Yfiler™ Plus PCR Amplification Kit

PCR Setup: Swab Substrate

Catalog Numbers 4484678 and 4482730

Pub. No. MAN1001549 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: Swab substrate

- Detach each buccal swab head from the swab shaft before lysis.
- If you are using the heated lysis protocol, perform lysis in any of the following formats:
  - 1.5-mL tubes with a heat block (VWR™ Scientific Select dry heat block or similar)
  - PrepFiler™ 96-Well Processing Plates (Cat. No. 4392904)
  - Robbins Scientific™ Model 400 Hybridization Incubator or similar
  - Agilent™ Benchtop Rack for 200 µL Tubes/V Bottom Plates (metal) or similar (Cat. No. 410094)

    IMPORTANT! Do not use a plastic plate adaptor.
- For optimum performance, lyse the entire swab. If you need to preserve the sample, use half of the lysate prepared from the entire swab.

Prepare the sample lysate: Room temperature

This protocol may improve the performance of challenging or aged samples.

1. Add 400 µL of Prep-n-Go™ Buffer (Cat. No. 4471406) to 1.5-mL tubes or the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. 4392904).
2. Place the entire head of each swab into a tube or well, then let stand for 20 minutes at room temperature (20–25°C) to lyse the sample.
3. After 20 minutes, transfer the sample lysate into tubes or plates for storage, then discard the tubes or plate that contain the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate before transferring the lysate.

Proceed to "Prepare the amplification kit reactions: Swab substrate" on page 2 or "Store the sample lysate" on page 3.

Prepare the sample lysate: Heat protocol

This protocol may improve the performance of challenging or aged samples.

1. Preheat the heat block to 90°C, or preheat the oven with a metal plate adaptor to 99°C.
2. Add 400 µL of Prep-n-Go™ Buffer (for buccal swabs, Cat. No. 4471406) to 1.5-mL tubes or to the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. 4392904).
3. Into each tube or well, put the entire head of each swab. If you are using tubes, cap the tubes.
4. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
5. After 20 minutes, remove the tubes or plate from the heat block or oven, then let the lysate stand at room temperature for ≥15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate to tubes or plates for storage. Discard the tubes or plate that contain the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.

Proceed to "Prepare the amplification kit reactions: Swab substrate" on page 2 or "Store the sample lysate" on page 3.

Prepare the amplification kit reactions: Swab substrate

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. Add Prep-n-Go™ Buffer (Cat. No. 4471406) to the appropriate wells of a MicroAmp™ Optical 96-Well Reaction Plate.

Control well	Prep-n-Go™ Buffer		
	26-cycle protocol	27-cycle protocol	28- and 29-cycle protocols
Negative control	3 µL	3 µL	3 µL
Positive control	— (Do not add buffer)	1 µL	2 µL

2. 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 protocols
Test sample	3 µL of sample lysate	3 µL of sample lysate	3 µL of sample lysate
Positive control	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.

3. 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.

4. 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.

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

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

   The final volume in each well is 28 µL (reaction mix plus Prep-n-Go™ Buffer or sample lysate or positive control).

7. 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.

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

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

Store the sample lysate

1. Cap the sample lysate storage tubes or seal the sample lysate storage plate with MicroAmp™ Clear Adhesive Film (Cat. No. 4306311).

2. Store the sample lysate as needed.

Storage time	Temperature
<2 weeks	2–8°C
>2 weeks	–25°C to –15°C

Note: The effects of multiple freeze/thaw cycles on the lysate have not been fully evaluated. Therefore, multiple freeze/thaw cycles are not recommended.

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. MAN1001549 A

Revision	Date	Description
A	22 April 2025	New document for the Yfiler™ Plus PCR Amplification Kit; replaces Pub. No. 100030922. 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.