PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits

USER GUIDE

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For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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

The information in this guide is subject to change without notice.

Revision history: Pub. No. 4463349

Revision	Date	Description
E	17 March 2021	• Added touch/trace samples to the list of tested sample types for the PrepFiler™ Automated Forensic DNA Extraction Kit. • Updated the preparation procedure for the magnetic particles. • Noted that the ID NIMBUS® Presto workstation does not currently accommodate a 500-µL lysis volume.
D	7 January 2021	• Added the PrepFiler™ BTA Automated Forensic DNA Extraction Kit. • Removed procedures for the HID EVOlution™ systems. Referred users to the *PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin*. • Removed the "Experiments and Results" appendix. Referred users to the *PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin* and the *PrepFiler™ and PrepFiler™ BTA Forensic DNA Extraction Kits User Guide*.
C	December 2011	Updated Life Technologies contact information.
B	October 2011	Added “Experiments and Results” appendix, which contains the validation studies previously described in the following user bulletins: • *Validation of the PrepFiler™ Automated Forensic DNA Extraction Kit on the Tecan HID EVOlution™—Extraction System User Bulletin* (Pub. No. 4425127, 12/08) • *HID EVOlution™—Extraction System and HID EVOlution™—Combination System: Validation of PrepFiler™ Wash Buffer B and the Related Modifications to Worktable Layout and Scripts for DNA Extraction User Bulletin* (Pub. No. 4457144, 6/10)
A	May 2011	Updated user guide (Pub. No. 4393917) to include instructions for using the PrepFiler™ Automated Forensic DNA Extraction Kitwith the HID EVOlution™—Extraction System and HID EVOlution™—Combination System.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The PrepFiler™ Automated Forensic DNA Extraction Kit or PrepFiler™ BTA Automated Forensic DNA Extraction Kit is part of an integrated solution that includes proven PrepFiler™ reagents or PrepFiler™ BTA reagents for use in semi-automated, high-throughput workflows to provide reliable, simplified, and time-saving DNA extraction and purification.

The kits use magnetic particles with an optimized multi-component surface chemistry to deliver robust and reliable DNA yield from the tested forensic sample types listed in Table 1.

Table 1 Tested sample types

Kit	Sample types
PrepFiler™ Automated Forensic DNA Extraction Kit	Routine forensic sample types, including: • Body fluids (blood, saliva, semen) • Stains and swabs of body fluids • Hair roots • Touch/trace samples
PrepFiler™ BTA Automated Forensic DNA Extraction Kit	The most challenging forensic sample types, including: • Bone • Teeth • Adhesive substrates (such as cigarette butts, chewing gum, and tape lifts) • Touch/trace samples

Key features of both kits

- Improves the overall yield, concentration, and purity of DNA isolated from forensic samples, while enabling removal of PCR inhibitors.
- Improves the downstream genotyping success rate, to help ensure that critical and often limited casework samples can be successfully analyzed.
- Contains specially developed magnetic particles and reagents that optimize DNA binding and elution of DNA in a small volume.
- Offers streamlined protocols to process an extensive variety of forensic sample types.
- The PrepFiler™ BTA Automated Forensic DNA Extraction Kit includes PrepFiler™ BTA Lysis Buffer to successfully process more challenging samples.

Validated robotic workstations

After manual sample lysis (DNA extraction), the DNA purification procedures can be performed on a robotic workstation of your choosing. We have validated the robotic workstations listed in Table 2.

IMPORTANT! If you perform DNA purification on a different robotic workstation, we can provide support for the kit chemistry, but we cannot provide support for the workstation.

Table 2 Validated robotic workstations

Robotic workstation	Validated for use with...	For more information, see...
HID EVOlution™ — Extraction System	PrepFiler™ Automated Forensic DNA Extraction Kit	*PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin* (Pub. No. MAN0019298)
HID EVOlution™ — Combination System		
ID NIMBUS® Presto	PrepFiler™ Automated Forensic DNA Extraction Kit	*PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the ID NIMBUS® Presto Workstation User Bulletin* (Pub. No. MAN0019368)
	PrepFiler™ BTA Automated Forensic DNA Extraction Kit	

Compatible quantitation and PCR amplification kits

The extracted DNA is compatible with the kits listed in Table 3.

