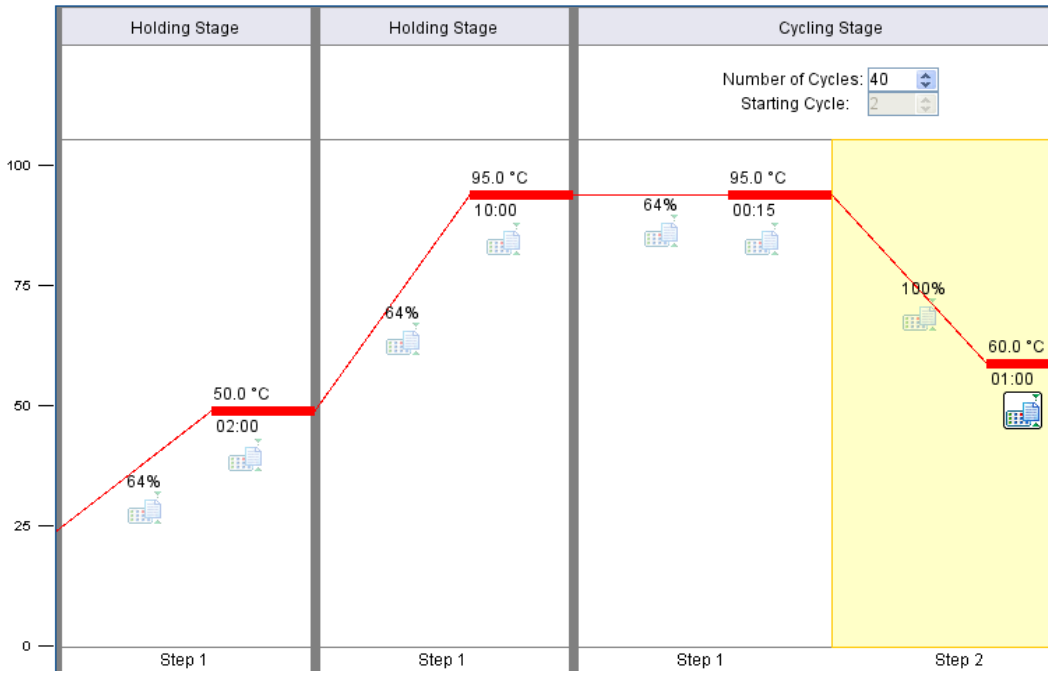


Figure 11 Quantifiler® Duo Kit thermal profile

Notes

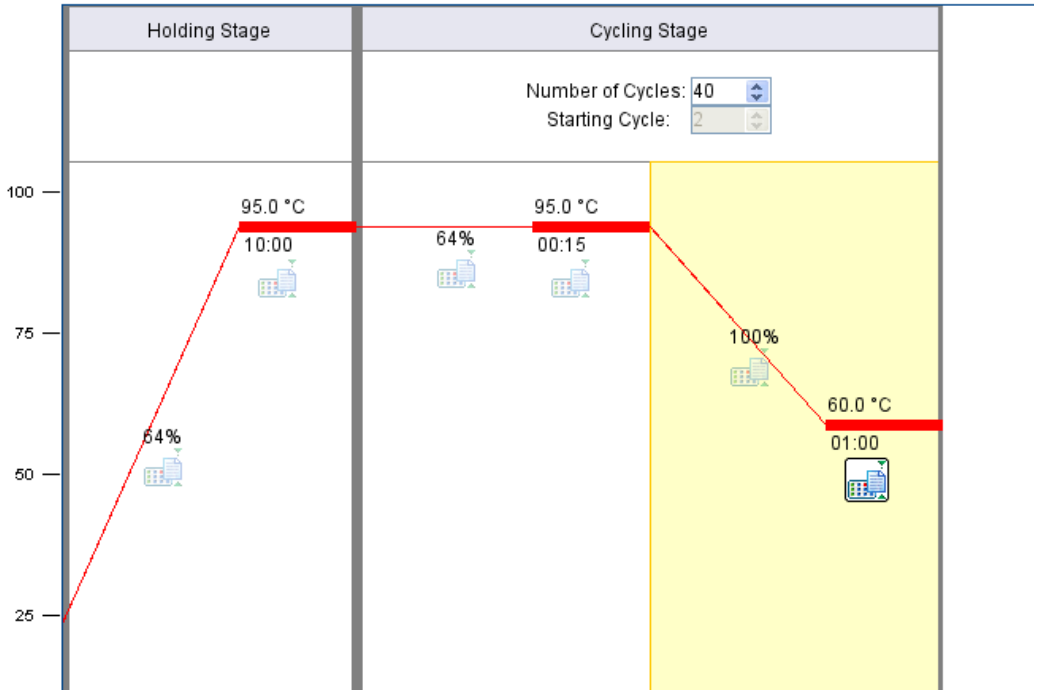
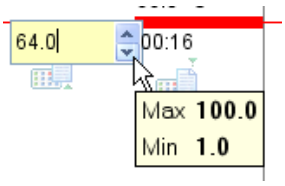


Figure 12 Quantifiler® Human and Male Kit thermal profile

3. To edit the run parameters, select **Add Stage**, **Add Step**, or **Delete Selected**. Click **Undo** to reverse an action and **Redo** to repeat an action. To undo all edits, click **Revert to Defaults**.



To change a ramp speed, temperature, or time, click the value you want to change to open the value field.

Use the up or down arrows to adjust the value. The value you select must be between the maximum and minimum values that are in the menu that opens when you click the value.

Note: To change other cycling parameters, perform a custom experiment: either click in the bottom left corner of the screen, then click **Custom assay** or, in the toolbar, select **Assays**, then select **Custom Assays** from the drop-down menu.

Notes

4. In either the Graphical View or Tabular View tab (Figure 13), verify that the reaction volume per well is 25 µL.

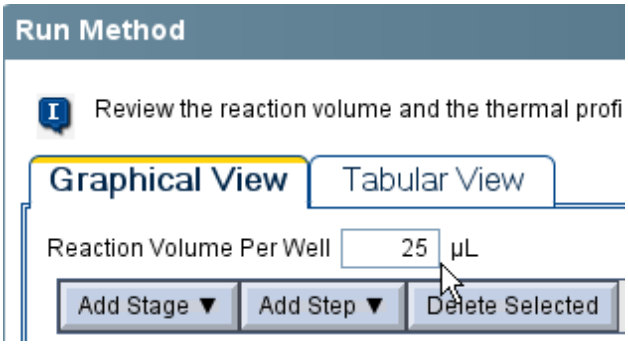


Figure 13 Reaction Volume Per Well

For more information on run parameters for Quantifiler® kits, refer to the *Applied Biosystems Quantifiler® Kits Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual* or the *Applied Biosystems Quantifiler® Duo DNA Quantification Kit User's Manual*.

Note: A 64% ramp up and a 100% ramp down are set by default for 9600 emulation mode.

Set notifications

You can specify that the software send e-mail notification of selected events to e-mail addresses that you specify.

Notes

1. In the Experiment Menu, select **Run ▸ Notification Settings** to open the screen (Figure 14).

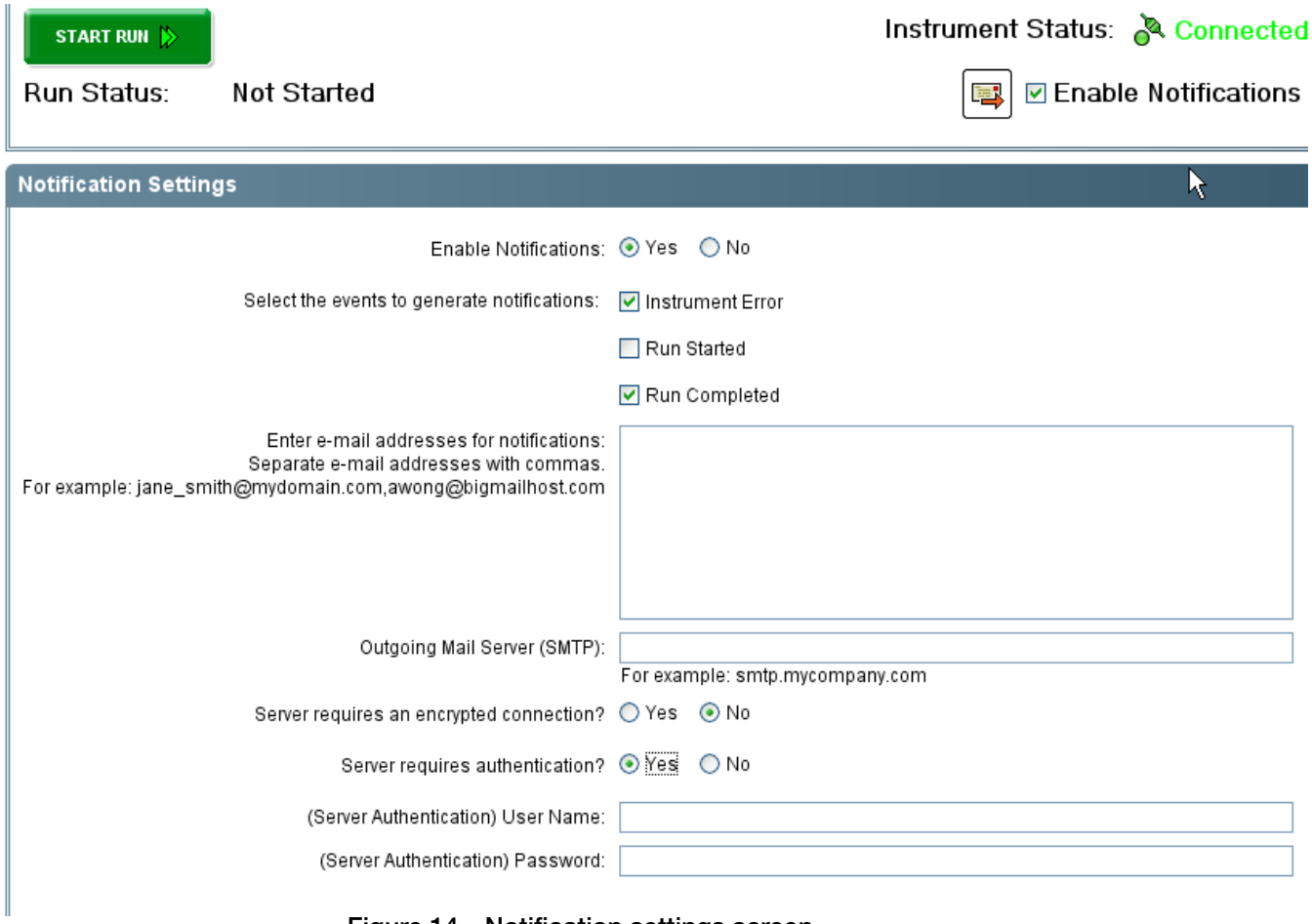


Figure 14 Notification settings screen

2. To send notifications:
   a. In the Run Status area, select the **Enable Notifications** check box.
   b. In the Notifications Settings area, select **Yes** for Enable Notifications. If you do not want the system to send notifications, select **No**.

