PRODUCT BULLETIN

Human identification

HID Real-Time PCR Analysis Software

Enhanced data analysis for QuantStudio 5 and 7500 Real-Time PCR Systems with Quantifiler kit

Applied Biosystems™ Quantifiler™ DNA Quantification Kits are used by human identification (HID) laboratories to aid forensic analysts in the processing of casework samples. The quantification results help guide selection of the most appropriate Applied Biosystems™ short tandem repeat (STR) kits in order to maximize the chances of obtaining optimal STR results on the first attempt.

To take full advantage of the data and capabilities offered by the Quantifiler kits and to maximize sample-processing efficiency, software designed specifically for the HID application is a must.

Applied Biosystems™ HID Real-Time PCR Analysis Software has been developed to provide an integrated solution for DNA quantification and introduces the capability to run the Quantifiler kits on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. The software introduces the ability to analyze the sample data with a virtual standard curve defined by the user. In addition, the software includes quantification and STR setup features, as well as data quality–review functionality, that integrate and streamline quantification and STR analysis steps. By providing predefined templates for Quantifiler and STR kit runs, sample setup time is minimized. Incorporating HID-specific data-quality assessment and STR setup tools into a single software package enables faster and better decisions.

Accuracy

- Designed and validated specifically for human identification applications on the Applied Biosystems™ kits
- Automatically provides sample quantity and quality information to help maximize data recovery
- Integrated STR reaction setup tools automatically calculate the sample normalization and STR reaction setup volumes based on the quantification data, minimizing the potential for errors

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Efficiency

- The virtual standard curve feature allows samples to be run in the place of standards, increasing throughput
- Intuitive, easy-to-navigate user interface streamlines run setup with predefined Quantifiler assay run templates
- Simple, faster sample processing that integrates the quantification and STR analysis setup steps with automated instrument calibration, data quality assessment, and sample normalization and/or STR reaction setup tools
- Faster run setup through optimized and validated robotic integration scripts for commonly used robotic platforms

Flexibility

- Adaptable analysis settings allowing for permutations of running and analyzing with traditional or virtual standard curves
- Enhanced capability to quantify HID samples on the Applied Biosystems™ QuantStudio 5 or 7500 instruments
- Optimized and validated for use with Quantifiler kits, or for performing custom assays by running in the “Custom Assay” mode
- Configurable run templates, experimental and data quality parameters, data display, and reporting features to fit lab-specific protocols
- Flexible for use with either instrument or as stand-alone analysis software on a user’s laptop

Virtual standard curve

HID Real-Time PCR Analysis Software allows for virtual standard curve functionality. This feature enables the user to define appropriate standard curve values (y-intercept and slope) for all targets applicable to the assay being run. The software allows for an enhanced data analysis pipeline that calculates the sample quantities using the traditional standards on the plate or virtual standard curve values (Figure 1).

Streamlined HID-specific workflow

The software provides HID-specific templates for Applied Biosystems™ Quantifiler™ Trio, HP, Duo, Human, Male, and Human and Male (hybrid plate) kits. Customize and set up a run by clicking one of the Quantifiler assay template icons on the home screen (Figure 2).

In each template, predefined experimental properties, targets, sample types, and standard concentrations simplify the quantification run setup process. To set up a run, simply enter or import the sample names and well positions of any unknown samples.

