Yfiler™ Plus PCR Amplification Kit

PCR Setup: Treated and Untreated Paper Substrates

Catalog Numbers 4484678 and 4482730

Pub. No. MAN1001550 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.

(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.

Sample preparation guidelines: Treated or untreated paper substrate

- Do not add water to the wells on the reaction plate before adding the punches. If you observe static issues with the paper discs, you can prepare and dispense the 25-µL reaction mix into the wells of the reaction plate before adding the punches.
- To facilitate optimum peak intensity, make a 1.2-mm punch as close as possible to the center of the sample. Increasing the size of the punch may cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2-mm Harris Micro-Punch™ on the card, hold the barrel of the Harris Micro-Punch™ (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch into the appropriate well on the reaction plate.
- For automated punching: For guidance, see the user guide for your automated or semi-automated disc punch instrument.
- For blood on untreated paper samples, add 2 µL of Prep-n-Go™ Buffer (for use with untreated paper substrates) on top of the 1.2-mm sample punch.

Prepare the amplification kit reactions: Treated or untreated paper substrates

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. 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.

Component	Amount per reaction		
	26-cycle protocol	27-cycle protocol	28- and 29-cycle protocol
Negative control	1.2 mm blank disc	1.2 mm blank disc	1.2 mm blank disc
Test sample	1.2 mm sample disc	1.2 mm sample disc	1.2 mm sample disc
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	3 µL of DNA Control 007	2 µL of DNA Control 007	1 µL of DNA Control 007

Note: If the peak heights are too high or too low for your optimized cycle number, the suggested volumes of positive control can be adjusted.

2. 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.

3. Pipet the required component volumes into an appropriately sized polypropylene tube.

Component	Amount per reaction
Master mix	10.0 µL
Primer set	5.0 µL
Low-TE buffer	10.0 µL

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

IMPORTANT! To overcome the PCR inhibition that is expected when amplifying unpurified samples, this kit is optimized for a final PCR reaction mix volume of 25 µL. Using a lower PCR reaction mix volume may decrease the ability of the kit chemistry to generate full STR profiles.

4. Vortex the reaction mix for 3 seconds, then briefly centrifuge.

5. Pipet 25 µL of the reaction mix into each well of a MicroAmp™ Optical 96-Well Reaction Plate.

6. Seal the plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film.

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

7. Vortex the plate at medium speed for 3 seconds.

8. Centrifuge the plate at 3,000 × *g* for ~20 seconds in a tabletop centrifuge with plate holders.

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. MAN1001550 A

Revision	Date	Description
A	22 April 2025	New document for the Yfiler™ Plus PCR Amplification Kit; replaces Pub. No. 100030920. 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.