IMPORTANT! Notifications cannot be sent unless the computer that performs the run is on an e-mail network.

Select notifications events and enter addresses

1. For "Select the events to generate notifications," select the check boxes for events that you want to generate e-mails. You can select:
   - **Instrument Error** – Notifies addressees that the run stopped before completion of the run
   - **Run Started** – Notifies addressees that the run began
   - **Run Completed** – Notifies addressees that the run is finished

Notes

2. In the "Enter email addresses for notifications" field, enter the e-mail address(es) (including you) to which notifications are sent. Use the format shown on the screen. Enter a comma between addresses.

Define the outgoing server (SMTP)

If you need information about the outgoing server to perform the steps listed below, contact your network system administrator.

1. In the Outgoing Server (SMTP) field, enter the name of the outgoing server. For example: **smtp.mycompany.com**
2. Select **Yes** next to "Server requires an encrypted connection?" if the outgoing server requires an encrypted connection. If no encrypted connection is required, select **No**.
3. If the outgoing server requires authentication to receive the e-mail from the instrument, select **Yes** next to "Server requires authentication?" Enter the authentication user name and password in the dialog box.

Start or stop the run

Note: You can set analysis parameters before or after you run a plate. If you prefer to set parameters before you run a plate, see Chapter 4, "Select Analysis Settings and Thresholds," for information on setting analysis parameters.

IMPORTANT! If the computer that performs the run is on a network, avoid excess use of the network during a run.

Start

To start a run:

- .In the Experiment Menu, select **Setup**, then select any screen, then click **Start Run** at the top right corner

  or

- In the Experiment Menu, select **Run**, select any screen, then click **Start Run** at the top left corner (Figure 15).

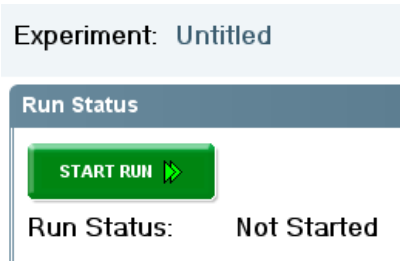


Figure 15 Start Run button

Stop

When you start a run, the green Start Run button becomes a red Stop Run button. Click the **Stop Run** button to stop the run immediately.

Notes

Monitor the run

During a run, you can access the amplification plot, temperature plot, and run method.

In the Experiment Menu, select **Run**, then click:

- **Amplification Plot** – To view amplification plots of reactions
- **Temperature Plot** – To view temperature plots of reactions
- **Run Method** – To view and edit the run method during the run

Save the results

After a run is complete, HID Real-Time PCR Analysis Software v1.0 automatically performs analysis and saves the initial results file. If you modify the plate (for example, if you remove a well from analysis and reanalyze the results), the software does not automatically save the changes. After reanalysis, the HID Real-Time PCR Analysis Software v1.0 prompts you to save the results.

After the run, see Chapter 5, "Enhance Data Analysis," to view and manage the results.

For more information

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

Notes

Notes

4 Select Analysis Settings and Thresholds

This chapter describes how to:

IMPORTANT! All default settings shown in this guide and in the software screens are for illustration only. For your experiments, set the parameters and thresholds according to your laboratory protocol.

Before analyzing data from a completed run, you must set values for the analysis parameters:

- HID flag thresholds
- C_T threshold, baseline start cycle and end cycle
- QC flag thresholds

The Analysis Settings screen also contains the area where you set the parameters for the Dilution Calculation tool to use in calculating a dilution scheme for downstream amplification.

Note: See "Edit dilution settings for individual samples" on page 55 for more information about settings in the Dilution Scheme area.

Open analysis settings

1. In the Experiment Menu, select **Analysis,** then select any one of the following data displays:
   - Amplification Plot
   - Standard Curve
   - Raw Data Plot
   - QC Summary
   - Multicomponent Plot

   Click **Analysis Settings** in the top right corner of the screen to display the Analysis Settings screen (Figure 16).

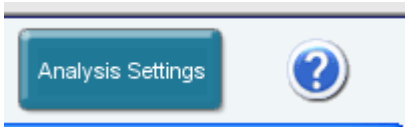


Figure 16 Analysis Settings button

Notes

Enter HID settings

1. Select the **HID Settings** tab (Figure 17) to view the Dilution Scheme area, the HID Flags area, and the HID Flag Settings area.

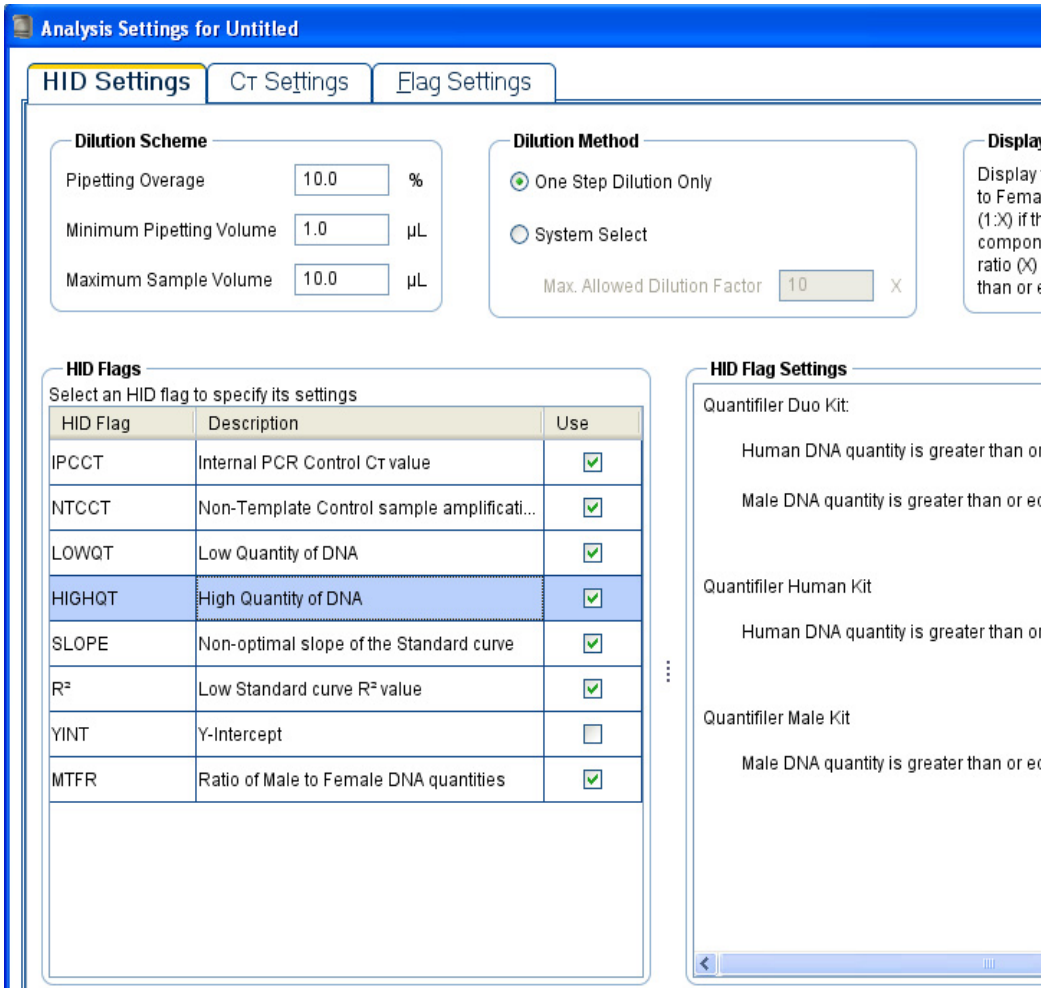


Figure 17 HID settings tab

Note: See "Edit dilution settings for individual samples" on page 55 for more information about settings in the Dilution Scheme area.

2. In the Use column in the HID Flags table, select the check box for each flag that you want to include in the analysis.

Note: You can use a flag to identify quality issues and help to interpret results for wells. Flags can indicate samples that may require further attention. You can exclude wells from data analysis. See "Exclude wells from analysis" on page 39 for instructions on excluding wells from analysis.

3. Enter threshold settings for the flag(s) that you select:

Notes

a. In the HID Flags table, select the flag of interest.

b. In the HID Flag Settings area, enter in the corresponding fields the value(s) that you want to use.

Repeat step a and step b until you enter settings (or view the default settings), for all the flags that you select.

Note: To save your HID flag settings for future use, save the experiment as a template before you start the run (see "Start or stop the run" on page 24.)

4. To analyze the data with new settings, click **Apply Analysis Settings**.

HIGHQT

The HIGHQT flag indicates that the well exhibits quantities above a threshold that you set.

IPCCT

The IPCCT flag indicates an unknown sample that has an IPC (Internal PCR Control) C_T value greater than the average of the IPC C_T values for all the standards plus the threshold that you set. In Quantifiler® kit experiments, IPC target amplification should be within an expected range. Low or no IPC amplification can indicate the presence of PCR inhibitors, incorrect experiment setup, or reagent or instrument failure.