Table 3 Quantitation and PCR amplification kits

Application	Kit
Quantitation	Quantifiler™ Human DNA Quantification Kit
	Quantifiler™ Duo DNA Quantification Kit
	Quantifiler™ HP DNA Quantification Kit
	Quantifiler™ Trio DNA Quantification Kit
PCR amplification	AmpFℓSTR™ Identifiler™ PCR Amplification Kit
	AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kits
	AmpFℓSTR™ MiniFiler™ PCR Amplification Kit
	AmpFℓSTR™ NGM™ PCR Amplification Kit
	AmpFℓSTR™ NGM SElect™ PCR Amplification Kit
	NGM Detect™ PCR Amplification Kit
	AmpFℓSTR™ Yfiler™ PCR Amplification Kit

Table 3 Quantitation and PCR amplification kits *(continued)*

Application	Kit
PCR amplification	Yfiler™ Plus PCR Amplification Kit
	GlobalFiler™ PCR Amplification Kit
	GlobalFiler™ IQC PCR Amplification Kit
	VeriFiler™ Plus PCR Amplification Kit

Validation studies

For detailed validation study information, see the documents referenced in Table 4. Unless otherwise indicated, the validation studies apply to both kits.

Table 4 Documentation for validation studies

Validation studies	Document
PrepFiler™ chemistry	*PrepFiler™ and PrepFiler™ BTA Forensic DNA Extraction Kits User Guide* (Pub. No. 4463348)
PrepFiler™ Wash Buffer B **Note:** We validated PrepFiler™ Wash Buffer B using the PrepFiler™ Automated Forensic DNA Extraction Kit, which is designed for automated purification on the HID EVOlution™ systems and shares the same chemistry as the PrepFiler™ Forensic DNA Extraction Kit.	*PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin* (Pub. No. MAN0019298)
HID EVOlution™—Extraction System and HID EVOlution™—Combination System **Note:** Validation studies performed on the HID EVOlution™ systems apply only to the PrepFiler™ Automated Forensic DNA Extraction Kit.	*PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin* (Pub. No. MAN0019298)
ID NIMBUS® Presto workstation	*PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the ID NIMBUS® Presto Workstation User Bulletin* (Pub. No. MAN0019368)

PrepFiler™ Automated Forensic DNA Extraction Kit: Contents and storage

The PrepFiler™ Automated Forensic DNA Extraction Kit is intended for semi-automated workflows, and contains the reagents required for the following procedures:

- Manual sample lysate preparation (DNA extraction)
- Automated DNA purification

The kit is sufficient for ≤960 samples, depending on the batch size and elution volume.

Table 5 PrepFiler™ Automated Forensic DNA Extraction Kit (Cat. No. 4463353)

Contents	Amount	Storage
PrepFiler™ Lysis Buffer	1 × 500 mL	18–25°C
PrepFiler™ Magnetic Particles	13 × 1.5 mL	
PrepFiler™ Wash Buffer A Concentrate	1 × 500 mL	
PrepFiler™ Wash Buffer B Concentrate	1 × 250 mL	
PrepFiler™ Elution Buffer	1 × 200 mL	

PrepFiler™ BTA Automated Forensic DNA Extraction Kit: Contents and storage

The PrepFiler™ BTA Automated Forensic DNA Extraction Kit is intended for semi-automated workflows, and contains the reagents required for the following procedures:

- Manual sample lysate preparation (DNA extraction)
- Automated DNA purification

The kit is sufficient for ≤960 samples, depending on the batch size and elution volume.

