Quantifiler™ Human DNA Quantification Kits PCR Amplification

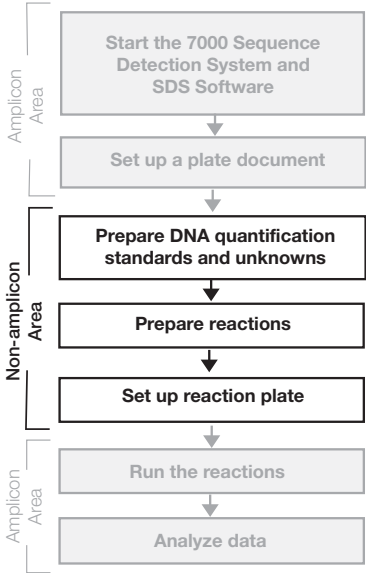
For safety and biohazard guidelines, refer to the "Safety" section in the Preface of the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual* (PN 4344790). For each chemical in **bold** type below, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product Overview

The Quantifiler™ Human DNA Quantification Kit and the Quantifiler™ Y Human Male DNA Quantification Kit are designed to quantify the total amount of amplifiable human (and higher primate) DNA or human male DNA in a human identity sample.

This quick reference card covers the protocol for the preparation of DNA standards and sample reactions. Refer to the Quantifiler kit's user's manual or the appropriate instrument quick reference card for instructions on setting up the plate document, running PCR, and analyzing data.

Workflow



Materials

Material	Source
Quantifiler Human DNA Quantification Kit	Applied Biosystems (PN 4343895)
Quantifiler Y Human Male DNA Quantification Kit	Applied Biosystems (PN 4343906)
Glycogen, 20 mg (1 mL)	Roche Applied Science (PN 901 393)
Equipment	**Source**
96-Well Optical Reaction Plates	Applied Biosystems (PN 4306737)
Optical Adhesive Covers Starter Kit	Applied Biosystems (PN 4313663)
Optical Tubes	Applied Biosystems (PN 4316567)
Optical Caps	Applied Biosystems (PN 4323032)

Figure 1 The *Quantifiler™ Human DNA Quantification Kit* and *Quantifiler™ Y Human Male DNA Quantification Kit*.

Preparing the DNA Quantification Standards

The table below shows a sample dilution series.

IMPORTANT! Applied Biosystems recommends:

- Three-fold dilution series with eight concentration points.
- Minimum input volume of 10 µL DNA for dilutions.

Table 1-1 Standards dilution series - example

Standard	Concentration (ng/µL)	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	4X
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	3X
Std. 3	5.560	50 µL [Std. 2] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 2] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 4	1.850	50 µL [Std. 3] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 3] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 5	0.620	50 µL [Std. 4] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 4] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 6	0.210	50 µL [Std. 5] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 5] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 7	0.068	50 µL [Std. 6] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 6] + 20 µL $T_{10}E_{0.1}$ buffer	3X
Std. 8	0.023	50 µL [Std. 7] + 100 µL $T_{10}E_{0.1}$/glycogen buffer	10 µL [Std. 7] + 20 µL $T_{10}E_{0.1}$ buffer	3X

Standards Preparation Procedure

1. Label 8 microcentrifuge tubes: Std. 1 through Std. 8.
2. Dispense the required amount of diluent ($T_{10}E_{0.1}$ Buffer with or without glycogen) to each tube.

   Note: $T_{10}E_{0.1}$ buffer recipe:

   – 10 mM Tris-HCl (pH 8.0)
   – 0.1 mM Na2EDTA
   – 20 µg/mL glycogen (optional)
3. Prepare Std. 1:
   a. Vortex the Quantifiler Human DNA Standard 3 to 5 seconds.
   b. Using a new pipette tip, add the calculated amount of Quantifiler Human DNA Standard to the tube for Std. 1.
   c. Mix the dilution thoroughly by vortexing for 5 sec.
4. Prepare Std. 2 through 8:
   a. Using a new pipette tip, add the calculated amount of the prepared standard to the tube for the next standard.
   b. Mix the standard thoroughly.
   c. Repeat steps 4a and 4b until you complete the dilution series.

Prepare Reactions

IMPORTANT! While preparing the reactions, keep the 96-well reaction plate in its base and do not place it on the counter.

1. Calculate the volume of each component needed:

Component	Volume Per Reaction (µL)
Quantifiler Human Primer Mix or Quantifiler Y Human Male Primer Mix	10.5
Quantifiler PCR Reaction Mix	12.5

2. Prepare the reagents:
   - Thaw the primer mix completely, then vortex 3 to 5 seconds and centrifuge briefly before opening the tube.
   - Swirl the **Quantifiler PCR Reaction Mix** gently before using. Do not vortex.
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 23 µL of the PCR mix into each reaction well.
6. Add 2 µL of sample, standard, or control to the appropriate wells. For plate setup examples, see the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual*.

   IMPORTANT! Applied Biosystems recommends running duplicates of the 8 DNA quantification standards for each assay.
7. Seal the reaction plate with the Optical Adhesive Cover.

   IMPORTANT! Make sure the corners are sealed properly.
8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.
9. Place the compression pad over the Optical Adhesive Cover with the gray side down, the brown side up, and the holes positioned directly over the reaction wells.
10. Run the reactions on the appropriate Applied Biosystems instrument. Refer to the Quantifiler kit's user's manual or the appropriate instrument quick reference card for the protocol and data analysis.

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