Quantifiler™ Human and Y Human Male DNA Quantification Kits

USER GUIDE

for use with:
Quantifiler™ Human DNA Quantification Kit Quantifiler™
Quantifiler™ Y Human Male DNA Quantification Kit

Catalog Numbers 4343895 and 4343906
Publication Number 4344790
Revision H

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

ThermoFisher SCIENTIFIC

Manufacturer: Thermo Fisher Scientific | 7 Kingsland Grange | Warrington, Cheshire WA1 4SR | United Kingdom

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 4344790

Revision	Date	Description
H	24 August 2018	Updated branding and trademarks, no technical changes.
G	January 2014	Update format, no technical changes.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. AmpliTaq Gold is a trademark of Roche Molecular Systems, Inc. Microsoft and Excel are trademarks of Microsoft Corporation.

Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

1 Overview

Product overview

Purpose

The Quantifiler™ Human DNA Quantification Kit (Quantifiler™ Human Kit) (Cat. no. 4343895) and the Quantifiler™ Y Human Male DNA Quantification Kit (Quantifiler™ Y Kit) (Cat. no. 4343906) are designed to quantify the total amount of amplifiable human (and higher primate) DNA or human male DNA in a sample. The results from using the kits can aid in determining:

- If sufficient human DNA or human male DNA is present to proceed with short tandem repeat (STR) analysis
- How much sample to use in STR analysis applications

Product description

The Quantifiler™ Kits contain all the necessary reagents for the amplification, detection, and quantification of a human-specific DNA target or a human male-specific DNA target.

The reagents are designed and optimized for use with the following instruments and software:

- ABI PRISM™ 7000 Sequence Detection System and SDS Software v1.0
- Applied Biosystems™ 7900HT Sequence Detection System (no automation module) and SDS Software v2.0.

See Chapter 6, "Data Analysis and Results" for validation studies performed using the Applied Biosystems™ 7500 Real-Time PCR System with SDS Software v1.2.3 and the ABI PRISM™ 7000 Sequence Detection System with SDS Software v1.2.3.

Chemistry overview

Assay overview

The DNA quantification assay combines two 5′ nuclease assays:

- A target-specific (human DNA or human male DNA) assay
- An internal PCR control (IPC) assay

Target-specific assay components

The target-specific assay consists of:

- Two primers for amplifying human DNA or human male DNA
- One TaqMan™ MGB probe labeled with FAM™ dye for detecting the amplified sequence

About the targets

Table 1 provides information about the targets of PCR amplification in the Quantifiler™ Human Kit and the Quantifiler™ Y Kit.

Table 1 Targets of Quantifiler™ Kits

Kit	Gene Target	Location	Amplicon Length	Region Amplified	Ploidy
Quantifiler™ Human Kit	Human telomerase reverse transcriptase gene (hTERT)	5p15.33	62 bases	Nontranslated region (intron)	Diploid†
Quantifiler™ Y Kit	Sex-determining region Y gene (SRY)	Yp11.3	64 bases	Nontranslated region	Haploid†

† Single-copy target

IPC assay components

The IPC assay consists of:

- IPC template DNA (a synthetic sequence not found in nature)
- Two primers for amplifying the IPC template DNA
- One TaqMan™ MGB probe labeled with VIC™ dye for detecting the amplified IPC DNA

About the probes

The TaqMan™ MGB probes contain:

- A reporter dye (FAM™ dye or VIC™ dye) linked to the 5′ end of the probe
- A minor groove binder (MGB) at the 3′ end of the probe
- This modification increases the melting temperature (Tm) without increasing probe length (Afonina *et al.*, 1997; Kutyavin *et al.*, 1997), which allows the design of shorter probes.
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe
- Because the quencher does not fluoresce, Life Technologies sequence detection systems can measure reporter dye contributions more accurately.

3. Based on wavelength, the grating separates the light into a predictably spaced pattern across the CCD camera.
4. During the run, the CCD camera detects the fluorescence emission between 500 nm and 660 nm from each well.
5. The SDS software obtains the fluorescence emission data from the CCD camera and applies data analysis algorithms.

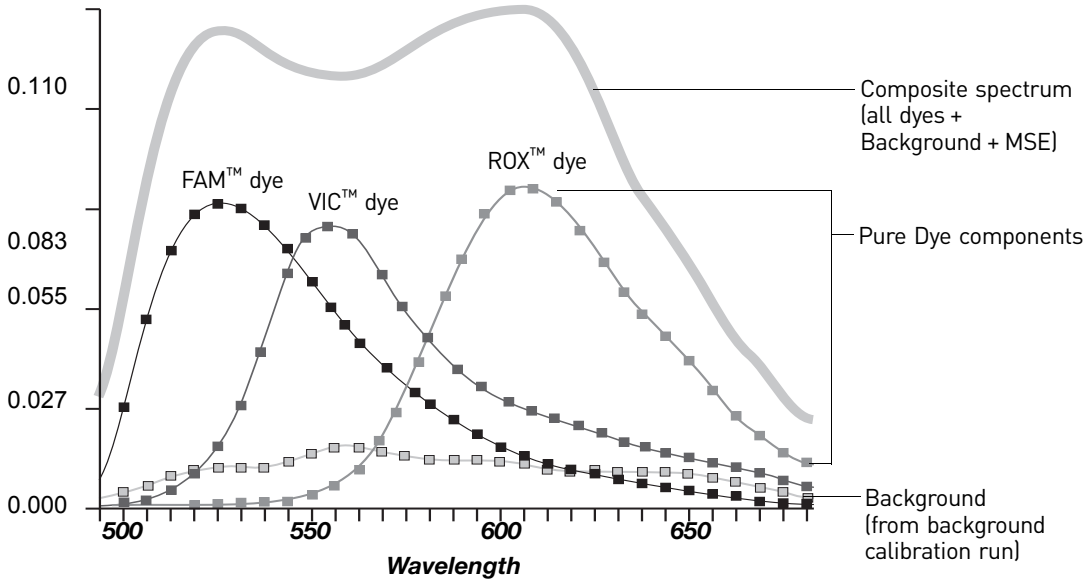
SDS software overview

This section describes how the SDS software analyzes raw run data from real-time runs. Raw data consists of the spectral data between 500 nm to 660 nm collected by the SDS software during a sequence detection run.

