Quantifiler™ Human DNA Quantification Kit
Quantifiler™ Y Human Male DNA Quantification Kit

- **The Quantifiler kits produce reliable and reproducible results, helping to select the appropriate short tandem repeat (STR) analysis method, reducing costs and saving time**
- **The Quantifiler Y Human Male DNA Quantification Kit is the first commercially available kit for male DNA quantification, a valuable tool for sexual assault sample analysis**
- **Pre-formulated Internal PCR Control (IPC) in each reaction allows quick identification of samples that may be inhibited or that do not contain human DNA**
- **Both Quantifiler kits utilize the same, simple protocol that requires fewer steps, minimizing hands-on time, and reducing manual error**
- **Both Quantifiler kits deliver specific, robust quantification of samples with DNA concentrations of 0.023µg/L to > 50µg/L**

Introduction

DNA quantification forensic samples ensure optimal STR analysis. Quantifying the amount of amplifiable human DNA and human male DNA prior to subsequent STR analysis is fast, easy, and reliable, thanks to two reagent kits from Applied Biosystems.

The Quantifiler Human DNA Quantification Kit and the Quantifiler Y Human Male DNA Quantification Kit provide reliable, reproducible, and highly sensitive real-time PCR quantification results for human identity samples.

Optimized and validated for use with the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System, the Quantifiler kits provide an ideal quantification solution to meet the stringent requirements of the human identification community. The Quantifiler Y Human Male DNA Quantification Kit provides additional value when analyzing samples that may contain mixtures of female and male DNA, by specifically amplifying a target only found on the Y chromosome.

Applied Biosystems offer the most complete, integrated system for the quantitation and STR analysis, including validated reagent kits, instruments, analysis software, technical training, and customer support services. Leading the way, the Human Identification group at Applied Biosystems offers the best scientific solutions continuing to enable laboratories to process more samples more efficiently. No other company delivers the depth of products, expertise, and applications support that are available for human identification applications.

Reliable. Reproducible. Real-Time.

The Quantifiler kits are optimized for use with the same simple protocol, utilizing the 5' nuclease assay (real-time PCR) and TaqMan® probe-based technology for maximum reliability and ease of use (see Figure 1). During the real-time PCR assay, the accumulating fluorescent-labeled PCR product is monitored as amplification occurs, allowing the analyst to view the cycle-

to-cycle increase in the fluorescent signal (see Figure 2). Since the Quantifiler kits are based on well characterized PCR technology that determines the amount of DNA present in a sample, the results provide an accurate assessment of how downstream STR analysis will perform.

Because Applied Biosystems quantification kits and AmpF*ℓ*STR kits utilize the same PCR technology, the Quantifiler kits provide the most accurate indication of subsequent STR analysis available.

With one mouse click the real-time PCR amplification results are obtained using the Quantifiler kits, the 7000 and 7500 System Software automatically calculates the amount of total amplifiable DNA present in each sample. The calculation is determined by the cycle number at which the amount of amplified product crosses the noise threshold in each reaction. For each sample, the cycle number is then compared against a standard curve generated from a series of DNA standards, translating the cycle number into a DNA concentration. The Quantifiler Human DNA Standard is included in each kit and is simultaneously amplified with forensic samples on the same reaction plate. The values can then be used to select the appropriate amount of amplifiable DNA that can be added to subsequent STR amplification. The Quantifiler kits enable balanced STR results to be obtained the first time reducing the test cost and time to reportable result.

Streamline Workflow. Ensure Superior Results

The Quantifiler kits include all reagents necessary for the amplification, detection, and quantification of 400 reactions using the Applied Biosystems 7500 Real-Time PCR System or the ABI PRISM 7000 Sequence Detection System. Each kit contains three reagent components:

- Quantifiler PCR Mix
- Quantifiler Human Primer Mix or Quantifiler Y Male Primer Mix
- Quantifiler Human DNA Standard

Both Quantifiler kits utilize the same reagents and allow the user to select the appropriate Quantifiler Primer Mix, which contains a pre-formulated mixture of primers and probes that amplify either the Human Telomerase Reverse Transcriptase gene (Quantifiler Human Kit) or the SRY gene (Quantifiler Y Kit). By amplifying both systems in a single tube, the Quantifiler kits provide an indicator of potential sample inhibition, and allow for quick identification of samples that do not contain human DNA.

