Yfiler™ Plus PCR Amplification Kit

PCR Setup: Extracted DNA

Catalog Numbers 4484678 and 4482730

Pub. No. MAN1001548 Rev. A

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0030230). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Yfiler™ Plus PCR Amplification Kit (100-reaction Cat. No. 4484678 or 500-reaction Cat. No. 4482730) is a 6-dye, short tandem repeat (STR) multiplex assay that amplifies 27 Y-STR loci in a single reaction.

The kit is optimized to allow amplification from extracted DNA or direct amplification from some types of single-source samples.

Before you begin

Place this guide in the laboratory area where you perform PCR setup procedures.

Effect of DNA quantity on results

If too much DNA is added to the PCR reaction, the increased amount of PCR product that is generated can result in the following:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the capillary electrophoresis instrument ("off-scale" data). Off-scale data are a problem because:
  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause a corresponding stutter peak to appear higher in relative intensity, therefore increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation ("pull-up").
- Incomplete +A nucleotide addition.

To address these problems, rerun the amplification reaction using less DNA.

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR reaction could be extremely low. Unbalanced amplification of the alleles can occur because of stochastic fluctuation.

Methods of quantifying DNA

Kit	Detects	How it works
Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Degraded DNA	• Uses 5′ nuclease assays with multiple-copy target loci, for improved detection sensitivity:[1] – The human-specific target loci are multiple-copy, and dispersed on various autosomal chromosomes – The primary quantification targets have relatively short amplicons (75–80 bases), to improve the detection of degraded DNA samples • Uses features that maximize consistency of quantification: – Genomic targets have conserved primer- and probe-binding sites – Minimal copy number variation between different individuals and population groups • Contains a Large Autosomal target with a longer amplicon (>200 bases) to help determine if a DNA sample is degraded
Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Human male DNA • Degraded DNA	

[1] The detection sensitivity of the Quantifiler™ HP Kit and the Quantifiler™ Trio kit is improved over the Quantifiler™ Duo Kit.

Note: For information on the Quantifiler™ kits, see the *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354).

(Before first use of the kit) Thaw reagents

Thaw the master mix and primer set.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set and allelic ladder from light when not in use.

IMPORTANT! Thawing is required only before first use of the kit. After first use, the reagents are stored at 2–8°C and do not require subsequent thawing. Do not refreeze the reagents.

Prepare the amplification kit reactions

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set and allelic ladder from light when not in use.

1. Vortex the master mix and primer set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by briefly centrifuging the tubes or tapping the bottles on the bench.
2. Pipet the required component volumes into an appropriately sized, clear (non-colored), polypropylene tube.

Component	Amount per reaction
Master mix	10.0 µL
Primer set	5.0 µL

Note: Include volume for extra reactions to provide excess volume for the loss that occurs during reagent transfers.

3. Vortex the reaction mix for 3 seconds, then briefly centrifuge.
4. Pipet 15 µL of the reaction mix into each well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.
5. *(If needed)* Adjust the sample input amount and volume.
   - If the total sample input amount is >1.0 ng, dilute with low-TE buffer to obtain a 10-µL input volume.
   - If the total sample volume is <10 µL, bring to volume with low-TE buffer to obtain a 10-µL input volume.
6. Prepare the samples and controls as shown in the following table, then add to the appropriate wells of a MicroAmp™ Optical 96-Well Reaction Plate or to each MicroAmp™ tube.

Component	Amount per reaction
	30-cycle protocol
Negative control	10 µL of low-TE buffer
Test sample	DNA to a total amount of 1.0 ng
Positive control	DNA Control 007 to a total amount of 1.0 ng **Note:** DNA Control 007 contains 2 ng/µL of human male genomic DNA.

The final reaction volume (sample or control plus reaction mix) is 25 µL.

7. Seal the plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.

IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps for the plate, which may not provide a consistent seal across all wells.

8. Vortex the plate or tubes at medium speed for 3 seconds.
9. Centrifuge the tubes or plate at 3,000 × *g* for ~20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

Perform PCR and capillary electrophoresis

To perform PCR amplification and capillary electrophoresis (CE), see the *Yfiler™ Plus PCR Amplification Kit— PCR Amplification and CE Quick Reference* (Pub. No. MAN1001551) or the *Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0030230).

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1001548 A

Revision	Date	Description
A	22 April 2025	New document for the Yfiler™ Plus PCR Amplification Kit; replaces Pub. No. 100030921. The following changes were made: • Compatible instruments, compatible software, and materials required were updated (throughout the document). • Copy edits and formatting changes were made to align with current documentation style (throughout the document).
B	27 December 2016	Content was reorganized. Non-technical changes only.
A	9 December 2014	New document for the Yfiler™ Plus PCR Amplification Kit.