Composite spectrum

Figure 6 shows a composite fluorescence spectrum from a single well containing the passive reference, one probe labeled with FAM™ dye and a nonfluorescent quencher, and one probe labeled with VIC™ dye and a nonfluorescent quencher. The example shows how the overlapping component dye spectra contribute to the composite spectrum.

Figure 6 Example of a composite spectrum



Processing multicomponent data

During the multicomponent transformation, the SDS software uses algorithms to determine the contribution of each dye:

- An algorithm removes the background component stored in the background calibration file to eliminate the contribution of background fluorescence in the raw data.
- The software uses the extracted pure dye standards to express the composite spectrum in terms of the pure dye components.
- Then, an algorithm applies matrix calculations to determine the contributions of each component dye to the composite spectrum.

How C_T values are determined

To determine the C_T value, the SDS software uses the R_n values collected from a predefined range of PCR cycles called the baseline (the default baseline occurs between cycles 6 and 15 on the 7000 SDS and between cycles 3 and 15 on the 7900HT SDS):

1. The software generates a baseline-subtracted amplification plot of ΔR_n versus cycle number.
2. An algorithm defines the cycle where the ΔR_n value crosses the threshold setting (the default threshold setting is 0.2) as the threshold cycle (C_T).

Relationship of threshold cycles to initial template amount

The following equation describes the exponential amplification of the PCR:

$$X_n = X_m(1 + E_X)^{n-m}$$

where:

Xn = number of target molecules at cycle n (so that n > m)

Xm = number of target molecules at cycle m

EX = efficiency of target amplification (between 0 and 1)

n – m = number of cycles elapsed between cycle m and cycle n

Amplicons designed and optimized according to our guidelines (amplicon size <150 bp) have amplification efficiencies that approach 100%. Therefore EX = 1 so that:

$$\begin{aligned} X_n &= X_m(1 + 1)^{n-m} \\ &= X_m(2)^{n-m} \end{aligned}$$

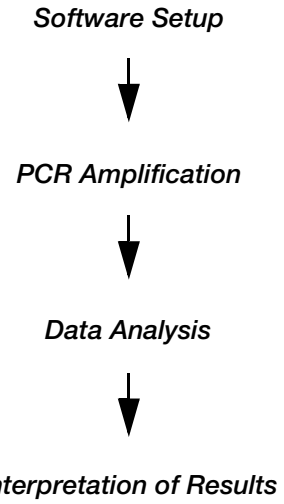
To define the significance in amplified product of one thermal cycle, set n – m = 1 so that:

$$\begin{aligned} X_n &= X_m(2)^1 \\ &= 2X_m \end{aligned}$$

Therefore, each cycle in the PCR reaction corresponds to a two-fold increase in product. Likewise, a difference in C_T values of 1 equates to a two-fold difference in initial template amount.

Procedural overview

Use of the Quantifiler™ Kits involves the following workflow:



Materials and equipment

Kit contents and storage

Each Quantifiler™ Kit contains materials sufficient to perform 400 reactions at a 25-µL reaction volume.

Table 2 Quantifiler™ Kits contents

Reagent	Contents	Quantity	Storage
Quantifiler™ Human Primer Mix or Quantifiler™ Y Human Male Primer Mix	Forward and reverse primers to amplify human DNA or human male DNA target Probe to detect human DNA or human male DNA target IPC system primers, template, and probe	3 tubes, 1.4 mL each	–15 to –25 °C
Quantifiler™ Human DNA Standard	200 ng/µL purified DNA standard	1 tube, 120 µL	–15 to –25 °C
Quantifiler™ PCR Reaction Mix	AmpliTaq Gold™ DNA Polymerase, dNTPs with dUTP, Passive Reference, and optimized buffer components	1 tube, 5 mL	2 to 8 °C

Additional storage guidelines for primer mixes

Follow the additional guidelines for storing the primer mixes:

- Minimize freeze-thaw cycles.
- Keep protected from direct exposure to light. Excessive exposure to light may affect the fluorescent probes.

Equipment and materials not included

Table 3 through Table 5 list required and optional equipment and materials not supplied with the Quantifiler™ Kits.

Table 3 Equipment

Equipment	Source
Applied Biosystems™ 7900HT Real-Time PCR System (no automation)	Contact your local Life Technologies sales representative.
ABI PRISM™ 7000 Sequence Detection System	
Tabletop centrifuge with 96-well plate adapters (optional)	major laboratory supplier (MLS)

Table 4 User-supplied materials

Material	Source
Quantifiler™ Human DNA Quantification Kit	Life Technologies (Cat. no. 4343895)
Quantifiler™ Y Human Male DNA Quantification Kit	Life Technologies (Cat. no. 4343906)
Glycogen, 20 mg (1 mL)	Roche Applied Science (Cat. no. 901 393)
High-Throughput Setup	
96-Well Optical Reaction Plates	Life Technologies (Cat. no. 4306737)
Optical Adhesive Covers Starter Kit (20 covers, 1 compression pad, 1 applicator)	Life Technologies (Cat. no. 4313663)
Optical Adhesive Covers (100 covers)	Life Technologies (Cat. no. 4311971)
MicroAmp™ Splash Free Support Base	Life Technologies (Cat. no. 4312063)

Material	Source
Mid-to-Low-Throughput Setup	
MicroAmp™ Optical Tubes (8 tubes/strip, 125 strips)	Life Technologies (Cat. no. 4316567)
MicroAmp™ 96-Well Tray/Retainer Set	Life Technologies (Cat. no. 403081)
Optical Caps (8 caps/strip, 300 strips)	Life Technologies (Cat. no. 4323032)
Compression pad from Optical Adhesive Covers Starter Kit **Note:** Not necessary if using Optical Caps	Life Technologies (Cat. no. 4313663)

Table 5 Documents

Document	Life Technologies Pub. no.
ABI PRISM™ 7000 Sequence Detection System User Guide	4317596
Applied Biosystems™ 7900HT Sequence Detection System User Guide	4317596

2 Software Setup

Plate document types

You can use the SDS software to create two types of plate document files.