Applied Biosystems strongly recommends that you base the threshold setting on validation data produced by your laboratory. For the Quantifiler® Duo DNA Quantification kit, IPC C_T values for all standards are very similar. In contrast, for the 50 ng/µL Standard of Quantifiler® Human and Human Male DNA Quantification kits, the IPC C_T value tends to be more than the value for the other quantification standards. The higher IPC C_T value of this standard can increase the average for all standards.

During validation of the HID Real-Time PCR Analysis Software v1.0 by your laboratory, Applied Biosystems recommends that you evaluate the 50 ng/µL standard IPC C_T value. You can set a C_T threshold that is higher to account for the higher IPC C_T value of the 50 ng/µL standard. As a result, the software flags only samples that have potential PCR inhibition or that do not amplify as expected.

LOWQT

The LOWQT flag indicates that the well exhibits quantities below a threshold that you set.

NTCCT

This flag refers to the C_T value of the NTC (non-template control). No amplification of human and/or male target(s) should occur in NTC wells.

MTFR flag and M:F ratio display

The MTFR (Male to Female Ratio) is expressed as 1:X. A well is flagged if X is greater than the threshold that you set. For example, if you set the MTFR flag threshold at 1:10, then a sample containing 5 ng/µL of male DNA and more than 55 ng/µL of human DNA generates an MTFR flag. The flag for this condition is a yellow triangle (⚠) in the Plate Layout or Well Table tab, and a red octagon (🛑) in the Analysis Summary (see Chapter 5, Enhance Data Analysis).

Notes

Samples that generate the MTFR flag are labeled "Thresholds Not Met" in the Analysis Summary area of the QC Summary tab. The MTFR flag indicates samples that might require Yfiler kit amplification due to low quantities of male DNA relative to female DNA. Autosomal amplification of these samples may result in partial to no profile for the secondary (male) contributor.

In contrast, the M:F ratio display does not have an associated flag. The M:F ratio is also expressed as 1:X and is displayed in the M:F ratio column of the well table only if X is greater than or equal to the threshold that you set for the M:F ratio display.

The M:F ratio display threshold is expressed as 1:X where X must be less than or equal to the X value for the MTFR flag. For example, if you set the M:F ratio display to 1:1, then the MTFR flag must be set to 1:≥1. Samples with ratios greater than the MTFR flag display the MTFR flag and display the calculated M:F ratio. The M:F Ratio Display function alerts you to male and female mixtures before STR analysis.

Table 1 Results of example M:F and MTFR settings

Male DNA (ng/µL)	Female DNA (ng/µL)	Male:Female ratio	HID setting		M:F ratio display?	MTFR flag?
			M:F Ratio display (1:X) X =	MTFR flag (1:X) X =		
1	1	1:1	1	1	Yes	No
1	2	1:2	1	1	Yes	Yes
1	1	1:1	1	2	Yes	No

SLOPE Indicates the PCR amplification efficiency for the experiment. The amplification efficiency is calculated using the slope of the regression line in the standard curve. The standard wells are flagged if the slope is not between the minimum and maximum values that you set.

The standard curve is derived from a serial dilution set of standards containing a range of known quantities. Results from amplifications of these standards are used to generate a curve.

A slope of −3.3 indicates 100% amplification efficiency. Refer to the *Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual* and the *Quantifiler® Duo DNA Quantification Kit User's Manual* for more information on the standard curve and slope.

R2 This flag indicates the regression coefficient calculated from the regression line of the standard curve. The R^2 value indicates the closeness of fit between the standard curve regression line and individual C_T data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.

Notes

YINT The Y-intercept value of the standard curve indicates the expected C_T value for a sample with a quantity of 1 (for example, 1 ng/µL). The YINT flag can assist in evaluating standard performance and serial dilution preparation. Your laboratory can perform validation studies to determine a range for the Y-intercept and you can set the HID Flag values for each Quantifiler® kit and the HID Flag values for each target (human and male) in the Quantifiler® Duo assay. A YINT flag may indicate incorrectly prepared standard concentrations, degraded standard, or other preparation errors.

View/Edit C_T settings

Select the **C_T Settings** tab to view and edit the settings for C_T (Figure 18).

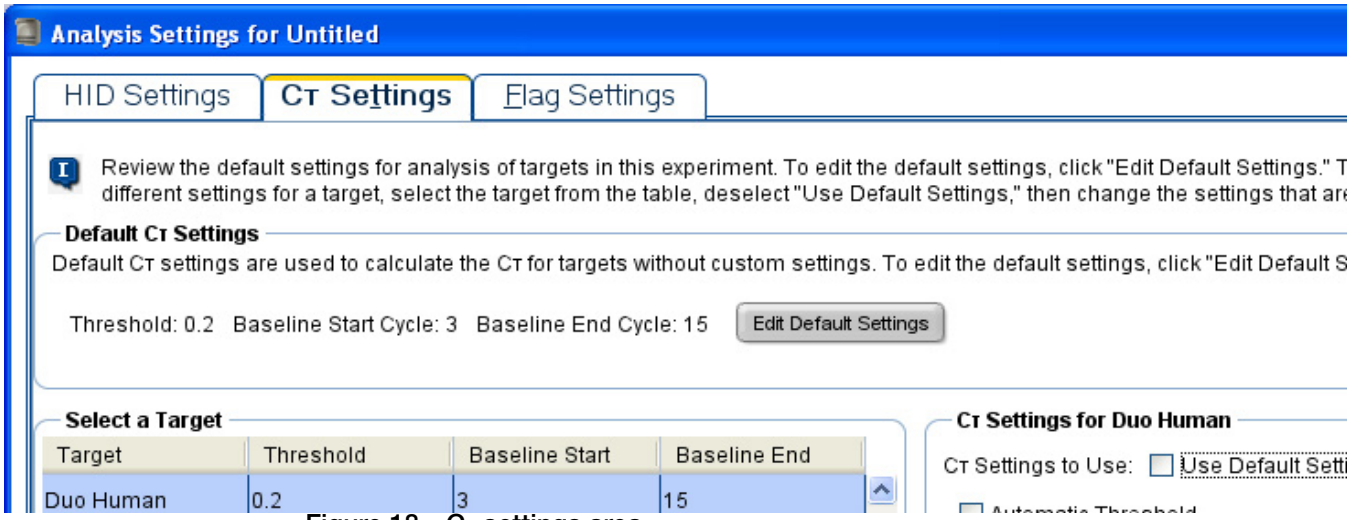


Figure 18 C_T settings area

The default settings are:

- Manual C_T Threshold = 0.2
- Manual Baseline Start Cycle = 3
- Manual Baseline End Cycle = 15

To change these settings, click **Edit Default Settings**, then enter the new values. To analyze the data with new settings, click **Apply Analysis Settings**.

Notes

Enter Flag settings

1. Select the **Flag Settings** tab to view and define instrument, sample, and data collection flags (Figure 19). Flags not used in the analysis are gray.

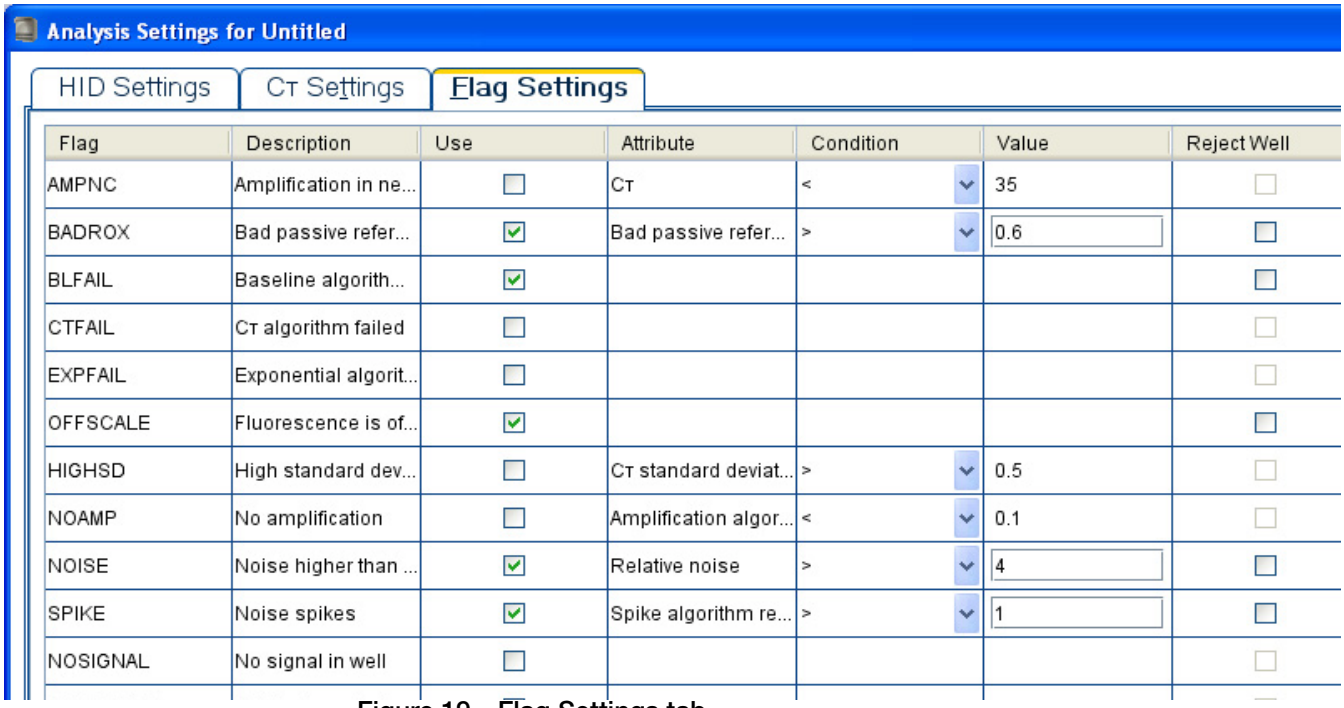


Figure 19 Flag Settings tab

2. In the Use column, select each flag that you want to include in the analysis.
3. Select the condition (<, >, or =) in the Condition column drop-down lists and enter the corresponding values in the Value column to specify the conditions that generate a flag.
4. To omit from the analysis the wells that have a flag, select the corresponding **Reject Well** check boxes.
5. To analyze the data with new settings, click **Apply Analysis Settings**.

