

# Quantifiler® Duo DNA Quantification Kit

- Simultaneously quantitates total human and human male DNA in a single, highly sensitive real-time PCR reaction
- Internal PCR control allows rapid identification of samples which may contain PCR inhibitors
- Target amplicons reliably predict STR performance including a realistic estimate of the amount of amplifiable DNA present in degraded samples
- Guides selection of the optimal STR chemistry (autosomal, Y-STR or miniSTR) for each sample
- Minimizes sample consumption and streamlines workflow
- Optimized and validated as part of a complete DNA testing system with the Applied Biosystems 7500 Real-Time PCR System and AmpFℓSTR® PCR Amplification Kits to provide high quality STR profiling results



## Introduction

Forensic analysts routinely encounter a variety of challenging biological samples, many of which contain mixtures of male and female DNA, and/or have been exposed to environmental insults. To aid selection of the most appropriate STR chemistry and maximize the chances of obtaining an interpretable STR profile in the first attempt, it is beneficial to efficiently determine the relative quantities of male and female DNA and detect the presence of PCR inhibitors in a sample at an early stage in the analysis process. TaqMan® real-time PCR assays, like the Quantifiler® Human DNA Quantification Kit and the Quantifiler® Y Human Male DNA Quantification Kit, have proven useful in addressing this need while providing numerous advantages over hybridization-based assays.

The Quantifiler® Duo DNA Quantification Kit improves and expands upon the trusted Quantifiler® technology, enabling forensic laboratories to simultaneously obtain a quantitative and qualitative assessment of total human and human male DNA in a single, highly sensitive real-time PCR reaction. This guides selection of the optimal STR chemistry (autosomal, Y-STR or miniSTR) and streamlines the workflow while increasing downstream analysis success rates. The Quantifiler® Duo kit has been optimized to predict and improve performance with the AmpFℓSTR® PCR Amplification Kits, and can be easily integrated into the forensic DNA workflow to maximize recovery of interpretable STR profiles from a wide range of forensic biological specimens including sexual assault and challenging samples.

Proven Technology – Enhanced

Based on the same 5’ nuclease assay (real-time PCR) and TaqMan® probe-based technology as the previous generation of Quantifiler® kits, the Quantifiler® Duo kit benefits from all the advantages afforded by this technology (Figure 1), but provides more comprehensive and predictive information in a single reaction.

The Quantifiler® assays use single-copy targets, which provide close correlation with the single-copy targets in STR kits, as well as superior accuracy and reproducibility compared to multi-copy targets (which can be affected by variability between individuals).

In the Quantifiler® Duo kit, the incorporation of a new human target and longer amplicons further improves the prediction of STR amplification performance (Table 1). Amplicon lengths of 140 bp and 130 bp, for the human and male targets respectively, better reflect the lengths of STR amplicons generated during downstream amplification and help to prevent overestimation of DNA concentration in degraded samples. The similar amplicon lengths for all three markers help produce a balanced PCR assay, avoiding preferential amplification of one target over another.

To further enhance the reliability of the assay, the Quantifiler® Duo kit uses a standard curve generated from pooled human male genomic DNA in contrast to the cell line source used in previous generation Quantifiler® kits. Using a DNA standard that is more similar in nature to the samples to which it will be compared improves the reliability of the DNA concentration estimate and the downstream amplification success rate.

Single Reaction – Multiple Functions

Each Quantifiler® Duo kit includes all reagent components necessary for the amplification, detection, and quantification of 400 samples in 25 µL reactions using the Applied Biosystems 7500 Real-Time PCR System.

Advantages of TaqMan® Real-Time PCR Assays for Forensic DNA Quantification

- Gold-standard technology widely adopted in forensic laboratories
- Highly specific for desired PCR products
- Robust and reproducible with a wide dynamic range
- Highly sensitive with low background noise enabling detection down to single molecule levels
- Provides a reliable and predictive estimate of the amount of amplifiable DNA present for downstream STR analysis
- Ability to detect the presence of PCR inhibitors with an internal PCR control
- Minimal hands-on time accelerates time-to-result

Figure 1. Advantages of TaqMan® Real-Time PCR assays for forensic DNA quantification.

