applied biosystems by life technologies™

Quantifiler® HP and Trio DNA Quantification Kits

Pub. No. 4485356 Rev. A

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Quantifiler® HP and Trio DNA Quantification Kits User Guide* (Pub. no. 4485354). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product overview

This document describes the Quantifiler® HP DNA Quantification Kit (Cat. no. 4482911) and Quantifiler® Trio DNA Quantification Kit (Cat. no. 4482910). The Quantifiler® HP Kit is designed to quantify the total amount of amplifiable human DNA in a sample. The Quantifiler® Trio Kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample.

Guidelines for calculating the standards dilution series

We recommend a ten-fold dilution series with 5 concentration points, for example, 0.005 ng/µL, 0.050 ng/µL, 0.500 ng/µL, 5.000 ng/µL, and 50.000 ng/µL. You can add an optional 100 ng/µL standard point if needed. However, you may see an increase in the IPC C_T for the 100 ng/µL standard. See the user guide for a standards dilution series example.

Use a minimum input volume of 10 µL DNA for dilutions (to ensure accuracy of manual pipetting).

Prepare the DNA quantification standards

When using Quantifiler® THP DNA Dilution Buffer, you can store the prepared DNA quantification standards in low-binding tubes for up to 2 weeks at 2 to 8°C. To prepare the DNA quantification standards dilution series:

1. Label five microcentrifuge tubes: Std. 1, Std. 2, Std. 3, and so on.
2. Dispense the required amount of diluent (Quantifiler® THP DNA Dilution Buffer) to each tube.
3. Prepare Std. 1:
   a. Vortex the Quantifiler® THP DNA Standard 3 to 5 seconds.
   b. Using a new pipette tip, add the appropriate volume of Quantifiler® THP DNA Standard for your dilution series to the tube for Std. 1.
   c. Mix the dilution thoroughly.
4. Prepare Std. 2 through 5:
   a. Using a new pipette tip, add the appropriate volume of the prepared standard to the tube for the next standard.
   b. Mix the standard thoroughly.
   c. Repeat steps a and b for each subsequent standard until you complete the dilution series.

life technologies™

Prepare the reactions

While preparing the reactions, keep the 96-well optical reaction plate or optical 8-tube strip in its base and do not place it directly on the bench top to protect it from scratches and particulate matter. To prepare the reactions:

1. Calculate the volume of each component needed to prepare the reactions, using the appropriate table below.

   For the Quantifiler®HP and Trio DNA Quantification Kits:

Component	Volume Per Reaction (µL)
Primer Mix	8
Reaction Mix	10

   Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

2. Prepare the reagents:
   - Thaw the Primer Mix completely, then vortex 3 to 5 seconds and centrifuge briefly before opening the tube.
   - Gently vortex the Reaction Mix before using.
3. Pipette the required volumes of components into an appropriately sized polypropylene tube.
4. Vortex the PCR mix 3 to 5 seconds, then centrifuge briefly.
5. Dispense 18 µL of the PCR mix into each reaction well or tube.
6. Add 2 µL of sample, standard, or control to the applicable wells or tubes.

   Note: We recommend running duplicates of each sample of the DNA quantification standards for each reaction plate.
7. Seal the reaction plate with the Optical Adhesive Cover, or the strip tube with the optical 8-cap strip.
8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.

   Note: If a tabletop centrifuge with 96-well plate adapters is not available, visually inspect the plate for bubbles, and lightly tap the plate to remove bubbles in wells.

Run the plate on the 7500 Real-Time PCR instrument

Before you run the reactions, power on the computer, 7500 Real-Time PCR instrument, and software. Create an experiment for the run. To run the plate on the 7500 Real-Time PCR instrument:

1. Press the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is correctly aligned in the holder.
3. Load the 96-well optical plate with the notched A12 position at the top-right of the tray.
4. Close the tray door.
5. Apply pressure to the right side of the tray and at an angle to close the tray door.
6. In the HID Real-Time PCR Analysis Software, open the experiment that you set up for the run.
7. Click **Start Run**.

Analyze the experiment

Analyze a run after it is complete and reanalyze after you make any changes to the experiment, such as sample names.