Plate Document Type	File Extension	Description
SDS document	*.sds	Primary file to use when performing a run. Required for all experiments.
SDS template	*.sdt	File that already contains run parameters that are commonly used in plate documents, such as detectors, thermal cycler conditions, and so on. Streamlines the creation of the SDS document (*.sds) file.

Example plate document setup

You can arrange the reactions in any well of the reaction plate, but you need to set up the plate document so that it corresponds exactly to the arrangement of the standards and unknown samples in the wells of the reaction plate. Table 6 shows one example of arranging reactions when running two Quantifiler™ Kits on one 96-well reaction plate:

- Wells A1 through D12 (gray) correspond to reactions using the Quantifiler™ Human DNA Quantification Kit (Quantifiler™ Human Kit)
- Wells E1 through H12 (white) correspond to reactions using the Quantifiler™ Y Human Male DNA Quantification Kit (Quantifiler™ Y Kit)

For each Quantifiler™ Kit assay, there are eight DNA quantification standards and two reactions for each standard. See "Prepare the DNA quantification standard" on page 51 for more information about the DNA quantification standards.

Table 6 Example plate setup of reactions with two kits

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6
B	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
C	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
D	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
E	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6
F	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
G	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
H	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN

Table 7 shows another example of arranging reactions when running two Quantifiler™ Kits on one 96-well reaction plate if you are using repeat pipettors:

- Wells A1 through D6 (gray) correspond to reactions using the Quantifiler™ Human Kit
- Wells A7 through H12 (white) correspond to reactions using the Quantifiler™ Y Kit

For each Quantifiler™ Kit assay, there are eight DNA quantification standards and two reactions for each standard. See "Prepare the DNA quantification standard" on page 51 for more information about the DNA quantification standards.

Table 7 Example plate setup of reactions using repeat pipettors

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	UNKN	UNKN	UNKN	UNKN	Std 1	Std 1	UNKN	UNKN	UNKN	UNKN
B	Std 2	Std 2	UNKN	UNKN	UNKN	UNKN	Std 2	Std 2	UNKN	UNKN	UNKN	UNKN
C	Std 3	Std 3	UNKN	UNKN	UNKN	UNKN	Std 3	Std 3	UNKN	UNKN	UNKN	UNKN
D	Std 4	Std 4	UNKN	UNKN	UNKN	UNKN	Std 4	Std 4	UNKN	UNKN	UNKN	UNKN
E	Std 5	Std 5	UNKN	UNKN	UNKN	UNKN	Std 5	Std 5	UNKN	UNKN	UNKN	UNKN
F	Std 6	Std 6	UNKN	UNKN	UNKN	UNKN	Std 6	Std 6	UNKN	UNKN	UNKN	UNKN
G	Std 7	Std 7	UNKN	UNKN	UNKN	UNKN	Std 7	Std 7	UNKN	UNKN	UNKN	UNKN
H	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN

Set up a plate document

Overview

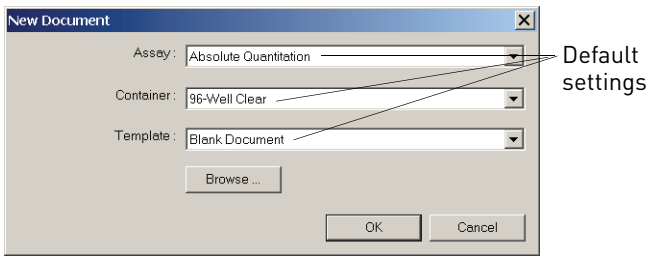
Setting up a plate document to run Quantifiler™ Kit assays involves:

1. Create a blank plate document (page 29)
2. Create detectors (the first time only, page 30)
3. Add detectors to the plate document (page 32)
4. Apply detectors for standards (page 32)
5. Apply detectors for unknown samples (page 34)
6. Add sample names for unknown samples (page 34)
7. Set thermal cycler conditions (page 35)
8. Save the plate document (page 37)

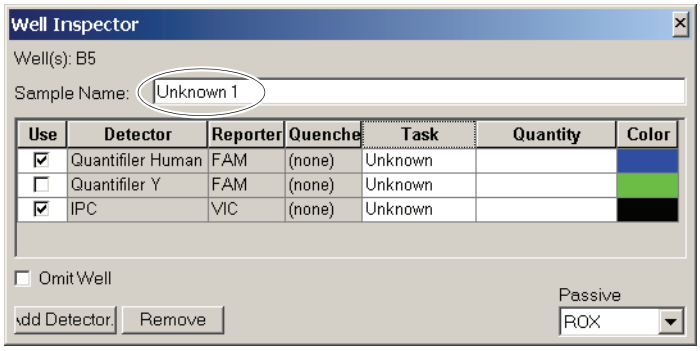
Create a blank plate document

To create a blank plate document:

1. If the SDS software is not already started, select **Start ▸ Programs ▸ ABI Prism 7000 ▸ ABI Prism 7000 SDS Software**.
2. In the SDS software, select **File ▸ New** to open the New Document dialog box.



For example:



Note: Samples with identical sample names are treated as replicates by the SDS software. Results for replicate reactions are grouped together automatically for data analysis.