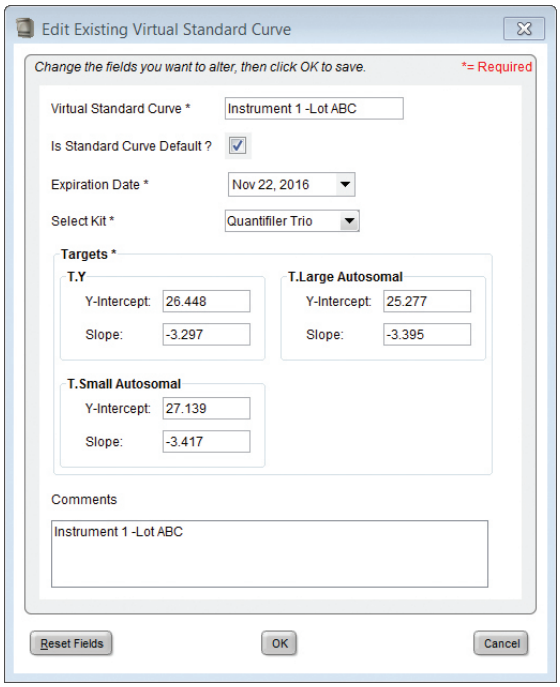

Figure 1. Enhanced data analysis using virtual standard curve functionality.

Figure 2. The home screen provides direct access to HID-specific templates for all Quantifiler DNA Quantification Kits.

The intuitive, easy-to-navigate user interface further streamlines the workflow (Figure 3). The Plate Setup, Run, and Analysis functions are accessed in the Experiment Menu in the left navigation pane. Detailed experimental parameters and results are displayed conveniently on the right pane. For advanced data analysis, there are multiple ways to view and filter data, including plate map and table views, amplification plots, standard curve plots, multicomponent plots, and raw data views that support fast QC, sample data review, and troubleshooting.

Rapid sample quality assessment

The software employs a Quantifiler assay–specific quality control flag to facilitate data analysis and more informed decision-making for STR analysis by identifying samples that require further analysis. Quality flags alert you to standard curve performance or serial dilution preparation issues, as well as samples that contain potential PCR inhibition (internal positive control (IPC) C_t flag), high or low quantities of DNA, or male-and-female mixtures (Figure 4).

Raising the bar in quality

In addition to sample quantity and flagging samples that do not meet data quality requirements, the software automatically calculates the level of DNA degradation (Degradation Index)* and PCR inhibition (IPC C_t) as well as male:female mixture ratios.* This provides a more comprehensive view of sample quality to help determine the optimal approach to maximize data recovery during STR analysis (Figure 5). More informed, up-front decisions can be made, such as whether to amplify using an STR kit with a high number of miniSTRs or Y-STRs, to add more DNA to the reaction to improve allele recovery, or to perform additional clean-up or sample dilution prior to STR analysis to remove or minimize potential inhibitors.

Validated for the Windows 10 platform

HID Real-Time PCR Analysis Software is designed and validated for use on the Microsoft™ Windows™ 10 (32- and 64-bit) platform.

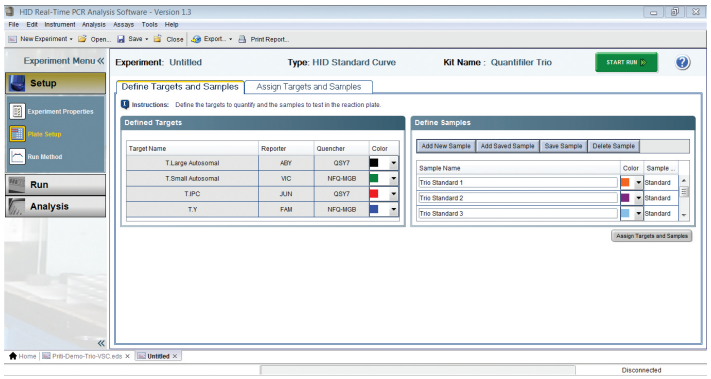

Figure 3. The easy-to-navigate user interface streamlines the workflow with easy access to Setup, Run, and Analysis menus in the left navigation pane.

