GlobalFiler™ Express PCR Amplification Kit

PCR Setup: Untreated Paper Substrate

Catalog Numbers 4476609 and 4474665

Pub. No. MAN1001556 Rev. A

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *GlobalFiler™ Express PCR Amplification Kit User Guide* (Pub. No. MAN0030237). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The GlobalFiler™ Express PCR Amplification Kit (200-reaction Cat. No. 4476609 or 1,000-reaction Cat. No. 4474665) is a 6-dye, short tandem repeat (STR) multiplex assay that amplifies 21 autosomal STR loci, 1 Y STR locus, 1 Y insertion/deletion (Y indel) locus, and Amelogenin. The kit is optimized to allow direct amplification 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 and prepare the master mix

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.

1. Thaw the master mix, primer set, and master mix additive.
2. Vortex the master mix, primer set, and master mix additive 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. Add the required volume of master mix additive to the master mix tube.

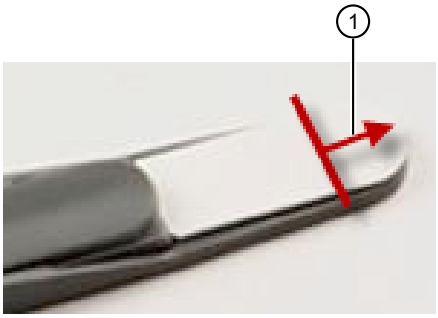
Kit	Master mix additive
200 reactions	80 µL
1,000 reactions	390 µL

4. Gently invert the master mix tube 10 times, then briefly centrifuge the tube or tap the bottle on the bench.
5. Label the master mix tube cap with a (+) to indicate that the master mix additive has been added.
6. Discard the master mix additive tube.

Sample preparation guidelines: Untreated paper substrate

- 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.

- If you are using a Bode Buccal DNA Collector™, make a 1.2-mm punch as close as possible to the tip of the DNA collector to enable optimum peak intensity. A larger 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.



① Location of punch with a Bode Buccal DNA Collector™

Prepare the amplification kit reactions: Untreated paper 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. 4467079) to the appropriate wells of a MicroAmp™ Optical 96-Well Reaction Plate.

Control or sample well	Prep-n-Go™ Buffer		
	25- and 26-cycle protocols	27-cycle protocol	28-cycle protocol
Negative control	3 µL	3 µL	3 µL
Test sample	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		
	25- and 26-cycle protocols	27-cycle protocol	28-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.

3. Vortex the master mix with master mix additive and the 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 with master mix additive	6.0 µL
Primer set	6.0 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 12 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 12 µL of the reaction mix into each well of the MicroAmp™ Optical 96-Well Reaction Plate.
7. Ensure that the punches are immersed.
8. 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.

9. Vortex the plate at medium speed for 3 seconds.
10. 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 *GlobalFiler™ Express PCR Amplification Kit—PCR and CE Quick Reference* (Pub. No. MAN1001553) or the *GlobalFiler™ Express PCR Amplification Kit User Guide* (Pub. No. MAN0030237).

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. MAN1001556 A

Revision	Date	Description
A	25 April 2025	New document for the GlobalFiler™ Express PCR Amplification Kit; replaces Pub. No. 4480795. 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).
C	6 October 2016	Branding and legal information was updated. No technical changes.
B	23 July 2013	Baseline for the revision (Quick Reference for the GlobalFiler™ Express PCR Amplification Kit).