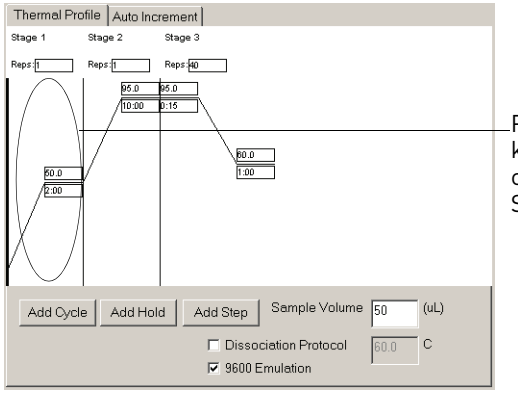
Set thermal cycler conditions

Before running a Quantifiler™ Kit assay, you need to make two changes to the default thermal cycler conditions:

- Thermal profile
- Sample volume

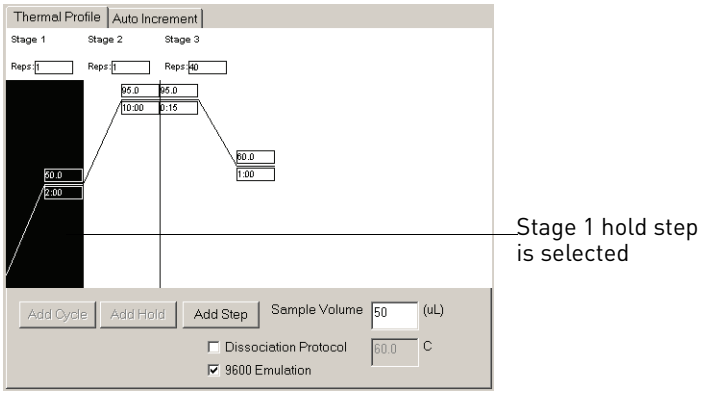
To set thermal cycler conditions:

1. In the plate document, select the **Instrument** tab.
2. Press the **Shift** key and click within the Stage 1 hold step (50 ·C for 2 minutes) to select it.

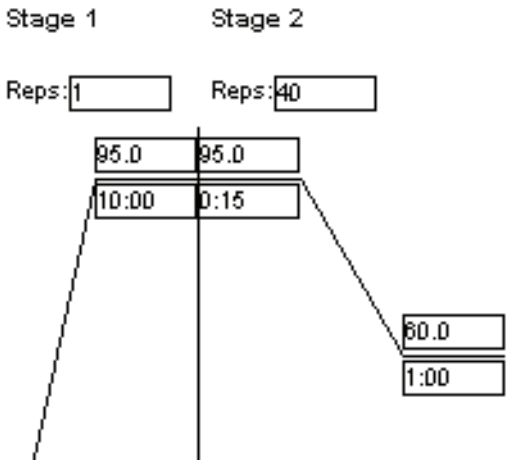


Press the **Shift** key while you click within the Stage 1 hold step

3. After the hold step is selected, press the Delete key.

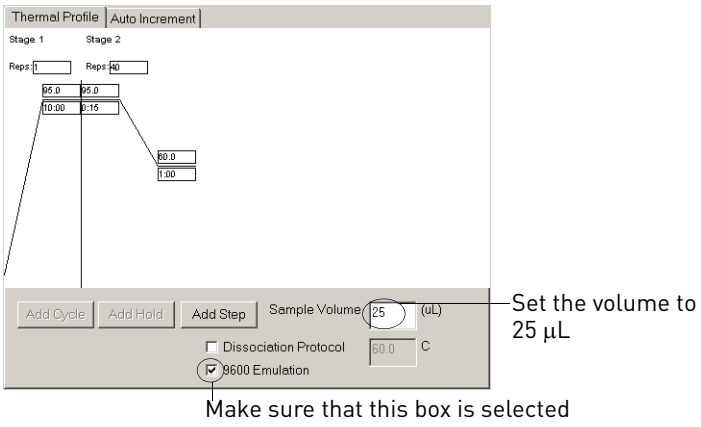


4. Make sure that the thermal profile appears as follows:



5. Change the Sample Volume to 25 (µL) and make sure that the 9600 Emulation box is selected.

   Note: Selecting the 9600 Emulation box reduces the ramp rate.



Save the plate document

Before running the reaction plate, save the plate document as an SDS Document (*.sds) file.

Note: To save the plate document as a template, see "Set up a plate document template" on page 37.

To save the plate document:

1. Select **File ▸ Save**.
2. Select the location for the plate document.
3. Enter a file name.
4. For Save as type, select **SDS Documents (*.sds)**.
5. Click **Save**.

Set up a plate document template

Purpose

A plate document template reduces the time required to set up a plate document. This section describes how to create an SDS Template Document (*.sdt) for running Quantifiler™ Kit assays.

Template settings

In addition to plate document settings (assay and container), templates can contain:

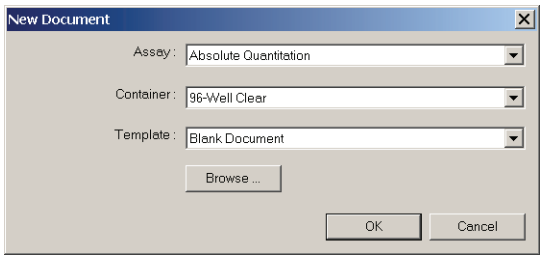
- Assay-specific detectors
- Well assignments for quantification standards, with detectors, tasks, and quantity
- Well assignments for unknown samples, with detectors and tasks
- Instrument settings: thermal cycler conditions and reaction volume settings

Creating a plate document template

This procedure assumes that you have created the detectors for running reactions using the Quantifiler™ Kits (page 30).

To create a plate document template:

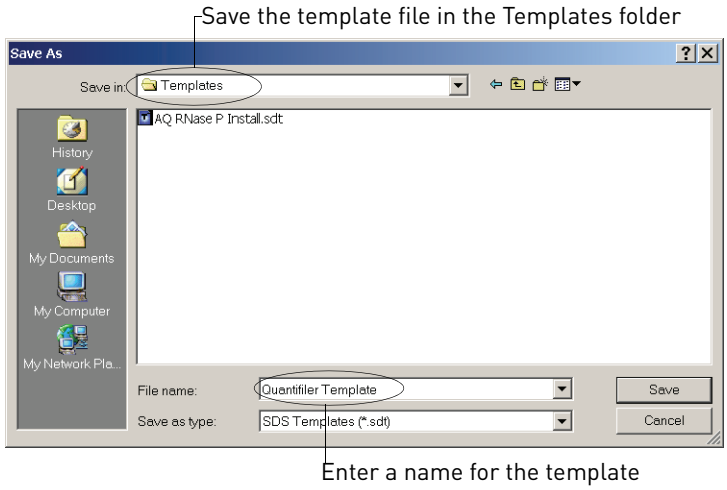
1. If the SDS software is not already started, select **Start ▸ Programs ▸ ABI Prism 7000 ▸ ABI Prism 7000 SDS Software**.
2. Select **File ▸ New**, complete the New Document dialog box, then click **OK**.