Table 2 explains the flags.

Table 2 QC flags

Flag	Description
AMPNC	Amplification in non-template control
BADROX	Bad passive reference signal
BLFAIL	Baseline algorithm failed
CTFAIL	C_T algorithm failed
EXPFAIL	Exponential algorithm failed

Notes

Table 2 QC flags *(continued)*

Flag *(continued)*	**Description**
OFFSCALE	Fluorescence is offscale
HIGHSD	High standard deviation in replicate group
NOAMP	No amplification
NOISE	Noise higher than others in plate
SPIKE	Noise spikes
NOSIGNAL	No signal in well
OUTLIERRG	Outlier in replicate group
THOLDFAIL	Thresholding algorithm failed

For more information

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

Notes

5 Enhance Data Analysis

This chapter describes how to:

View the analysis results

View flagged wells

To view the results of the data analysis:

1. In the Experiment Menu, select **Analysis** ▸ **QC Summary** to open the QC Summary screen.
2. In the QC Summary area, select the **Analysis Summary** tab to display areas that list the HID-specific flags that you selected to include in the data analysis and indicate the number of wells that meet/do not meet the threshold that you set (Figure 20).

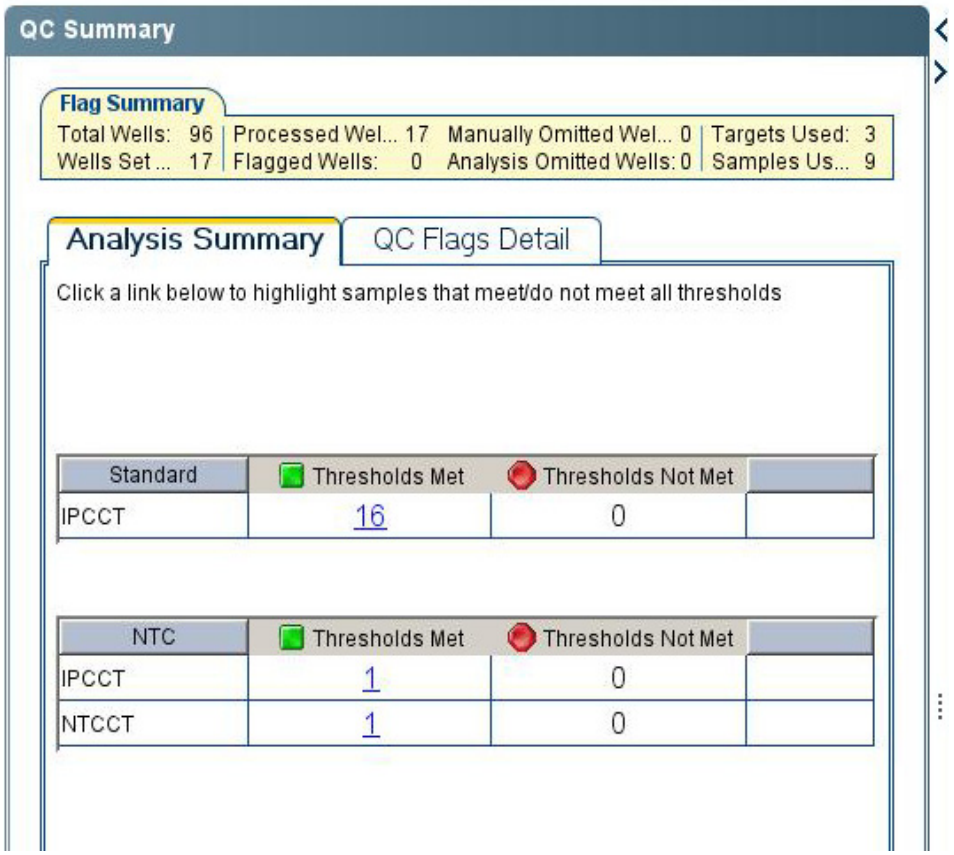


Figure 20 Analysis Summary tab

In the Standard Curve bar, a green square (■) indicates that a value for Slope, R^2, or Y-Intercept meets the threshold. A red octagon (●) indicates a value that does not meet the threshold. In the Standard Curve, Standard, NTC, and Unknown bars, hyperlinked numbers in the All Threshold Met or Thresholds Not Met columns indicate the number of wells that meet/do not meet the thresholds for a flag value.

Standard curve

The Standard Curve bar contains the SLOPE, R2, and Y-Intercept flags. Click the column heading for a red octagon (●) to display the standard curve(s). This graphical view simplifies the identification of wells that require further analysis using your laboratory protocol.

Notes

Standard

The Standard bar reports the IPCCT flags for all the wells on the plate that you designated as sample type Standard. Click the number in the Thresholds Not Met column to view the well(s) that do not meet the IPCCT threshold in the plate layout or well table format. You can use the amplification, multi-component, or the raw data plot(s) to troubleshoot the data for these wells. You can examine the wells that meet the threshold by clicking the number in the All Threshold Met column.

NTC (non-template control)

The NTC bar reports the IPCCT and NTCCT flags for all the wells on the plate that you designated as sample type NTC (non-template control). Click the number in the Thresholds Not Met column to view the well(s) that do not meet the IPCCT or NTCCT threshold in the plate layout or well table format. You can use the amplification, multi-component, or raw data plot(s) to troubleshoot the data for these wells. You can examine the wells that meet the threshold by clicking the number in the All Threshold Met column.

Unknown

The Unknown bar reports the IPCCT, HIGHQT, LOWQT, and MTFR flags for all the wells on the plate that you designated as sample type Unknown (note that the MTFR flag is available only in Quantifiler® Duo kit experiments). The HIGHQT, LOWQT, and MTFR (male to female ratio) flags indicate that the quantity of DNA or ratios of male to female DNA in unknown samples might require additional attention. Numbers below the flag indicate the number of wells that do not meet the threshold.
Click the number in the Thresholds Not Met column to view the well(s) that do not meet a threshold in plate layout or well table format. You can use the amplification, multi-component, or raw data plot(s) to troubleshoot the data for these wells. You can examine the wells that meet the threshold by clicking the number in the All Threshold Met column.

Instrument-related flags

In addition to the areas listed above, a message might be displayed to indicate that one or more of the instrument-related flags is generated by a potential problem with the instrument. The message prompts you to select the **QC Flags Details** tab to view the flags.

Notes

Interpret QC flag information

QC Flags Detail

1. In the QC Summary screen, select the **QC Flags Detail** tab (Figure 21) to view all QC flags (both general and HID)Click a flag to select all affected wells in the plate layout, and to open a brief description of the flag and wells in a box below the list.

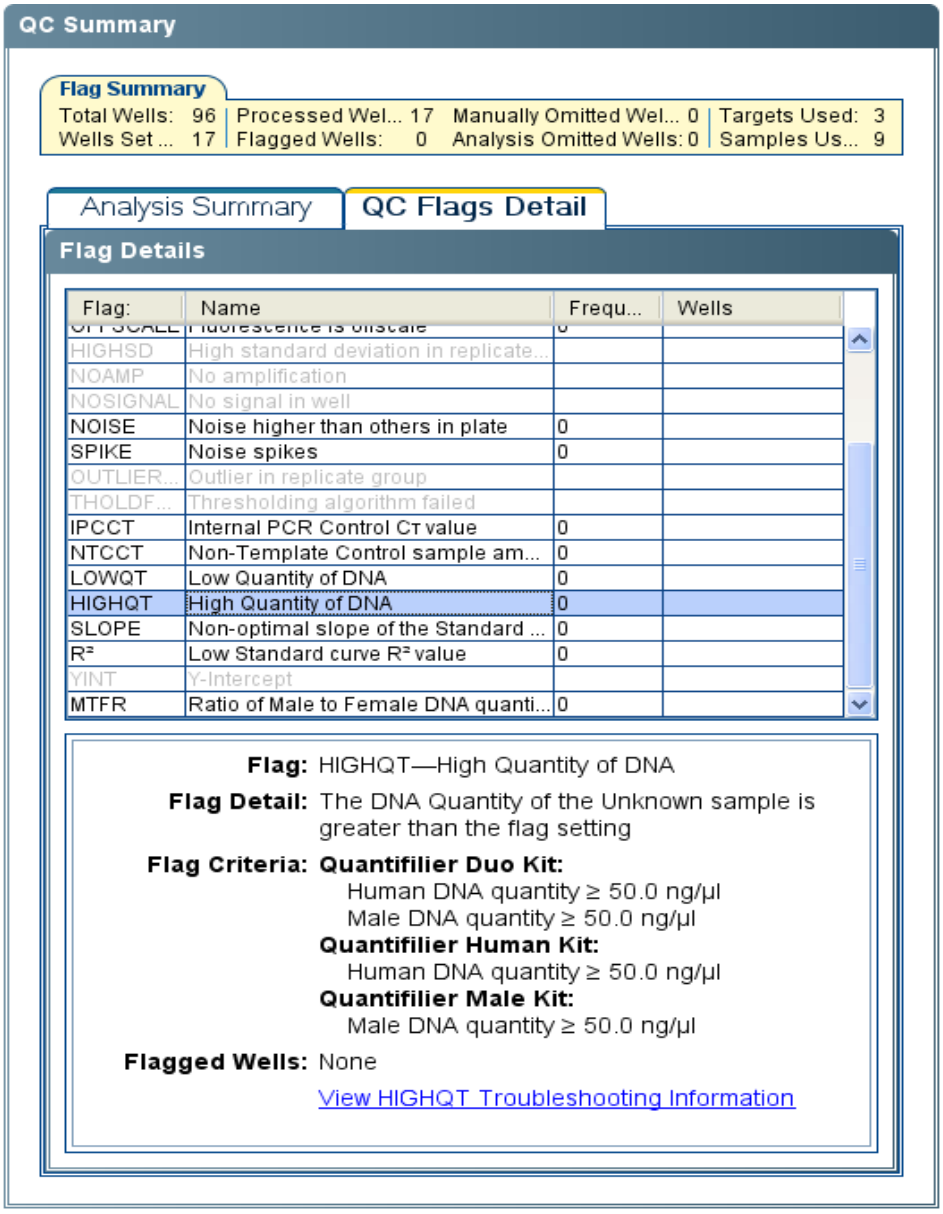


Figure 21 QC Flags detail tab

Also in the QC Flags Details description box is a hyperlink to online Help for troubleshooting the flag and the criteria used for analysis (see Chapter 4, "Select Analysis Settings and Thresholds," for more information about these flags).