Table 6 PrepFiler™ BTA Automated Forensic DNA Extraction Kit (Cat. No. 4463354)

Contents	Amount	Storage
PrepFiler™ Lysis Buffer	1 × 300 mL	18–25°C
PrepFiler™ BTA Lysis Buffer	1 × 250 mL	
PrepFiler™ Magnetic Particles	13 × 1.5 mL	
PrepFiler™ Wash Buffer A Concentrate	1 × 500 mL	
PrepFiler™ Wash Buffer B Concentrate	1 × 250 mL	
PrepFiler™ Elution Buffer	1 × 200 mL	
Proteinase K	8 × 0.85 mL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Required materials for manual lysis (excluding bone or tooth samples)

Table 7 Manual lysis in LySep™ columns

Item	Source[1]
DL-dithiothreitol [DTT; molecular biology grade; ≥98% (HPLC), ≥99% (titration)]	MLS
General purpose thermal shaker, capable of temperatures ≤70°C and speeds ≤900 rpm	MLS
Vortexer, variable-speed	MLS
PrepFiler™ LySep™ Columns	4468323
PrepFiler™ Capless Microtubes, 1.5-mL	A48397
Laboratory microcentrifuge capable of 13,000–16,000 × *g*	MLS

[1] Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

Table 8 Manual lysis in spin/filter tubes

Item	Source[1]
DL-dithiothreitol [DTT; molecular biology grade; ≥98% (HPLC), ≥99% (titration)]	MLS
General purpose thermal shaker, capable of temperatures ≤70°C and speeds ≤900 rpm	MLS
Vortexer, variable-speed	MLS
Nonstick, RNase-free Microfuge Tubes, 1.5 mL; certified DNase- and RNase-free (250 tubes) If you use an equivalent tube, select tubes that allow you to observe the tube contents.	AM12450, or equivalent
Laboratory microcentrifuge capable of 13,000–16,000 × *g*	MLS
If substrate removal is required	
PrepFiler™ Spin Tubes and Filter Columns, ethylene oxide-treated (300 tubes and 100 columns)	A36853

[1] Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

Required materials for manual lysis of bone or tooth samples

Table 9 Manual lysis in screw-cap tubes

Item	Source[1]
DL-dithiothreitol [DTT; molecular biology grade; ≥98% (HPLC), ≥99% (titration)]	MLS
General purpose thermal shaker, capable of temperatures ≤70°C and speeds ≤1,100 rpm	MLS
Vortexer, variable-speed	MLS
Laboratory centrifuge capable of 10,000 × *g*	MLS
Screw-cap tubes and caps, 2-mL	MLS

[1] Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

Required materials for the automated purification run

Table 10 Reagent preparation

Item	Source
Ethanol (Molecular biology grade; 95% or 190 proof) **Note:** Open a new bottle when preparing the PrepFiler™ Wash Buffer A and Wash Buffer B solutions.	MLS
Clean containers to store the prepared Wash Buffer A and Wash Buffer B solutions; we use: Nalgene™ Square PETG Media Bottles with Closure: Sterile, Shrink-Wrapped Trays	342020-0500 or 342020-1000

PrepFiler™ Automated Forensic DNA Extraction Kit: Workflow

Standard workflow

Determine sample size or input amount

Select a lysis protocol

Before each use: Prepare the lysis reagents

Prepare sample lysate

Select one of the following protocols:

- Standard LySep™ column protocol (300 µL)
- Standard spin/filter tube protocol (300 µL)

Chapter 6, “Prepare for the automated purification run”

PrepFiler™ BTA Automated Forensic DNA Extraction Kit: Workflow

Standard workflow

Chapter 2, “Prepare BTA samples for lysis”

Determine sample size or input amount

Select a lysis protocol

Before each use: Prepare the lysis reagents

Prepare sample lysate

Select one of the following protocols:

- Standard 2-mL screw-cap protocol (230 µL)
- Standard LySep™ column protocol (230 µL)
- Standard spin/filter tube protocol (230 µL)

Chapter 6, “Prepare for the automated purification run”

2 Prepare BTA samples for lysis

Note: This chapter provides pre-lysis preparation procedures for some sample types that will be processed with the PrepFiler™ BTA Automated Forensic DNA Extraction Kit. For all other sample types, follow your laboratory's sample preparation protocols.

Prepare samples for lysis: Bone or tooth

1. Clean the bone or tooth sample to remove any adhered tissue.
2. Prepare a uniform bone or tooth powder using standard laboratory procedures.

Prepare samples for lysis: All other sample types

Follow the appropriate procedure to prepare the sample for lysis.