3. Apply the desired template settings to the plate document:
   - Add detectors to the plate document (page 32)
   - Apply detectors for standards and for unknown samples (page 32 and page 34)
   - Set thermal cycler conditions (page 35)
4. Select **File ▸ Save As** and complete the Save As dialog box:
   a. For Save as type, select **SDS Templates (*.sdt)**.
   b. Locate and select the Templates folder within the software folder:

      X:Program Files ▸ ABI Prism 7000 ▸ Templates, where X is the hard drive on which the SDS software is installed.

      Saving the template file in the Templates folder makes the template available in the Template drop-down list of the New Document dialog box (see step 2 in "Create a plate document from a template" on page 38).
   c. For File name, enter a name for the template. For example, enter **Quantifiler Template**:



   d. Click **Save**.

Create a plate document from a template

After you create a template, you can use it to create a plate document:

1. If the SDS software is not already started, select **Start ▸ Programs ▸ ABI Prism 7000 ▸ ABI Prism 7000 SDS Software**.
2. Select **File ▸ New** and in the New Document dialog box and make the following selections:
   - For Assay, select **Absolute Quantitation**.
   - For Container, select **96-Well Clear**.
   - For Template, select an appropriate template from the list.

   Note: If the template is not available in the list, click **Browse** to locate and select an appropriate template.

3. Complete the plate document setup:
   - Add detectors to the plate document (page 32)
   - Apply detectors for standards and for unknown samples (page 32 and page 34)
   - Set thermal cycler conditions (page 35)

   Note: The tasks that you perform vary according to which settings were defined in the template.

4. Save the plate document (page 37).

   For Save as type, select **SDS Documents (*.sds)**.

Section 2.2 7900HT SDS Software Setup

Overview

Purpose

During software setup, you start up the Applied Biosystems™ 7900HT Real-Time PCR System and set up a plate document for DNA quantification using the Quantifiler™ Kits.

Configuration

The Quantifiler™ Kits are supported using the following configuration of the 7900HT Real-Time PCR System for real-time data collection and analysis:

- 96-well reaction plates
- Manual setup
- Sequence Detection Systems (SDS) software v2.0

Note: Use of the robotic microplate handler and/or 384-well reaction plates is not supported.

Start the 7900HT Real-Time PCR System

Overview

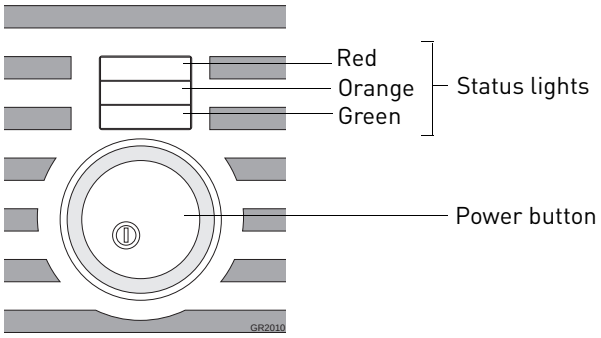
Starting the Applied Biosystems™ 7900HT Real-Time PCR System involves:

1. Powering on the computer.
2. Powering on the instrument.
3. Starting the SDS software.

Start the 7900HT System

To start the 7900HT System:

1. Press the power buttons on the computer and on the monitor.
2. In the login screen, enter the User Name and Password.
3. Press the power button below the status lights on the front of the instrument.



At startup, the instrument:

- Emits a high-pitched tone, indicating that the system is initialized
- Cycles the status lights (red ▸ orange ▸ green), indicating that the instrument is active

4. Select **Start ▸ Programs ▸ Applied Biosystems ▸ SDS 2.0**.

   At startup, the software attempts to establish communication with the 7900HT instrument. If the connection is successful, the software displays 

in the status bar.

About plate documents

How plate documents are used

Running a reaction plate on the 7900HT Real-Time PCR System requires creating and setting up a plate document using the SDS software. A plate document is a representation of the arrangement of samples (standards and unknowns) and reagents on the reaction plate. The SDS software uses the plate document to:

- Coordinate the instrument operation, such as thermal cycling and data collection
- Organize and store the data gathered during the run
- Analyze the data from the run

Plate document types

You can use SDS software to create two types of plate document files.

Plate Document Type	File Extension	Description
Single plate document	*.sds	Primary file to use when performing a run. Required for all experiments.
Template plate document	*.sdt	File that already contains run parameters that are commonly used in plate documents, such as detectors, thermal cycler conditions, and so on. Streamlines the creation of the SDS document (*.sds) file.

Example plate document setup

You can arrange the reactions in any well of the reaction plate, but you need to set up the plate document so that it corresponds exactly to the arrangement of the standards and unknown samples in the wells of the reaction plate. Table 8 shows one example of arranging reactions when running two Quantifiler™ Kit assays on one 96-well plate:

- Wells A1 through D12 (gray) correspond to reactions using the Quantifiler™ Human Kit
- Wells E1 through H12 (white) correspond to reactions using the Quantifiler™ Y Kit

Note: For each Quantifiler™ Kit assay, there are eight DNA quantification standards and two reactions for each standard. See "Prepare the DNA quantification standard" on page 51 for more information about the DNA quantification standards.

Table 8 Example arrangement of reactions with two kits

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6
B	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
C	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
D	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
E	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6
F	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
G	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN
H	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN

Table 9 shows another example of arranging reactions when running two Quantifiler™ Kits on one 96-well reaction plate if you are using repeat pipettors:

- Wells A1 through D6 (gray) correspond to reactions using the Quantifiler™ Human Kit
- Wells A7 through H12 (white) correspond to reactions using the Quantifiler™ Y Kit

For each Quantifiler™ Kit assay, there are eight DNA quantification standards and two reactions for each standard. See "Prepare the DNA quantification standard" on page 51 for more information about the DNA quantification standards.