Notes

For more information about how to view and edit the information about samples, see "Change the appearance of a plot" on page 41.

Exclude wells from analysis

You can exclude wells from analysis. To view data from individual wells on the Amplification analysis plot, in the Experiment Menu, select one of the following screens:

- **Amplification** – Amplification vs. cycle and amplification vs. well
- **Standard curve** – C_T vs. quantity of standards, flagged samples, and unflagged samples
- **Multicomponent plot** – Fluorescence vs. cycle of all reaction components
- **Raw data plot** – Amplitude vs. filter
- **Multiple plots view** – Amplification, Standard curve, Multicomponent, and Raw data plots in one pane

Exclude wells from analysis

1. In the Experiment Menu, select **Analysis.** Click any Analysis screen. If no data are displayed, click **Analyze.**
2. Omit wells using the well table or plate layout:
   - To use the well table, select the **View Well Table** tab, then select the **Omit** check boxes corresponding to the wells to exclude from the analysis (Figure 22).

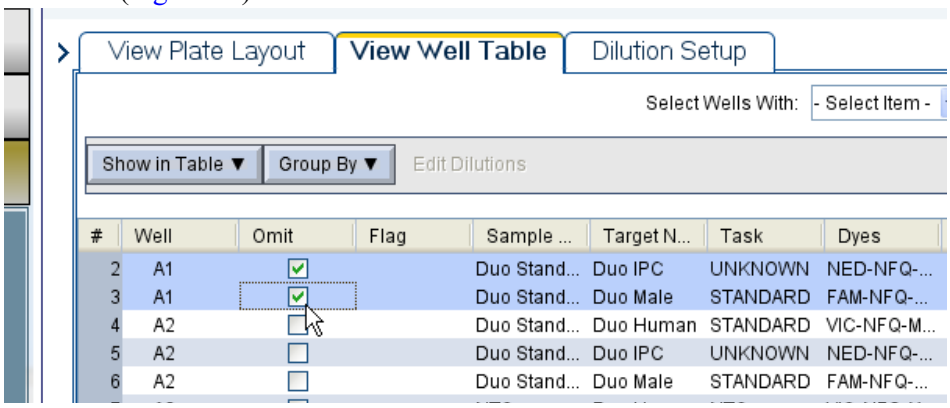


Figure 22 Omit wells using the well table

or

Notes

- To use the plate layout, select the **View Plate Layout** tab (Figure 23).

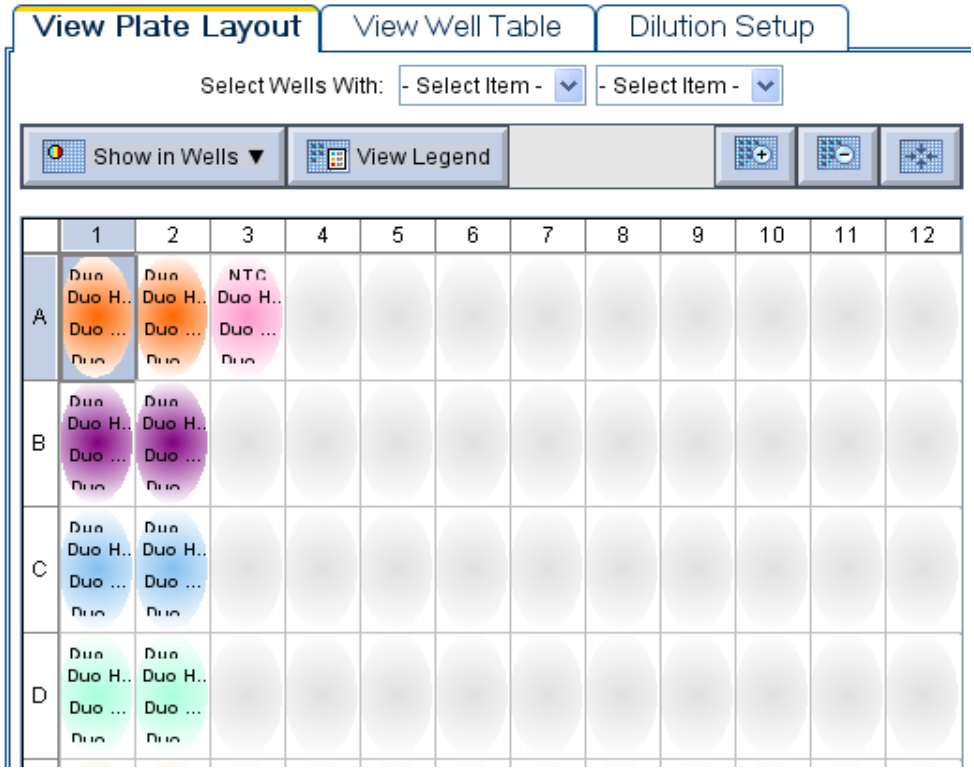


Figure 23 View Plate Layout tab

Right-click the well(s) to omit, then select **Omit** (Figure 24).

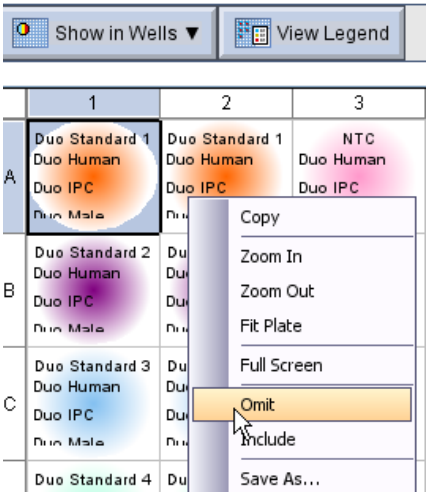


Figure 24 Omit wells using the plate layout

3. Click **Analyze** to reanalyze the experiment data with the omitted well(s) excluded from the analysis.

4. Review the data that are analyzed without the omitted well(s).

Notes

Change the appearance of, print, and save plots

Change the appearance of a plot

You can change the appearance of, print, and save any of the analysis plots.

1. In the Experiment Menu, select **Analysis,** then click the name of a plot of interest.
2. In the plot screen, locate the icon bar above the plot (Figure 25):

Figure 25 Plot modification icons

Click (Hide) to hide the plot legend.

3. To change the appearance of a plot, click (Edit Plot Properties) to open the Plot Properties dialog box. Three tabs are displayed (Figure 26).

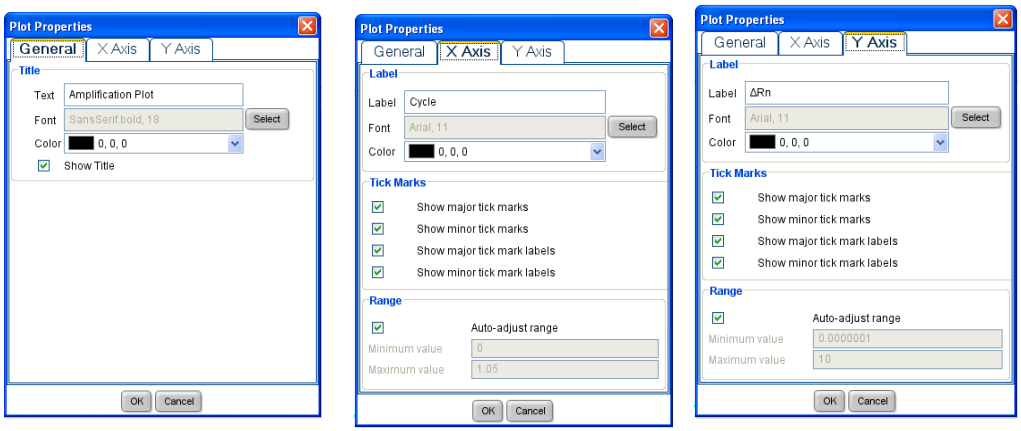


Figure 26 Plot Properties areas

4. Select the appropriate tab to enter the values you want to use to plot the data.
5. Click **OK** to apply the changes.

Print or save a plot

Click (Print) to print the plot.

Click (Save) to save the plot as a *.jpg file.

For more information

For more information on:

- Analysis methodology, refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.
- Troubleshooting data and standard curve metrics, refer to the *Applied Biosystems Quantifiler® Kits Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual* or the *Applied Biosystems Quantifiler® Duo DNA Quantification Kit User's Manual*.

Notes

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

Notes

6 Export and Report Results

This chapter describes how to:

Overview After the HID Real-Time PCR Analysis Software v1.0 completes analysis and after you review the data, you can generate a customized report in *.pdf files, then save or print the report.