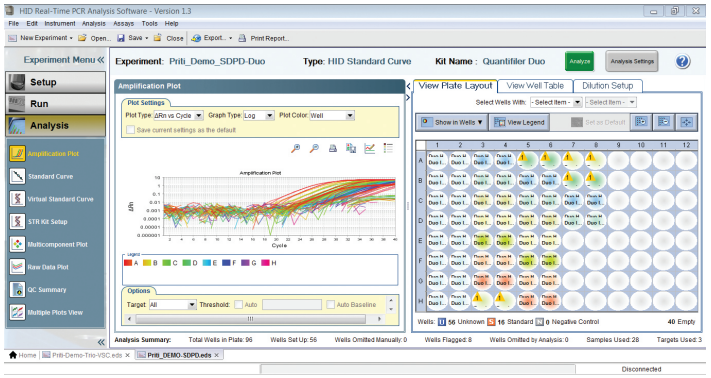

Figure 4. The Analysis Summary provides a quick overview of samples that meet or do not meet thresholds, with hyperlinks to view more detailed information on flagged samples in the well table and plate layout screens.

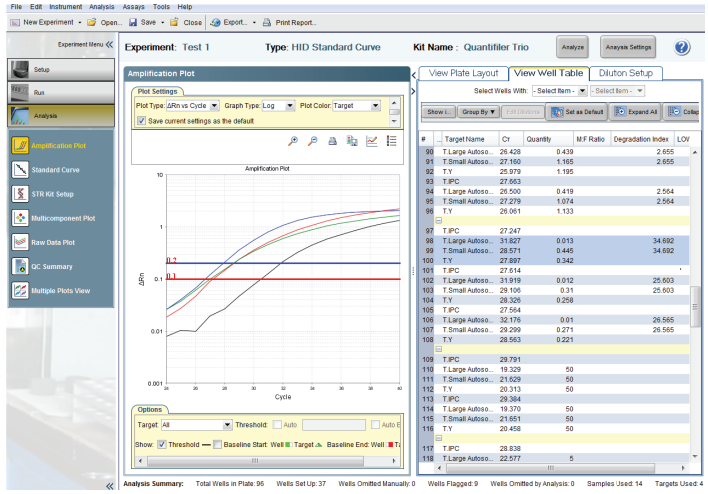

Figure 5. Sample quality information such as the level of DNA degradation and PCR inhibition, as well as the ratio of male to female DNA in mixed samples, is automatically provided.

* For use with Quantifiler Trio and HP kits.

Dilution Calculation Tool

This feature provides instructions based on user preferences to normalize samples prior to STR amplification. Define the dilution scheme parameters, including pipetting overage (%), and minimum pipetting and maximum sample volumes; also choose either the one-step or two-step dilution method as well as a desired dilution factor to meet the laboratory's protocols (Figure 6).

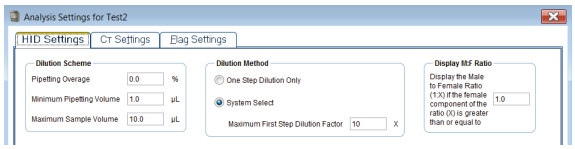

Figure 6. The Dilution Calculation Tool enables users to easily normalize samples.

STR Kit Reaction Setup Tool

This tool utilizes customizable STR kit and sample dilution information to generate sample dilution and STR reaction setup worksheets (Figure 7). Customizable parameters include a list of PCR master mix components, component and sample volumes, target DNA concentration, and pipetting overage allowances. Select the STR kit(s) for downstream analysis in the well table, and the sample dilution and STR kit parameters are automatically applied. Dilution preferences or DNA target concentration can be edited in real time for each sample. This tool makes STR reaction setup quick and easy, and minimizes the potential for errors.

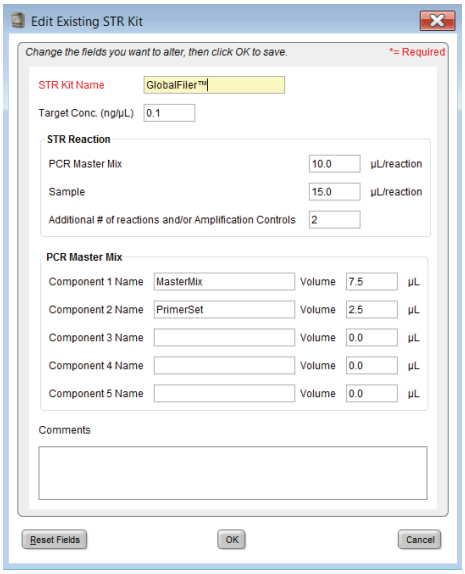

Figure 7. The STR Kit Reaction Setup Tool guides users to set up downstream STR reactions in a simple and easy manner.