Table 9 Example arrangement of reactions using repeat pipettors

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	UNKN	UNKN	UNKN	UNKN	Std 1	Std 1	UNKN	UNKN	UNKN	UNKN
B	Std 2	Std 2	UNKN	UNKN	UNKN	UNKN	Std 2	Std 2	UNKN	UNKN	UNKN	UNKN
C	Std 3	Std 3	UNKN	UNKN	UNKN	UNKN	Std 3	Std 3	UNKN	UNKN	UNKN	UNKN
D	Std 4	Std 4	UNKN	UNKN	UNKN	UNKN	Std 4	Std 4	UNKN	UNKN	UNKN	UNKN
E	Std 5	Std 5	UNKN	UNKN	UNKN	UNKN	Std 5	Std 5	UNKN	UNKN	UNKN	UNKN
F	Std 6	Std 6	UNKN	UNKN	UNKN	UNKN	Std 6	Std 6	UNKN	UNKN	UNKN	UNKN
G	Std 7	Std 7	UNKN	UNKN	UNKN	UNKN	Std 7	Std 7	UNKN	UNKN	UNKN	UNKN
H	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN

Set up a plate document

Overview

Setting up a plate document involves:

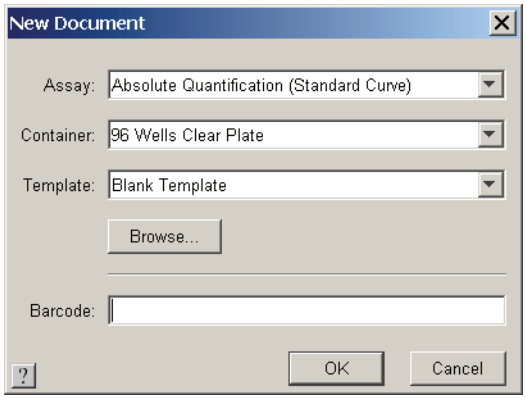
1. Create a blank plate document (page 43)
2. Create detectors (page 43)
3. Copy detectors to the plate document (page 45)
4. Apply detectors for standards (page 45)
5. Apply detectors for unknown samples (page 46)

6. Apply detectors for unknown samples (page 46)
7. Set thermal cycler conditions (page 47)
8. Save the plate document (page 48)

Create a blank plate document

To create a blank plate document:

1. If the SDS software is not already started, select **Start** ▸ **Programs** ▸ **Applied Biosystems** ▸ **SDS 2.0**.
2. Select **File** ▸ **New**, complete the New Document dialog box, then click **OK**.

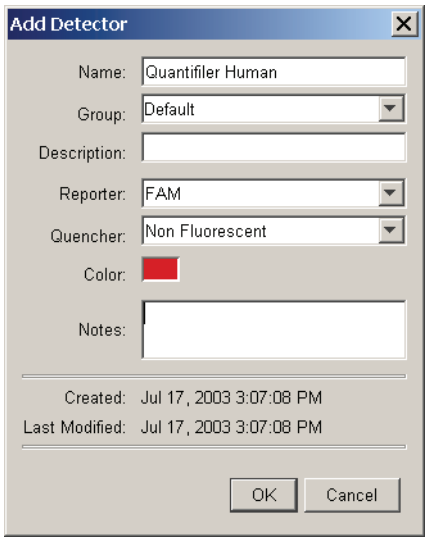


Create detectors

Before you set up the plate document, you need to create detectors in the SDS software for running Quantifiler™ Kit assays. After the detectors are created, you do not need to create detectors for subsequent runs of Quantifiler™ Kit assays and you can skip to "Copy detectors to the plate document" on page 45.

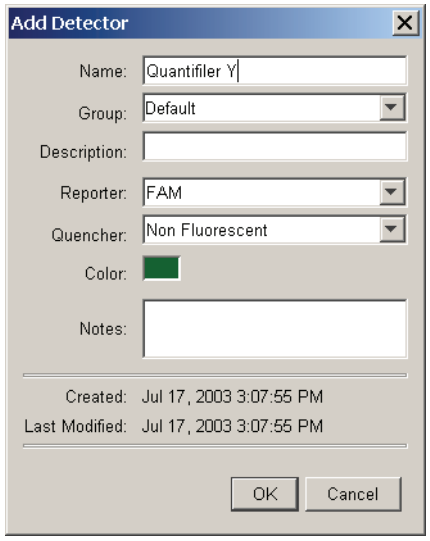
To create detectors:

1. With a new plate document open, select **Tools** ▸ **Detector Manager**.
2. Create a detector for the Quantifiler™ Human kit:
   a. In the lower left part of the Detector Manager, click **New**, then complete the dialog box:



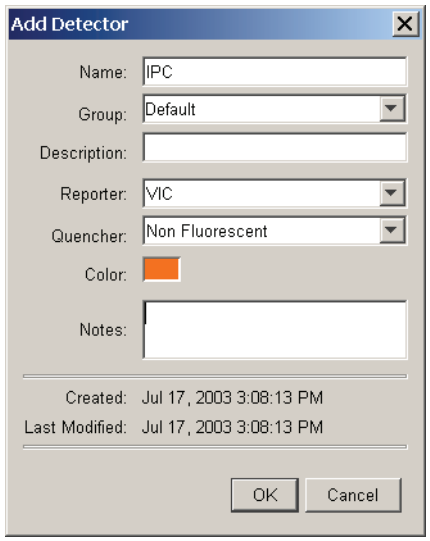
b. Click **OK** to return to the Detector Manager.

3. Create a detector for the Quantifiler™ Y Human Male Kit:
   a. In the Detector Manager, click **New** and complete the dialog box:



   b. Click **OK** to return to the Detector Manager.

4. Create a detector for the IPC assay:
   a. In the Detector Manager, click **New**, then complete the Add Detector dialog box:



   b. Click **OK** to return to the Detector Manager.