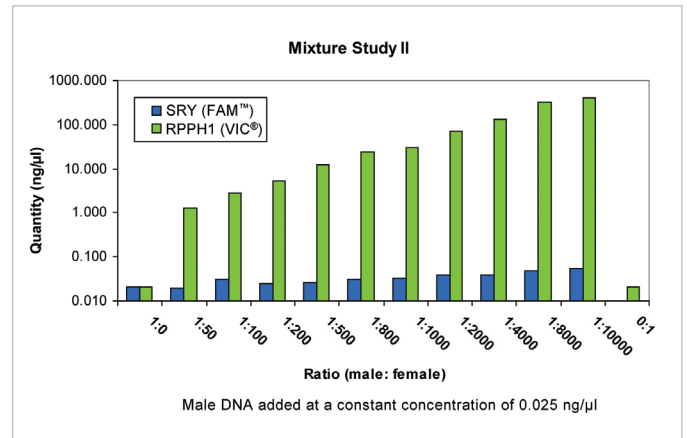
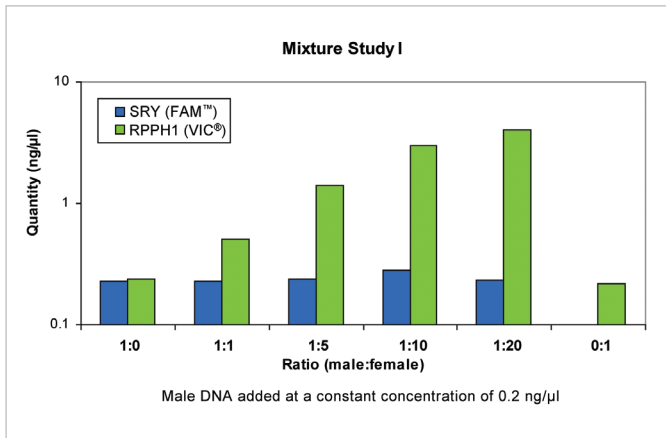
Target	Marker	Size	Dye
Human DNA	RPPH1 (Ribonuclease P RNA component H1)	140 bp	VIC®
Human Male DNA	SRY (Sex determining region Y)	130 bp	FAM™
Internal PCR Control	Artificial Template	130 bp	NED™

Table 1. Quantifiler® Duo kit configuration.

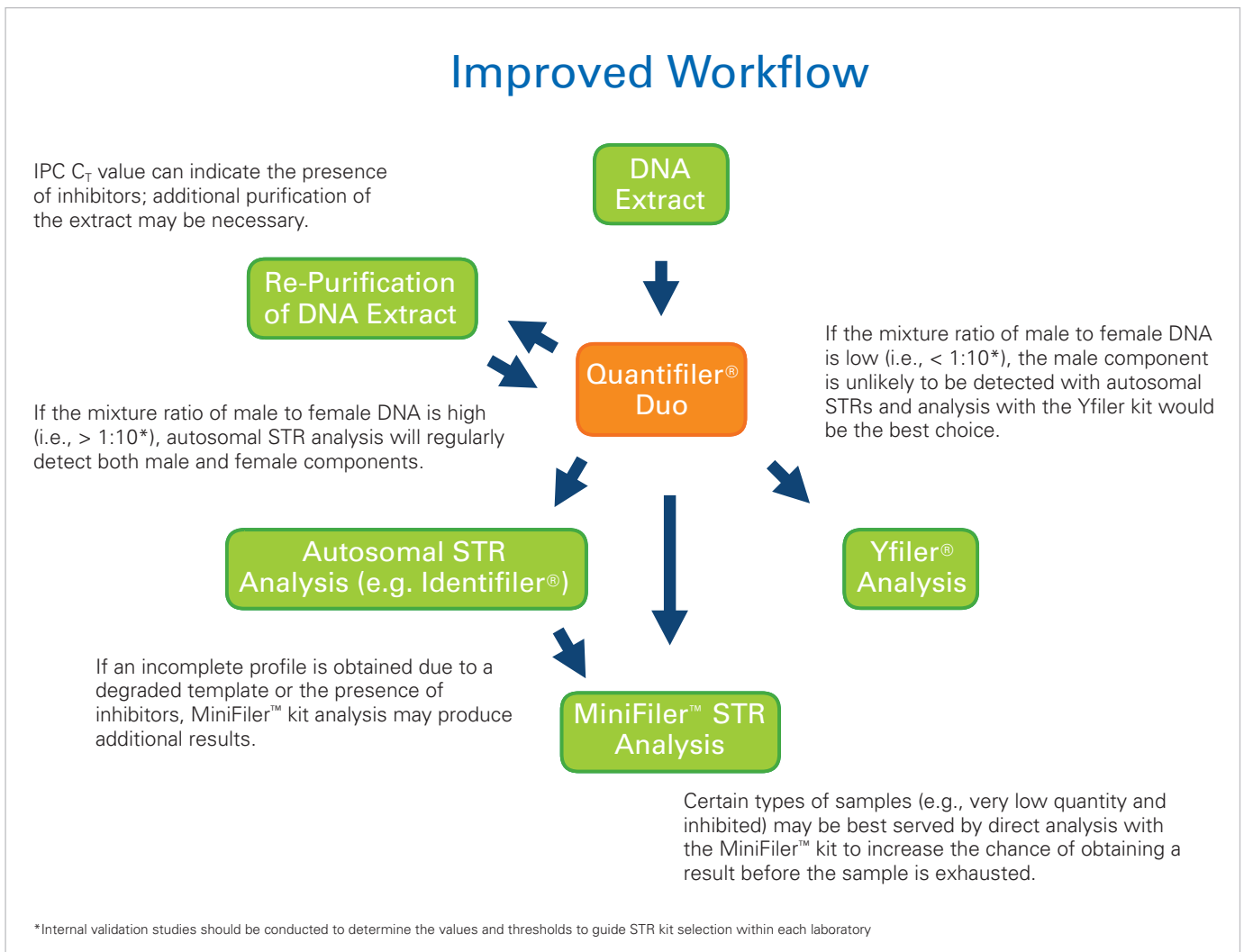
The Quantifiler® Duo Primer Mix is pre-formulated with primer pairs and probes that simultaneously amplify the ribonuclease P RNA component H1 (RPPH1) human target and the sex determining region Y (SRY) male-specific target. Additionally, a third primer and probe system along with an Internal PCR Control (IPC) template has been formulated into this reagent creating a triplex reaction. By amplifying all reactions with the IPC in a single tube, the Quantifiler® Duo kit enables detection of PCR inhibitors and allows for rapid identification of samples that do not contain human DNA. The Quantifiler® Duo kit also enables detection of minute quantities of human male DNA, even in the presence of a large excess of female DNA, and efficiently provides a reliable estimate of the male to female DNA mixture ratio (Figure 2). This indicates when Y-STR analysis using the Yfiler® kit may be required to obtain results for the male component.