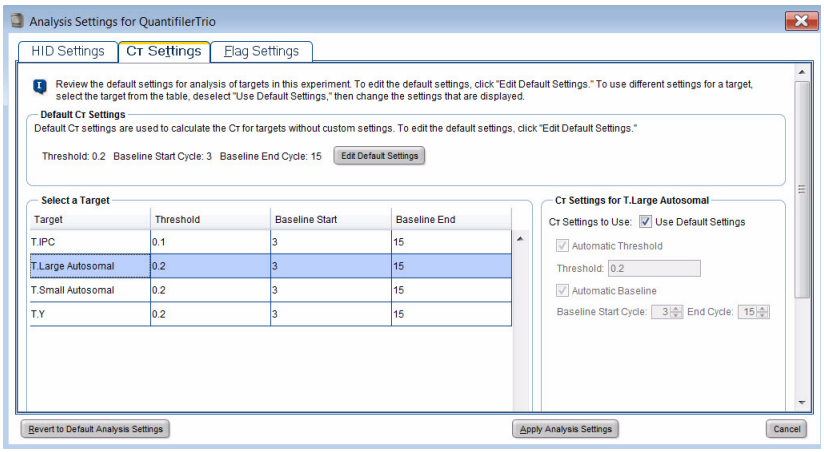
To analyze an experiment:

1. Open the experiment for analysis.
2. Verify the analysis settings:
   a. Click **Analysis Settings** in the upper-right corner of the window.
   b. Click the **C_T Settings** tab.

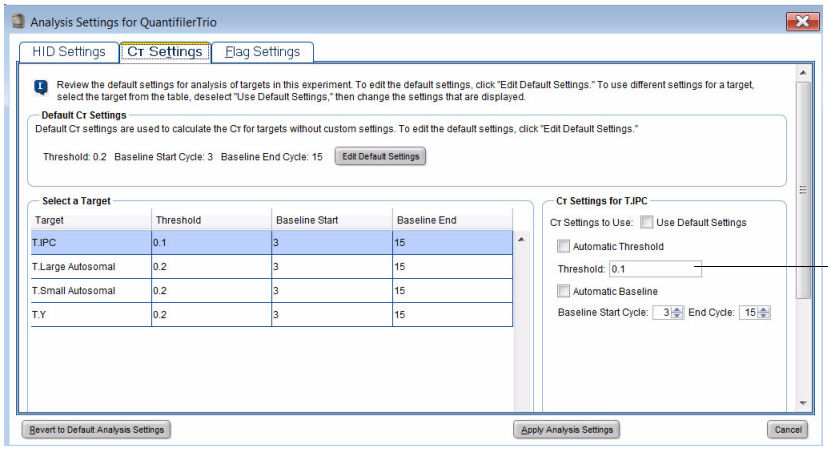
      The recommended C_T settings for each Quantifiler® kit are included in the experiment templates provided with the software. They are also listed in the *Quantifiler® HP and Trio DNA Quantification Kits User Guide*, and shown below. The recommended settings are those which were used in the validation experiments performed for each kit by Thermo Fisher Scientific.

   c. Verify that the settings are as shown below, then:
      - If the analysis settings are correct, click **Apply Analysis Settings**, *or*
      - If the analysis settings differ from those shown below, change them to match the settings, then click **Apply Analysis Settings**.

Large, small, and Y target threshold and baseline settings:



IPC target threshold and baseline settings:



For IPC threshold, 0.1 (see the note below)

Note: Quantifiler® HP and Trio Kits have been validated using the Manual Baseline method. Studies were also performed applying the Automatic Baseline method and the Manual Baseline method to evaluate potential differences between the methods for concentrations from 5 – 0.005 ng/µL. No statistically significant differences were observed within this range for C_T values generated using the Automatic Baseline and Manual analysis methods.

A value of 0.1 was used for the IPC Threshold during the developmental validation studies. Before using alternative baseline methods, (e.g. automatic) or thresholds, perform the appropriate internal validation studies.

3. Click **Analyze**.

View and export results

For information about how to view and export your results, see the *Quantifiler® HP and Trio DNA Quantification Kits User Guide* (Pub. no. 4485354).

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

29 April 2014