Copy detectors to the plate document

To copy detectors to the plate document:

1. If the Detector Manager is not already open, select **Tools ▸ Detector Manager**.
2. Select the Quantifiler™ Human, Quantifiler™ Y, and the IPC detectors by clicking them while pressing the **Ctrl** key.
   Note: If the detectors are not available, create them first (see page 43 for the procedure).
3. With the three detectors selected, click **Copy To Plate Document**.
4. Click **Done** to close the Detector Manager and return to the plate window.

Apply detectors for standards

You need to apply the detectors to the plate document for the wells on the reaction plate that contain DNA quantification standards. Repeat the procedure until you complete applying detector tasks, quantities, and sample names for all quantification standards.

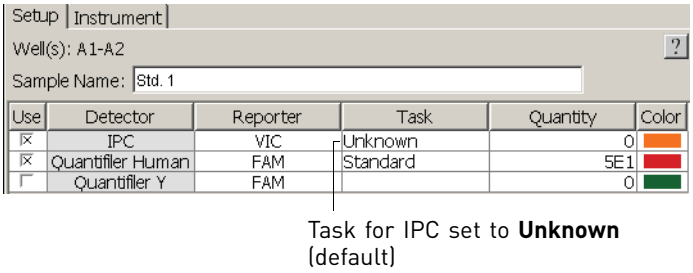
IMPORTANT! Set up detectors for each quantity and for each kit separately. For example, set up detectors for Std. 1 for the Quantifiler™ Human Kit first, and then for Std. 2 for the Quantifiler™ Human Kit, and so on, until you finish setting up the detectors for all wells containing quantification standards.

1. In the plate grid, press the **Ctrl** key while you select the wells that correspond to a specific quantification standard for one kit.
2. Complete the Well Inspector:
   a. Select the Use boxes for the applicable detectors:
      - IPC
      - Quantifiler™ Human *or* Quantifiler™ Y
   b. For the Quantifiler™ Human *or* Quantifiler™ Y detector:
      - Click **Unknown** in the Task column, then select **Standard** from the drop-down list.
      - Select the Quantity field and enter the quantity of DNA in the well.

      IMPORTANT! Although you do not enter units for Quantity, you must use a consistent unit (for example, ng/µL) for all standard quantities. The units used for standard quantities defines the quantification units for analysis results.

      Note: Leave the IPC detector Task for standard reactions set to Unknown. Quantity values are not needed for IPC detectors.
   c. Enter the Sample Name (for example, Std. 1, Std. 2, and so on).
   d. Make sure that **ROX** is selected for the Passive Reference.

For example:



Apply detectors for unknown samples

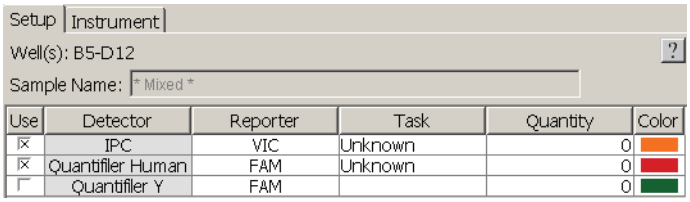
You need to apply detectors to the plate document for the wells on the reaction plate that contain unknown samples.

IMPORTANT! If you run reactions for the Quantifiler™ Human Kit and the Quantifiler™ Y Kit on the same plate, apply detectors for unknown samples for each kit separately.

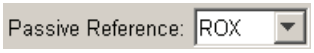
To apply detectors for unknown samples:

1. In the plate grid, press the **Ctrl** key and select the wells that contain unknown samples for one kit.
2. In the Well Inspector, select the Use boxes for the detectors in the selected wells:
   - IPC
   - Quantifiler™ Human *or* Quantifiler™ Y detector

   For example:



3. In the Well Inspector, make sure that **ROX** is selected for the Passive Reference.



Add sample names to unknown samples

Repeat this procedure to enter the names for all unknown samples.

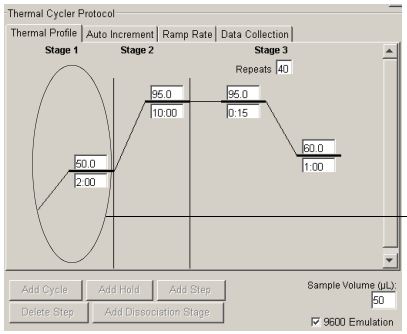
1. In the plate grid, select a reaction well containing an unknown sample.
2. In the Well Inspector panel, enter a name in the Sample Name field.

IMPORTANT! Samples with identical sample names are treated as replicates by the SDS software. Results for replicate reactions are grouped together automatically for data analysis.

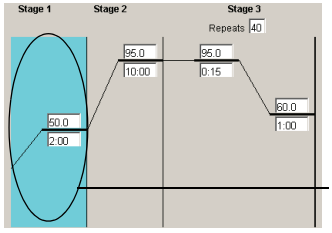
Set thermal cycler conditions

To set thermal cycler conditions:

1. In the plate window, select the **Instrument** tab.
2. Delete the Stage 1 hold step (50 ·C for 2 minutes):
   a. Press the **Shift** key and click within the Stage 1 hold step.



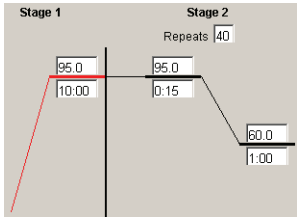
Press the Shift key and click within the Stage 1 hold step



Hold step is selected

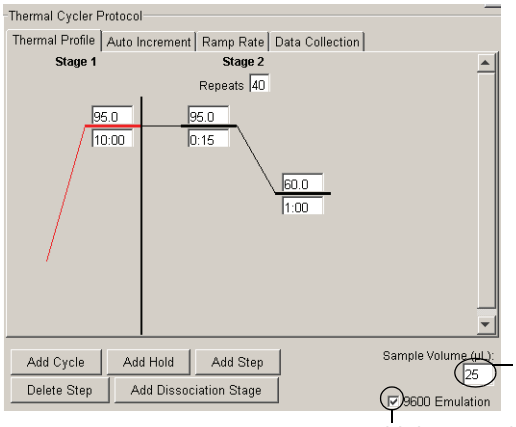
   b. After the hold step is selected, press the **Delete** key.