Configurable reporting features

A report in PDF format can be exported for inclusion in a case file. A report can also be customized and exported in Microsoft™ Excel™ or text format to perform additional analysis, if needed. To customize an Excel or text file, select the desired information and how it should be organized in the report, such as plate setup, results, amplification data, standard curve, STR dilution and reaction setup, and the file format (Figure 8).

Easy switch to Custom Assay mode

Users who need to perform custom assays can easily switch to the Custom Assay mode by simply clicking "Assays" on the top menu bar and selecting "Custom Assays". This enables you to use the software for different applications or configurations.*

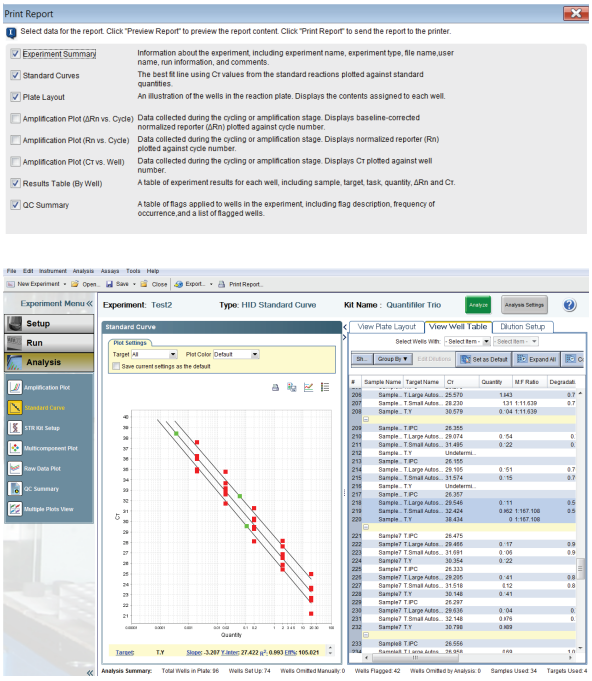

Figure 8. Customizable reporting features provide flexibility to select desired information for reporting in PDF format.

* Only 7500 and 7500 Fast real-time systems supported on custom mode.

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Simplified instrument calibration

In addition to simplifying sample analysis, step-by-step wizard-based instructions guide you through the system calibration process (Applied Biosystems™ ROI, Background, Optical, Pure Dye Calibration Kits, and TaqMan® RNase P Verification Plate). At the end of each calibration, the software automatically analyzes the calibration data and displays the result as Pass or Fail. Time-consuming manual calibration analysis is no longer required. The software automatically records the date and time of each calibration and notifies you when a calibration is due.

System requirements

Component	Recommended requirements
Computer	OS: Windows 10 Enterprise 2016 LTSB Processor: Intel™ Core™ i5-6440HW CPU @2.6 GHz, 16 GB RAM Installed memory: 16 GB
Monitor	• 1,280 x 1,024 pixel resolution for full-screen display • 19-inch • 32-bit color • UL listed
Instrument firmware	• 7500 real-time system: G2 v2.10 • QuantStudio 5 real-time system: 1.3.3 or later

Ordering information

Product	Cat. No.
HID Real-Time PCR Analysis Software—Single User License	A31150
HID Real-Time PCR Analysis Software—5 User License	A31152
HID Real-Time PCR Analysis Software—Single Upgrade User License	A31153
HID Real-Time PCR Analysis Software—5 Upgrade User License	A31154

Find out more at **thermofisher.com/quantifiler**

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