A Highly Informative Tool

The Quantifiler® Duo kit has been optimized and validated as part of a complete DNA testing system with the AmpFℓSTR® PCR Amplification Kits. This enables it to function as a critical component of an improved and integrated workflow for more effective processing of forensic casework samples. The workflow diagram in Figure 3 illustrates how the kit functions as a “sample assessment system” which can efficiently determine the ratio of male to female DNA and guide selection of the optimal AmpFℓSTR® PCR Amplification Kit (autosomal, Y-STR or miniSTR) to maximize recovery of interpretable STR profiles.



**Figure 2.** Mixture Study I demonstrates the Quantifiler® Duo kit's ability to provide a reliable estimate of the male to female DNA mixture ratio. Samples with a low male to female ratio (i.e. < 1:10\*) may require Y-STR analysis using the Yfiler® kit to obtain results for the male component. Mixture Study II demonstrates the ability to detect minute quantities of human male DNA in the presence of a large excess of female DNA.



**Figure 3.** The Quantifiler® Duo kit is a highly informative tool which can detect the presence of inhibitors, determine the ratio of male to female DNA, and guide selection of the optimal AmpF/STR® PCR Amplification Kit (autosomal, Y-STR or miniSTR) to maximize recovery of interpretable STR profiles.

## Designed For Forensic Samples

The Quantifiler® Duo kit components (Table 2) and amplification protocols have been developed to provide specific, robust amplification of forensic samples with the Applied Biosystems 7500 Real-Time PCR System. With a greater than 2,000-fold dynamic range of detection, the kit provides quantification of samples with DNA concentrations of 0.023 ng/μL to > 50 ng/μL. The highly sensitive assay has also demonstrated the ability to consistently detect DNA in samples with concentrations as low as 6 pg/μL. However, detection of <100 pg/μL using any amplification-based quantification method is subject to stochastic effects

which may result in some uncertainty in quantification results and prediction of amplification success. Any samples yielding quantification values of 100 pg/μL or less will require the maximum volume of DNA extract for STR amplifications targeting 1 ng regardless of the DNA concentration.

The Quantifiler® Duo kit quantifies 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 all commonly used DNA extraction methods.

## Validate with Confidence

Developmental validation experiments for the Quantifiler® Duo kit were performed by Applied Biosystems scientists according to the Revised Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDM). Complete protocols and a summary of the experiments performed along with results obtained for developmental validation are available in the Quantifiler® Duo DNA Quantification Kit User's Manual (P/N 4391294). Each laboratory using the Quantifiler® Duo kit should perform its own internal validation studies.

## Quality Counts

Each Quantifiler® Duo kit lot is subjected to extensive quality control testing to ensure reliability. 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 kit meet quality-assurance testing specifications.

## Experience Matters™

Applied Biosystems is the only company in the world that designs and validates its reagents, instruments, and data analysis software together as an integrated system for DNA testing.

Technical Service Scientists and Field Application Specialists are available to answer your technical questions, review data, provide training and make recommendations on how to perform assays and troubleshoot results. All of these resources help ensure success when using Applied Biosystems Human Identification solutions in your laboratory.

COMPONENT	COMPOSITION
Quantifiler® Duo Primer Mix	Primer pairs for amplification of RPPH1, SRY, and IPC. The TaqMan® probes for RPPH1, SRY, and IPC are labeled with VIC®, FAM™ and NED™ dyes, respectively. In addition, this reagent contains Internal PCR Control (IPC) template.
Quantifiler® Duo PCR Reaction Mix	AmpliTaq Gold® DNA Polymerase in buffer and salts
Quantifiler® Duo DNA Standard	Pooled human male genomic DNA
Quantifiler® Duo Dilution Buffer	10 mM Tris HCl buffer, pH 8.0 containing 0.1 mM EDTA

Table 2. Quantifiler® Duo kit components.

## ORDERING INFORMATION

Description	P/N
Quantifiler® Duo DNA Quantification Kit (400 Reactions)	4387746
Quantifiler® Duo DNA Quantification Kit User's Manual	4391294
Applied Biosystems 7500 Real-Time PCR System for Human Identification	4366605
HID Real-Time PCR Analysis Software v1.0	4413973

For Research, Forensic or Paternity Use Only. Not for use in diagnostic procedures.

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