3. Make sure that the thermal profile appears as follows:



4. Set the Sample Volume to **25 µL** and make sure that the 9600 Emulation box is selected.

5. Selecting the 9600 Emulation box reduces the ramp rate.



6. Make sure that the default settings are kept on the remaining tabs:
   - Auto Increment
   - Ramp Rate
   - Data Collection

Save the plate document

Before running the reaction plate, save the plate document as an ABI Prism SDS Single Plate (*.sds) file.

Note: To save the document as a template, see "Set up a plate document template" on page 48.

To save the plate document:

1. Select **File** > **Save As**.
2. For Files of Type, select **ABI Prism SDS Single Plate (*.sds)**.
3. Navigate to where you want to save the plate document file.
4. In the File Name field, enter a name for the plate document.
5. Click **Save**.

Set up a plate document template

Purpose

A plate document template reduces the time required to set up a plate document. This section describes how to create an SDS Template Document (*.sdt) set up for running Quantifiler™ Kit assays.

Template settings

In addition to plate document settings (assay and container), templates can contain:

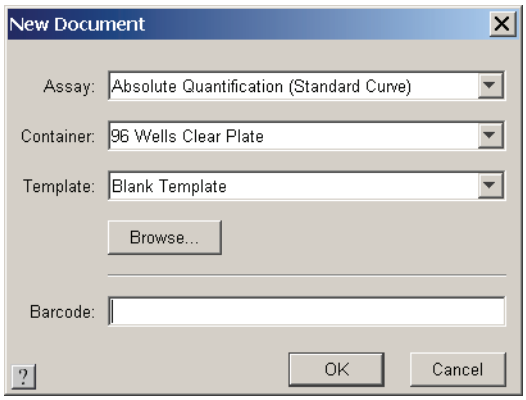
- Assay-specific detectors
- Well assignments for quantification standards, with detectors, tasks, and quantity
- Well assignments for unknown samples, with detectors and tasks
- Instrument settings: thermal cycler conditions and reaction volume settings.

Create a plate document template

This procedure assumes that you have created the detectors for running reactions using the Quantifiler™ Kits (page 43).

To create a plate document template:

1. If the SDS software is not already started, select **Start ▸ Programs ▸ Applied Biosystems ▸ SDS 2.0**.
2. Select **File ▸ New**, then complete the New Document dialog box:



3. Apply the desired template settings to the plate document:
   - Copy detectors (page 45)
   - Apply detectors for standards (page 45)
   - Apply detectors for unknown samples (page 46)
   - Set thermal cycler conditions (page 47)
4. Select **File ▸ Save As** and complete the Save As dialog box:
   a. For Files of Type, select **ABI Prism SDS Template Document (*.sdt)**.
   b. Locate and select the Templates folder within the software folder:
      X:Program Files ▸ Applied Biosystems ▸ 7900HTSDS ▸ Templates, where X is the hard drive on which the SDS software is installed.

      Note: Saving the template file in the Templates folder makes it available in the Template drop-down list of the New Document dialog box (see step 2 in "Create a plate document template" on page 49).
   c. Enter a name for the template. For example, enter **Quantifiler Template**.
   d. Click **Save**.

Create a plate document from a template

After you create a template, you can use it to create a plate document.

To create a plate document from a template:

1. If the SDS software is not already started, select **Start ▸ Programs ▸ Applied Biosystems ▸ SDS 2.0**.
2. Select **File ▸ New** and in the New Document dialog box and make the following selections:
   - For Assay, select **Absolute Quantitation**.
   - For Container, select **96-Well Clear Plate**.
   - For Template, select an appropriate template from the list.

   Note: If the template is not available in the list, click Browse to locate and select an appropriate template.
3. Complete the plate document setup:
   - Copy detectors (page 45)
   - Apply detectors for standards (page 45)
   - Apply detectors for unknown samples (page 46)
   - Set thermal cycler conditions (page 47)

   Note: The tasks that you perform vary according to which settings were defined in the template.
4. Save the plate document (page 48).

   Note: For Files of Type, select **ABI Prism SDS Single Plate (*.sds)**.

3 PCR Amplification

Prepare the DNA quantification standard

Required materials

- Pipettors
- Pipette tips
- Quantifiler™ Human DNA Standard

  Note: The same standard can be used for both Quantifiler™ Kits.

- $T_{10}E_{0.1}$ buffer:
  - 10 mM Tris-HCl (pH 8.0)
  - 0.1 mM Na_2EDTA
  - 20 µg/mL glycogen (optional)

  Note: If you use $T_{10}E_{0.1}$ buffer with glycogen, you can store the DNA quantification standards for up to 2 weeks at 2 to 8 °C.

Guidelines for calculating the standards dilution series

The standard dilution series example shown in Table 10 is suitable for general use.

We recommend:

- Three-fold dilution series with eight concentration points in the standard series for each assay
- Minimum input volume of 10 µL DNA for dilutions (to ensure accuracy of pipetting)

Standards dilution series example

Table 10 shows an example of one standards dilution series with the concentrations ranging from 50 ng/µL (Std. 1) to 0.023 ng/µL, or 23 pg/µL (Std. 8). A sample at the lowest concentration (2 µL per reaction) contains on average 14 to 16 copies of a diploid single-copy locus and 7 to 8 copies of a haploid single-copy locus.

Table 10 Standards dilution series example

Standard	Concentration (ng/µL)	Example Amounts	Minimum Amounts	Dilution Factor
Std. 1	50.000	50 µL [200 ng/µL stock] + 150 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [200 ng/µL stock] + 30 µL $T_{10}E_{0.1}$ buffer	4×
Std. 2	16.700	50 µL [Std. 1] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 1] + 20 µL $T_{10}E_{0.1}$ buffer	3×