You can also export and save data in:

- Excel (*.xls)
- Powerpoint (*.ppt)
- text (*.txt)

Export data

1. In the toolbar, click (Export) to open the Export Data screen, then select the **Export Properties** tab (Figure 27).

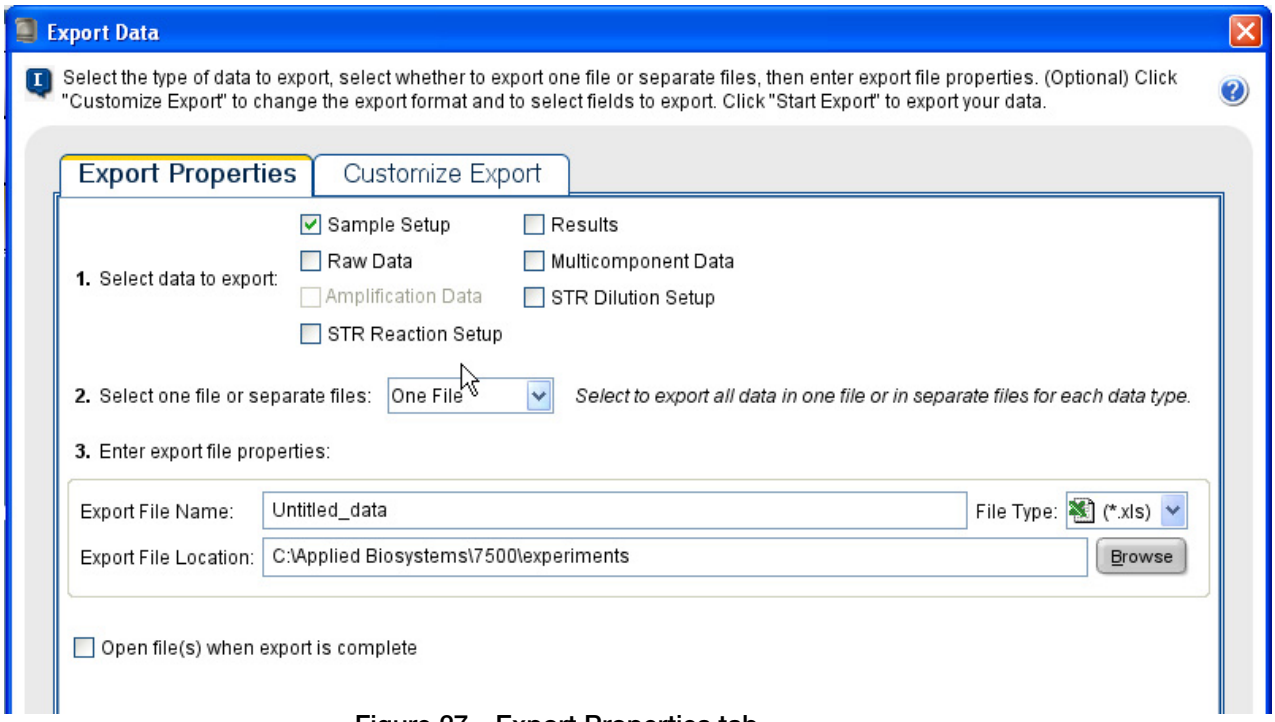


Figure 27 Export Properties tab

2. Select the type of data to export:
   - **Sample Setup** – Setup information such as well, sample name, and sample color
   - **Raw Data** – Raw fluorescence data for each filter, for each cycle
   - **Multicomponent Data** – Fluorescence data for each dye, for each cycle
   - **Amplification Data** – Data that was collected during the cycling or amplification stage
   - **STR Dilution Setup** – Sample dilution worksheet to prepare samples for amplification. For more information, see Chapter 7, "Generate Dilution and Reaction Worksheets for STR Setup."

Notes

- **Results** – Results of the analysis
- **STR Reaction Setup** – STR reaction setup worksheet to prepare samples for amplification. For more information, see Chapter 7, "Generate Dilution and Reaction Worksheets for STR Setup."

3. Select **Separate Files** or **One File** in the drop-down list.

4. Enter the export file properties. For:
   - **File name** – Enter the name of the report.
   - **File Type** – Select the type of file to which you want to send the data. Refer to the online Help for information on creating slides *.ppt slides.
   - **Export File Location** – Enter the filepath to the location where you want to store the report.

5. To customize the data:

   a. Select the **Customize Export** tab (Figure 28).

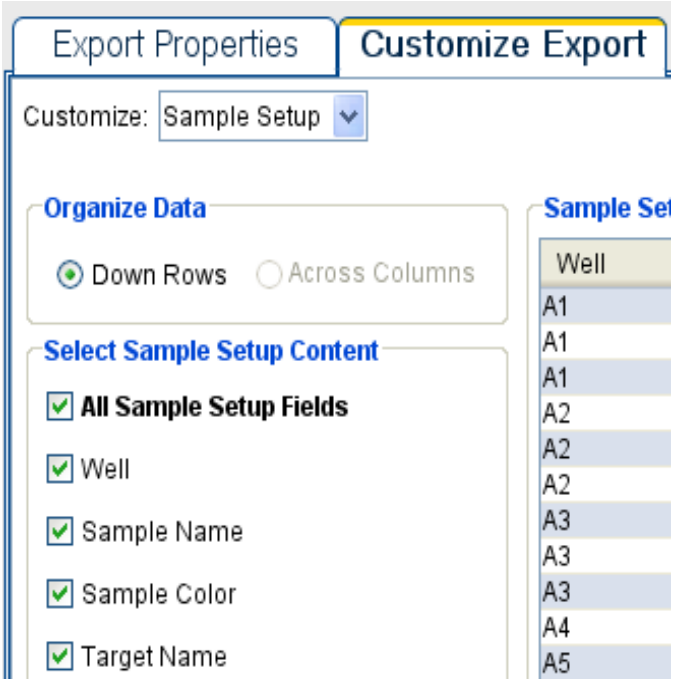


Figure 28 Customize Export tab

   b. Select the information that you want to export.

> **Note:** Sample setup should be exported as a .txt file only.

6. Click **Start Export** to export the data to the file(s) that you selected.

Notes

Print a report

1. In the toolbar, click **Print Report** to display the Print Report screen (Figure 29).

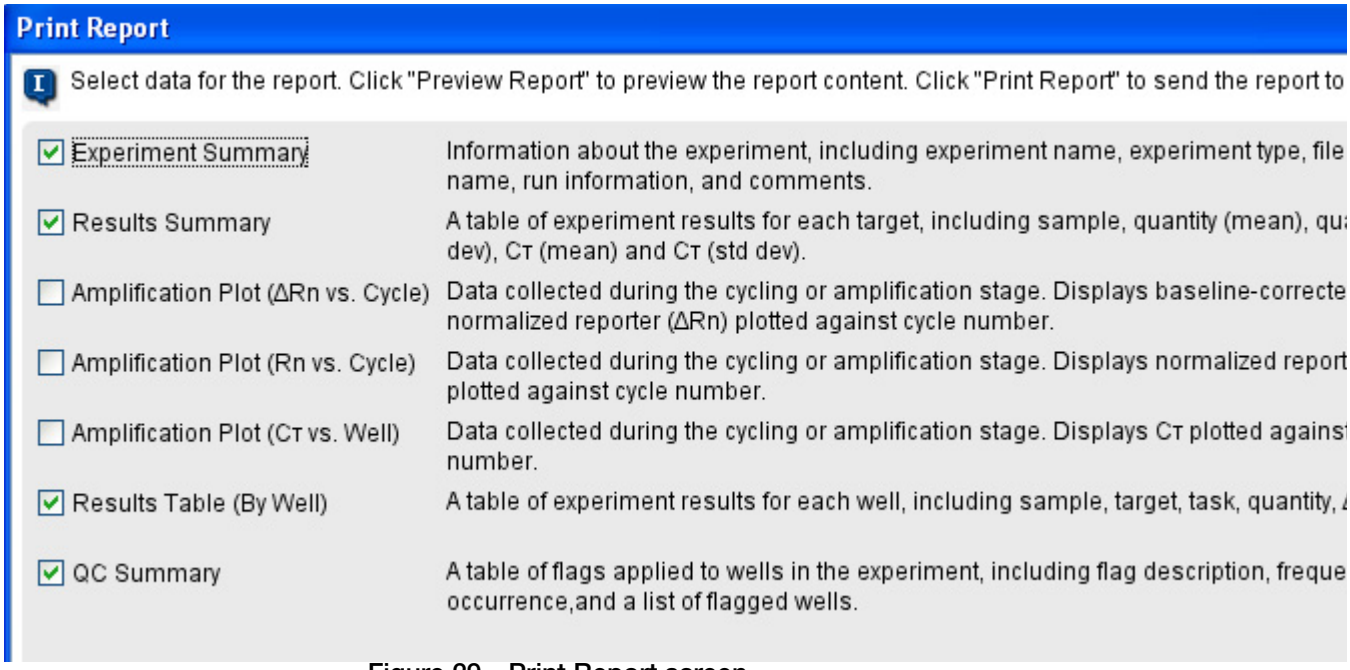


Figure 29 Print Report screen

2. Select the check box corresponding to each data topic that you want to include in the report.

Note: To print the plate layout, select **Analysis**, then select the **View Plate Layout** tab, then click **Show in Wells**. Select items in the drop-down list to display. Right-click the plate layout, then select **Print Preview** to view the data that display in the printed document. If you select many report items, not all items may display and print.

3. Click **Print Preview** or **Print Report** at the bottom of the screen.

IMPORTANT! To save the report to a file, you must click **Print Preview** before you print the report.

4. Select **Save** to save the report, or select **Print** to print the report.

Note: If you do not enter a name in the Experiment Name field of the Experiment Properties screen, the experiment name on the report is “Untitled.”

Notes

For more information

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

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7 Generate Dilution and Reaction Worksheets for STR Setup

This chapter describes how to:

Overview

After a run is complete, you can use the HID Real-Time PCR Analysis Software v1.0 to generate dilution and reaction worksheets for STR set up.

The software uses the AmpFℓSTR kit information you enter in the STR Kit Library and the default dilution settings you specify in Analysis Settings to generate dilution and reaction setup worksheets to perform calculations for the kit(s) that you select.