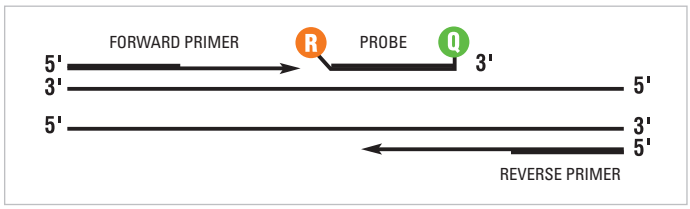
Each kit includes the Quantifiler Primer Mix specific for either the Quantifiler Human or Quantifiler Y Human Male Assay, 1 bottle of the Quantifiler PCR Reaction Mix, and 1 tube of the Quantifiler Human DNA Standard, sufficient for 400 reactions. The Quantifier Human Primer Mix and Quantifiler Y Male Primer Mix is formulated specifically to amplify the Human Reverse Transcriptase gene or SRY gene and is pre-formulated with the IPC system.

Fast and Easy Protocol

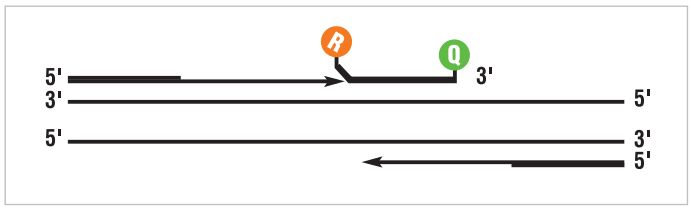
The Quantifiler kits minimize the labor required by reducing procedural steps and accelerating time-to-results. The simple protocol includes preparation of a DNA standard curve, setting up the PCR reactions, and clicking Start to begin amplification and detection. No additional steps are required.

Unlike other quantification methods, the Quantifiler kits protocol does not require lengthy incubation steps, tubes to open and close, sample-transfer steps, or mid-process mixing and washing. The entire quantification procedure can be completed in approximately thirty minutes of hands-on time, providing robust, reproducible results

Figure 1. Real-time PCR reaction.



1. Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' ends of a TaqMan probe, respectively.