Sample type	Sample preparation
Chewing gum	1. In a clean Petri disk, flatten the piece of gum into a pancake shape ~5-mm thick. 2. Cover the Petri dish, tape it closed, then place the dish in a –80°C freezer for ≥2 hours.
Cigarette butt	1. Remove the first 5 mm of filter paper from the end of the cigarette butt, making sure to remove all the filter fibers. 2. Cut the filter paper into 2–3 pieces.
Tape lift	Cut the tape with a razor blade as needed to fit into the labware that you will use for sample lysis.
Swab	Prepare according to your laboratory protocol.

3 Prepare for manual lysis

Determine sample size or input amount

Determine the appropriate sample size or input amount for use with your sample types. Examples of appropriate sample types and inputs are shown in Table 11 and Table 12. Optimal input amounts may be affected by factors such as sample age and substrate properties. Each lab should perform studies to independently validate input amounts.

Table 11 PrepFiler™ Automated Forensic DNA Extraction Kit: Example sample types and inputs

Sample type	Example sample input[1]
Liquid sample (blood, saliva)	≤40 µL
Fabric or paper substrate	≤25-mm^2 cutting or punch
Swab	≤1 swab
Hair root	≤5-mm cutting from root

[1] You do not need to use an entire sample punch or swab.

Table 12 PrepFiler™ BTA Automated Forensic DNA Extraction Kit: Example sample types and inputs

Sample type	Example sample input
Bone or tooth	≤50 mg of powdered bone or tooth
Envelope flap	≤1 × 1.5-cm cutting
Chewing gum	≤50 mg (~3 × 3 × 5-mm piece)
Cigarette butt	≤25-mm^2 cutting of cigarette filter paper **IMPORTANT!** Remove all filter material from the filter paper.
Tape lift	≤2-cm^2 cutting
Swab	≤1 swab

Select a lysis protocol

Select one lysis protocol from Table 13 or Table 14, based on your kit, sample type, sample size, and labware.

IMPORTANT! Use the same protocol to prepare all the samples that are included in the same automated run on your robotic workstation.

Table 13 Lysis protocols for the PrepFiler™ Automated Forensic DNA Extraction Kit

Sample type	Labware	Protocol
Contains no substrate *OR* Includes a substrate that can be submerged using 300 µL of lysis buffer	LySep™ columns	"Standard LySep™ column protocol (300 µL)" on page 20
	1.5-mL spin/filter tubes	"Standard spin/filter tube protocol (300 µL)" on page 22
Includes a substrate that requires more than 300 µL of lysis buffer to submerge your sample	LySep™ columns	"Large-sample LySep™ column protocol (500 µL)" on page 46
	1.5-mL spin/filter tubes	"Large-sample spin/filter tube protocol (500 µL)" on page 50

For the PrepFiler™ Automated Forensic DNA Extraction Kit, note the following:

- If you are unsure whether to use the standard or large-sample protocols, start with a standard protocol, add the recommended amount of lysis buffer, then switch to a large-sample protocol if needed.

  IMPORTANT! The large-sample (500-µL) protocols were evaluated for sensitivity and cross-contamination using LySep™ columns for lysis and the ID NIMBUS® Presto workstation for automated purification; however, a full validation has not been completed. The large-sample protocols are intended only for samples that are not submerged by the 300 µL of lysis buffer that is used in the standard protocols. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large-sample protocols may be lower than yields that are obtained using the standard protocols with comparable sample input amounts.

- The labware that you select for manual lysis is independent of the labware you select for the DNA eluate.

Table 14 Lysis protocols for the PrepFiler™ BTA Automated Forensic DNA Extraction Kit

Sample type	Labware	Protocol
Bone or tooth	2-mL screw-cap tubes	"Standard 2-mL screw-cap protocol (230 µL)" on page 26
Adhesive substrates (for example, chewing gum, cigarette butts, swabs, and tape lifts)	LySep™ columns	"Standard LySep™ column protocol (230 µL)" on page 28
	1.5-mL spin/filter tubes	"Standard spin/filter tube protocol (230 µL)" on page 31

Note: We have not developed large-sample protocols for the PrepFiler™ BTA Automated Forensic DNA Extraction Kit. Large-sample protocols for the PrepFiler™ BTA Automated kit may promote cross-contamination on robotic workstations because of the higher liquid volumes required. In addition, our studies showed no significant difference in yield between a 230-µL protocol and a 460-µL protocol for the PrepFiler™ BTA Automated kit.