Configure STR Library and default dilution settings

Configure the STR Kit Library

Most AmpFℓSTR Kits are listed in the STR Kit Library by default. To add or modify kit information:

1. In the toolbar, select **Tools** ▸ **AmpFℓSTR Kit Library** to open the Kit Dilutions Library screen (Figure 30).

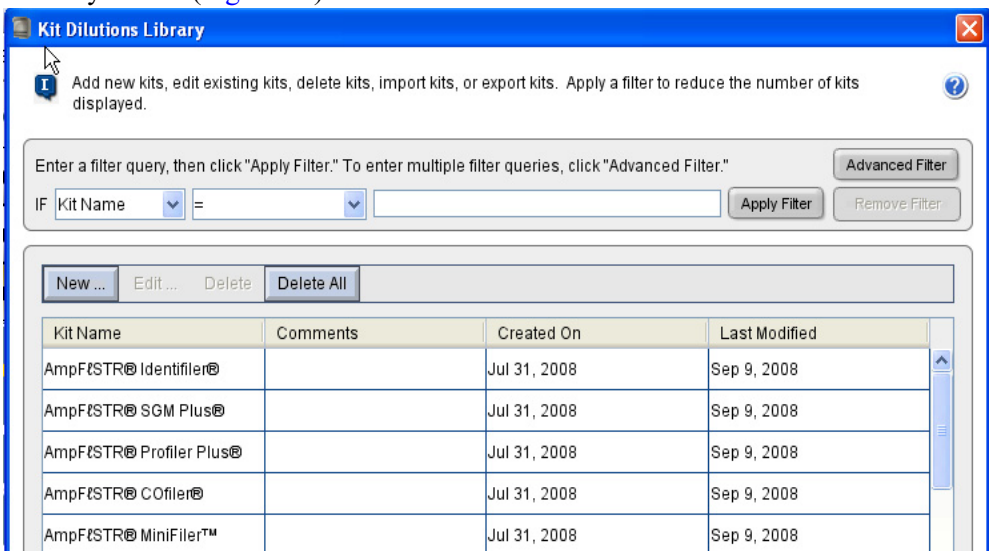

Figure 30 Kit library

2. Click **New** or **Edit** to open the Create New STR Kit screen (Figure 31).

Note: To remove an amplification kit from the list, select the kit, then select **Delete**.

Notes

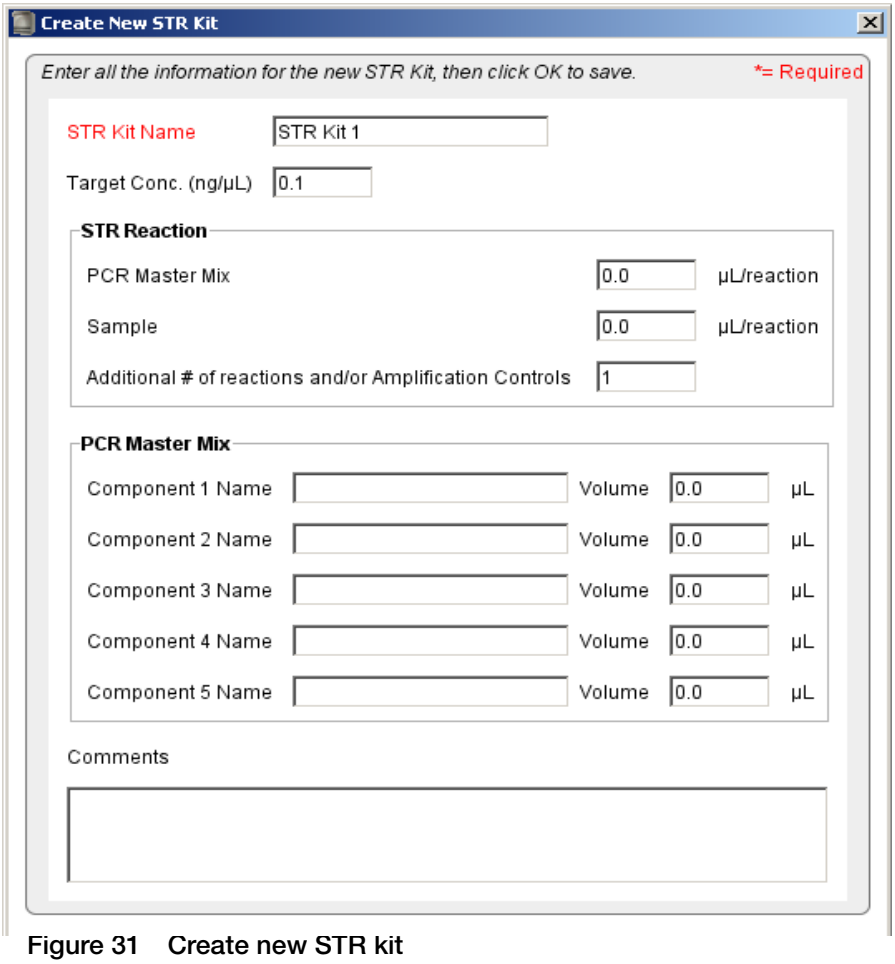

Figure 31 Create new STR kit

3. Enter settings:
   - **STR Kit Name** – The name of the kit that you are adding to the list.

     Note: Kit names must be unique. To use the same kit with different sample types or different input amounts of DNA, add the kit with a different name, such as Identifiler_1.5 ng.

   - **Target Conc.** – The amount of DNA that you want to use divided by the total sample volume per reaction. Examples:

Total DNA (ng)	Volume/reaction (µL)	Target Conc. (ng/µL)
0.5	10	0.05
1.0	10	0.1
1.0	20	0.05
2.0	20	0.1

   - **STR Reaction**
     - **PCR Master Mix** – Enter appropriate volumes (µL)

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- **Sample** – Enter appropriate volumes (µL)

The sum of the Master Mix volume and the sample volume must equal the total volume of the STR reaction. Example:

Sample (µL)	PCR Master Mix (µL)	Reaction volume (µL)
10	15	25
20	30	50

- **Additional # of Reactions and/or Amplification Controls** – Enter the number of additional STR reactions per amplifications to allow for pipetting overage.

IMPORTANT! Because not all kits allow for pipetting overage, you might need to enter more Additional Reactions to compensate for volume losses that occur during pipetting. Refer to your kit user manual (see page 58) for information about pipetting overage.

- **PCR Master Mix** – List each component of the STR Reaction Master Mix. Refer to your kit user's manual for more information.

4. Click **OK**.
5. Repeat steps 2 through 4 for all needed kits.
6. Verify that the kits to be used in the downstream STR reactions are listed, with correct information.

Note: You can also save a kit from an experiment into the library (for example, if you import an experiment from a system with a different library setup). See "Save STR Kit information from an experiment into STR Kit Library" on page 57.

Set default dilution settings

In Analysis Settings, you can specify default dilutions settings to apply to all samples (You can edit individual sample dilution settings after you associate an STR kit with an experiment).

1. In the Experiment Menu, select any analysis screen, then click **Analysis Settings.**
2. Select the **HID Settings** tab (Figure 32).

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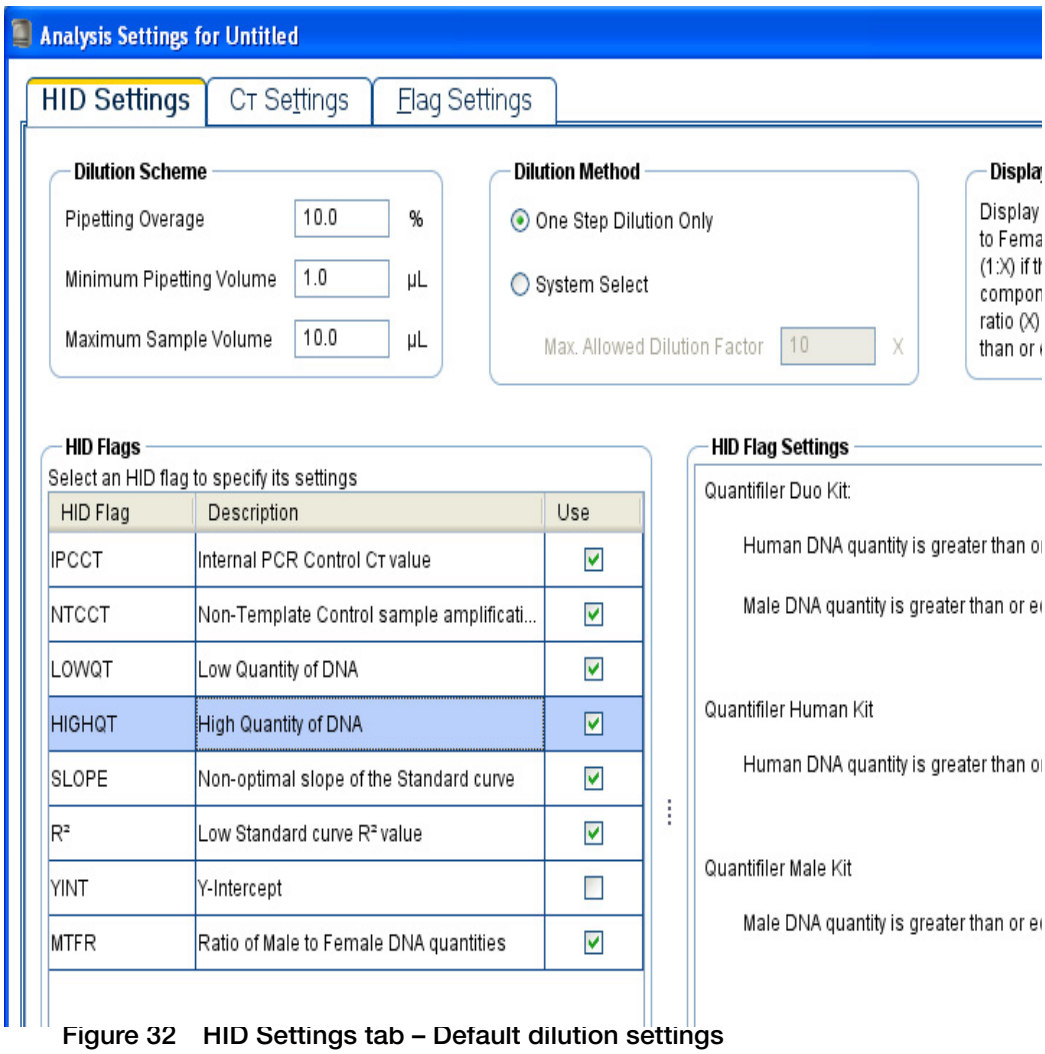

Figure 32 HID Settings tab – Default dilution settings

3. In the Dilution Method area:

   a. Select a dilution method:

      - **One Step Dilution** – Use a single dilution in all instances.
      - **System Select** – Use a dilution scheme that depends on your preferences, with a maximum of two dilutions.

   b. Enter in the Dilution Scheme area dilution scheme parameters according to your preferences or laboratory protocol.