2. Strand displacement: When the probe is intact, the reporter dye emission is quenched.

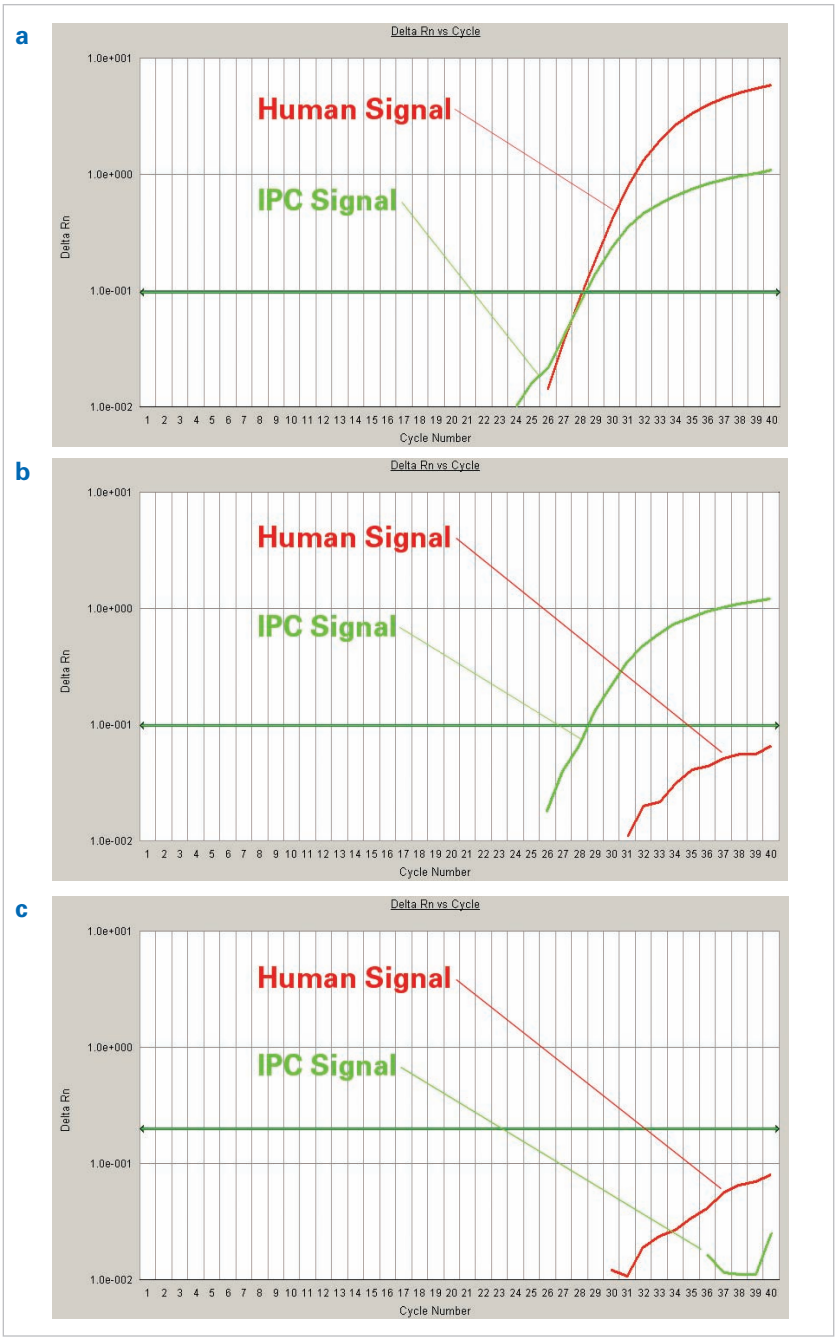


Figure 2. Data obtained using Quantifiler Kits.

2a. Example of a positive DNA sample.

2b. Example of a sample with no detectable DNA.

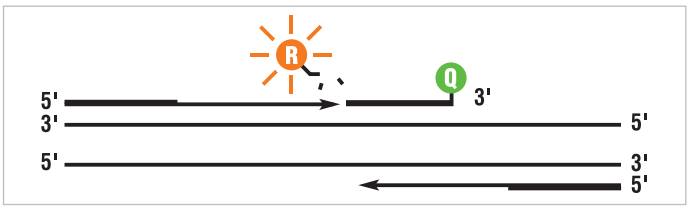
2c. Example of a sample that is inhibited.

for up to 80 samples and one set of standard curves (an 8 point standard curve run in duplicate). Because they use the same amplification and detection parameters, both kits can be run on a single plate, yielding results for up to 64 samples and two sets of standard curves (see Figure 3).

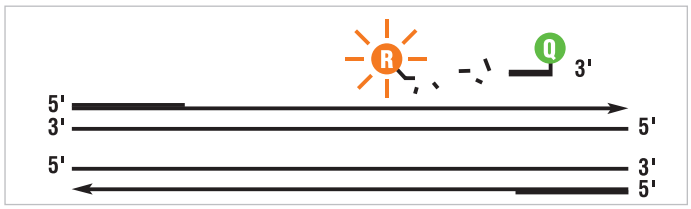
Minimal hands-on time allows analysts to focus on other aspects of their laboratory work, and reduces the possibility of errors that may occur with multiple handling steps. Users of the Quantifiler kits have greater confidence in the reliability of their DNA quantification results. The easy, three-step protocol for reaction setup—together with automated amplification, detection, and analysis procedures—makes DNA quantification a simple, streamlined procedure.

Validated Protocols for a Variety of Sample Types

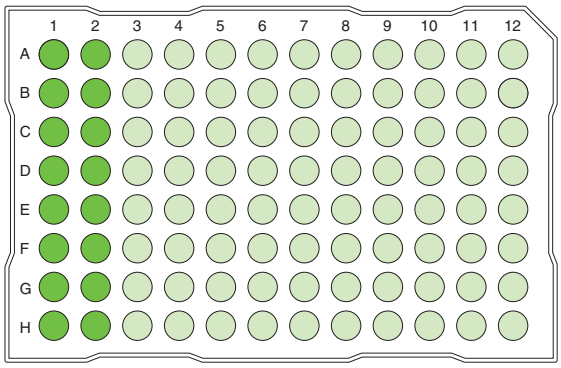
The Quantifiler kit components and amplification protocols are validated and optimized as a component of Applied Biosystems integrated systems for human identification applications. Stringent validation by the manufacturer ensures specific, robust amplification of human identity samples with DNA concentrations of 0.023µg/L to >50µg/L.



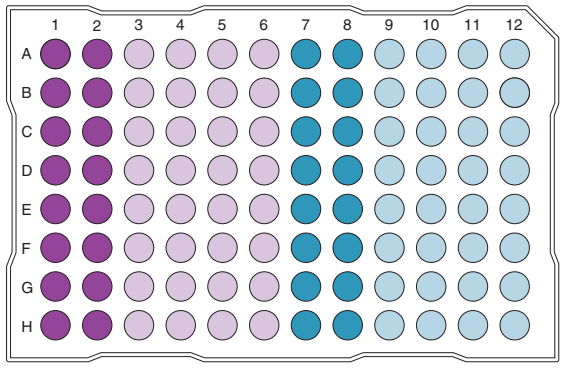
3. Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.



4. Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence.



Rows 1 – 2: Quantifiler Kit Standards
(8-point standard curve run in duplicate)
Rows 3 – 12: Quantifiler Kit Samples



Rows 1 – 2: Quantifiler Human Kit Standards
(8-point standard curve run in duplicate)
Rows 3 – 6: Quantifiler Human Kit Samples
Rows 7 – 8: QuantifilerY Kit Standards
(8-point standard curve run in duplicate)
Rows 9 – 12: QuantifilerY Kit Samples

Figure 3. Examples of possible plate setups using the Quantifiler and Quantifiler Y Human DNA Quantification Kits.

Experiments to evaluate the Quantifiler kits were performed at Applied Biosystems, according to the DNA Advisory Board (DAB) guidelines, "Quality Assurance Standard for Forensic DNA Testing Laboratories." Complete protocols and a summary of the experiments performed and results obtained for developmental validation are available in the Quantifiler Kits User's Manual. For information on Applied Biosystems developmental validation of the Quantifiler kits please reference the article "Developmental Validation of the Quantifiler Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples," Journal of Forensic Sciences, July 2005.

Both Quantifiler kits require minimal sample handling and quantify DNA from a wide variety of human identity sample types, including, but not limited to, buccal swabs, blood, semen, and tissue samples. The quantification protocol is compatible with several commonly used DNA extraction methods including Phenol-Chloroform, Chelex, and silica based chemistries. Once samples are extracted, loaded into the quantification tubes, and sealed, no further sample handling is required. This ensures sample integrity and reduces the risk of contamination.

Complete System Approach to Quantification

Applied Biosystems offers fully integrated reagents, instruments and analysis software for processing data generated in human identification laboratories. DNA samples quantified with the Quantifiler kits may be amplified and analyzed with the ABI PRISM Sequence Detection System that accommodates an individual laboratory's throughput needs. Each Sequence Detection System includes all of the software necessary to amplify, detect, and analyze samples processed using both Quantifiler kits.

Stringent Quality Control

Each Quantifiler kit lot is subjected to stringent quality control testing to help ensure reliability and reproducibility from lot to lot. Applied Biosystems develops and manufactures its products in accordance with ISO 9001 quality system requirements. Additionally, a Certificate of Analysis is available upon request, which confirms that the specific components of the kits meet quality-assurance testing specifications. The Quantifiler and Quantifiler Y Human Quantification Kits convey certain specific rights to perform paternity and forensics testing services in conjunction with an Authorized Thermal Cycler such as the Applied Biosystems 7500 Real-Time PCR System or the ABI PRISM 7000 Sequence Detection System.

Highly Trained Forensic Application Specialists

Applied Biosystems highly trained and experienced Forensic Application Specialists are available either by telephone or online to assist your laboratory with the implementation and use of the Quantifiler kits. Additional training on the use of the Quantifiler kits and Sequence Detection Systems can be provided on site by a Forensic Application Specialist with the purchase of a new Applied Biosystems 7500 Real-Time PCR System or ABI PRISM 7000 Sequence Detection System and a Quantifiler kit.

Applied Biosystems 7500 Real-Time PCR System and Quantifiler Kits.

Features and Benefits at a Glance

- Both Quantifiler kits meet the Human Identification community's requirements for highly sensitive quantification results
- Quantification results more accurately reflect the performance of DNA amplification that will be observed in downstream PCR-based applications
- An IPC is pre-formulated in each reaction, allowing you to quickly identify samples that may be inhibited or that do not contain human DNA
- Easy-to-follow protocol minimizes hands-on time and reduces the possibility of contamination
- Reliable and reproducible results, produced with a single mouse click
- Both Quantifiler kits have a greater than 2,000-fold dynamic range of detection delivering quantification of samples with DNA concentrations of 0.023ng/µL to > 50ng/µL. Quantifiler Y and Yfiler™ kits can be used together to obtain the most comprehensive information present in sexual assault samples

Ordering Information

Description	Rxns x Total Rxn Volume	P/N
Quantifiler™ Human DNA Quantification Kit	400 x 25 µL	4343895
Quantifiler™ Y Human Male DNA Quantification Kit	400 x 25 µL	4343906
Quantifiler™ Human DNA Kit User's Manual		4344790
ABI PRISM® 7000 Sequence Detection System (Laptop)		4349132
ABI PRISM® 7000 Sequence Detection System (Tower)		4349117
Applied Biosystems 7500 Real-Time PCR System (Laptop)		4366604
Applied Biosystems 7500 Real-Time PCR System (Tower)		4366605

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