      Enter the:

      - **Pipetting overage** – The percent to add to compensate for error in pipetting. If the sample concentration is less than the target concentration and the sample volume is limited, set the pipetting overage to zero to maximize the amount of DNA in the STR reaction.
      - **Minimum Pipetting Volume** – The minimum volume that you want to pipette.

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- **Maximum Sample Volume** – The maximum quantity of sample that you want to use.
- **Dilution Factor** – The maximum first dilution that you want to perform with the available DNA. For example, for 10-fold first dilutions, enter **10**.

The software displays target sample concentration based on maximum sample volume, number of replicates, sample volume per STR reaction, and pipetting overage that you set if the desired target concentration cannot be reached.

Add kits to an experiment

Add kits for the experiment

Before exporting worksheets, add kits to an experiment:

1. Open the experiment of interest.
2. In the Experiment Menu, select **STR Kit Setup**.
3. In the STR Kit Setup area, click **Add Kit to Experiment** to open the Kit Dilutions Library (Figure 33).

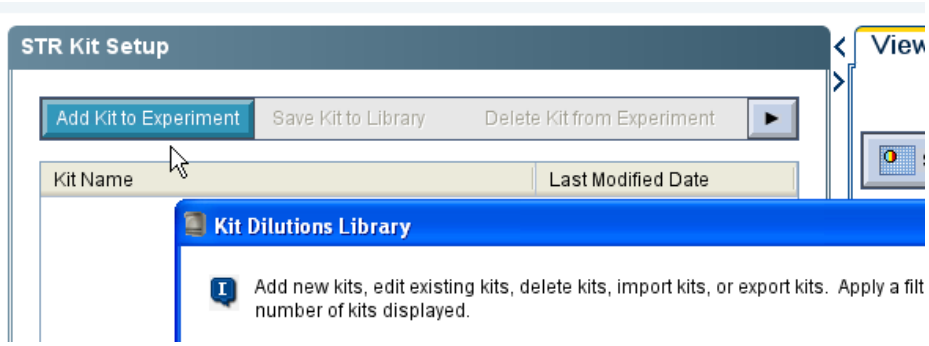

Figure 33 Add a kit to the experiment

4. Select the kit(s) to use in the experiment.
5. Repeat steps 2 through 4 until you select all the kits to use in the experiment.
6. To delete a kit from the experiment (not from the Kit Library), select the kit to delete, then click **Delete Kit from Experiment**.

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Select unknown samples for amplification

After adding kits to an experiment, select the unknown samples for amplification and associate samples with kits:

1. In the Experiment Menu, select any analysis screen, then select the **View Well Table** tab.
2. Select the check box corresponding to the unknown sample to use and the STR kit with which to use the sample.

   Note: The software automatically assigns the same kit for replicates.

3. Select the **Dilution Setup** tab to view the dilution scheme and the STR kit(s) that you selected for each sample.
4. Repeat steps 2 and 3 for each unknown sample and kit(s).

   Note: You cannot select an STR kit for standard or NTC sample types. Dilution calculations apply only to the unknown sample (Human or Male) target in the well(s), not to standards or NTCs.

Edit dilution settings for individual samples

If needed, edit the default dilution settings for samples:

1. Select the **View Well Table** tab.
2. Select the sample of interest.
3. In the toolbar at the top of the well table, click **Edit Dilutions** (Figure 34) to open the Edit Target Dilution Details screen (Figure 35).

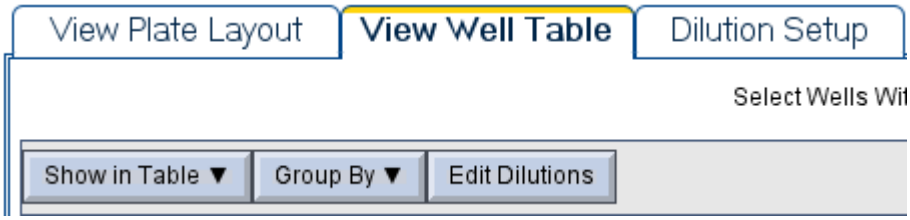

Figure 34 Edit Dilutions button

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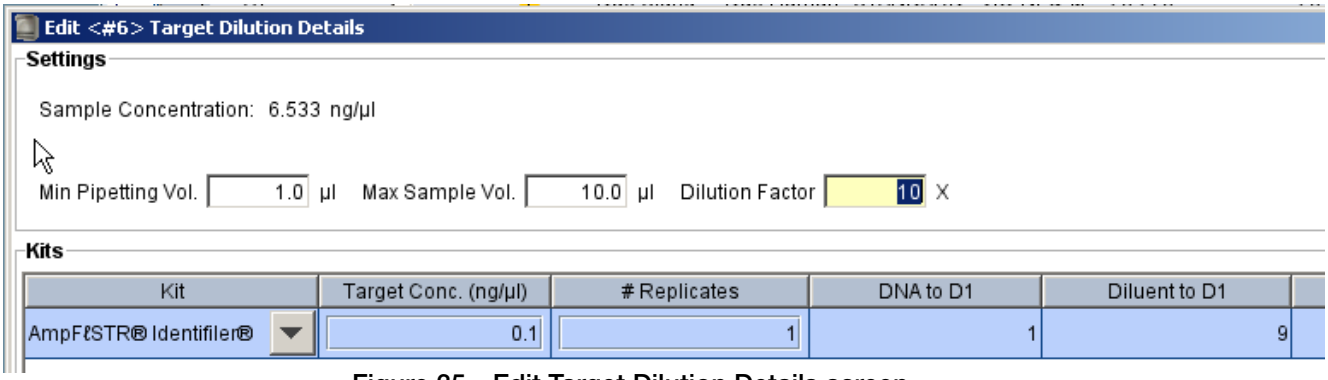

Figure 35 Edit Target Dilution Details screen

Note: If you quantify replicates, this screen displays the sample concentration or the mean sample concentration.

4. View or edit:
   - **Min. Pipetting Vol.** – The minimum quantity to pipette.
   - **Max. Sample Vol.** – The maximum volume of available sample.
   - **Dilution Factor** – For example, enter **10** for 10-fold dilutions.
   - **Target Conc.** – The amount of target DNA that you want to use divided by the total sample volume per STR reaction.
   - **# Replicates** – The number of identical reactions.

Note: The software displays target sample concentration based on maximum sample volume, number of replicates, sample volume per STR reaction, and pipetting overage that you set if the desired target concentration cannot be reached.

View the dilution scheme

View the dilution scheme to ensure settings are appropriate for the experiment:

1. In the Experiment Menu, select **Analysis.**
2. Click any plot to open a plot screen.

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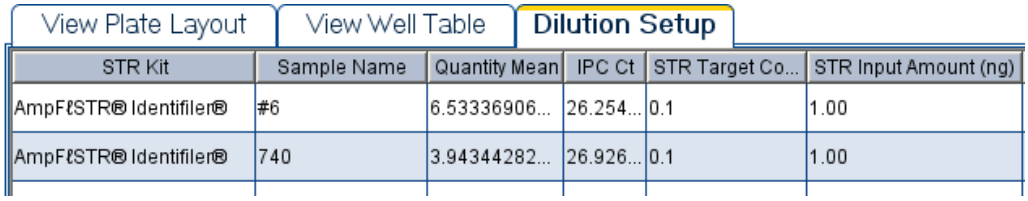
3. Select the **Dilution Setup** tab to open the Dilution Setup screen (Figures 36 and 37).



STR Kit	Sample Name	Quantity Mean	IPC Ct	STR Target Co...	STR Input Amount (ng)
AmpFℓSTR® Identifiler®	#6	6.53336906...	26.254...	0.1	1.00
AmpFℓSTR® Identifiler®	740	3.94344282...	26.926...	0.1	1.00

Figure 36 Dilution Setup tab (left)

STR Input Amount (ng)	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2	# of STR Rxn.
1.00	1.0	64.3	10.0	0.0	1
1.00	1.0	38.4	10.0	0.0	1

Figure 37 Dilution Setup tab (right)

4. Review the dilution setup settings for downstream reactions.

Export dilution and reaction worksheets

Export the STR Dilution Setup worksheet and the STR Reaction Setup worksheet as described in "Export data" on page 44.

Save STR Kit information from an experiment into STR Kit Library

You can save a kit from an experiment into the library (for example, if you import an experiment from a system with a different library setup).

Note: If the STR kit name you are saving from the experiment is already listed in the library, rename or delete the kit from the library before saving the kit information from the experiment.

To save kit information from an experiment to the library:

1. Open the experiment.
2. In the STR Kit Setup screen, select the kit to save.
3. Click **Save Kit to Library**.

Notes

For more information

For more information on analysis methodology, refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

For more information on AmpFℓSTR PCR amplification kits, refer to the:

- *AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual*
- *AmpFℓSTR® MiniFiler™ PCR Amplification Kit User's Manual*
- *AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual* (includes information about the COfiler® amplification kit)
- *AmpFℓSTR® SEfiler Plus™ PCR Amplification Kit User's Manual*
- *AmpFℓSTR® SGM Plus® PCR Amplification Kit User's Manual*
- *AmpFℓSTR® Yfiler® PCR Amplification Kit User's Manual*

Access the Help system by pressing **F1**, by clicking in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help ▸ Contents and Index**.

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A

Safety Information

This appendix covers:

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Examples

The following examples show the use of safety alert words:

IMPORTANT! The sample name, run folder name, and path name, *combined*, can contain no more than 250 characters.

MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

Notes

General instrument safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting stand-alone computers and monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.** These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

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