Before each use: Prepare the lysis reagents

1. If the PrepFiler™ Lysis Buffer contains precipitate, heat the lysis buffer for 15 minutes at 37°C, then vortex for 5 seconds.

   Note: This step applies to the PrepFiler™ Lysis Buffer that is included in both kits. Do not heat the PrepFiler™ BTA Lysis Buffer that is included in the PrepFiler™ BTA Automated Forensic DNA Extraction Kit.

2. Thaw or prepare a fresh 1.0 M solution of DL-dithiothreitol (DTT) in molecular-biology grade DNA-*free*™ water.

   To prepare fresh DTT: Dissolve 1.54 g of dithiothreitol (DTT, MW 154) in 10 mL of molecular-biology grade DNA-*free*™ water. Aliquots of the desired volume (for example, 100 µL or 500 µL) can be prepared ahead of time, then stored at –20°C for ≤6 months.

   Note: After completing lysis, discard unused DTT.

4 PrepFiler™ Automated Forensic DNA Extraction Kit: Prepare sample lysate

Standard LySep™ column protocol (300 µL)

Guidelines for handling LySep™ column/tube assemblies

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling the sample tubes. For example, change gloves after removing the column from the sample tube.
- Properly dispose of used PrepFiler™ LySep™ Columns. Used columns are potentially biohazardous.

Perform lysis (LySep™ columns, 300 µL)

1. Bring the thermal shaker temperature to 70°C.
2. Label a 1.5-mL PrepFiler™ Capless Microtube.

   Note: If you use barcodes to track samples, position the barcode on the tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ LySep™ Column into the labeled 1.5-mL PrepFiler™ Capless Microtube (together called the "column/tube assembly"), then carefully transfer the sample into the column.

4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ Lysis Buffer	300 µL	30 mL
DTT, 1.0 M	3 µL (use 5 µL for samples that contain semen)	300 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See “Before each use: Prepare the lysis reagents” on page 18.

[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 300 µL of the lysis buffer solution to each column/tube assembly.
6. Tightly cap the column/tube assembly.

Note: Do not place labels on tube caps; doing so can cause leakage.

7. Place the column/tube assembly in a thermal shaker, then incubate at 750–900 rpm for 40 minutes at 70°C.

Note: For most common sample and substrate types, we recommend a 40-minute incubation time. You can extend the incubation time for sample types that may be difficult to lyse, such as fixed stains or neat liquid semen. With the proper validation studies, the lysis time and temperature can be varied between 10–90 minutes and 50–80°C, respectively. We do not recommend times > 90 minutes or temperatures >80°C. We do not recommend overnight incubation because it may degrade DNA.

The lysis buffer can cause salt precipitation, especially with increased incubation times. Salt precipitation may lead to instrument crash, tip clogging, or tip filter wetting. See Appendix A, "Troubleshooting" for suggestions on preventing and/or dissolving precipitated salts.

Proceed to "Remove substrate (LySep™ columns, 300 µL) PrepFiler Auto Kit" on page 21.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (LySep™ columns, 300 µL)

1. Centrifuge the column/tube assembly at 10,000 × *g* for 2 minutes to transfer the lysate to the sample tube.
2. Check the volume of sample lysate. If the volume is <180 µL, see "The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 300-µL protocols" on page 44.

IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

3. Carefully remove the column from the sample tube.
   - If there is clear lysate remaining in the column, use a pipette to transfer the lysate to the sample tube.
   - Properly dispose of the column that contains the sample substrate.
4. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Capless Microtube.

IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

5. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

6. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Standard spin/filter tube protocol (300 µL)

Guidelines for handling spin/filter tubes

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling tubes. For example, change gloves after removing the filter column from the spin tube.
- To avoid leaks, ensure that the tubes are tightly sealed before vortexing or incubation.

IMPORTANT! Leaking tubes may result in DNA cross-contamination.

- Before opening, briefly centrifuge the tubes (~2 seconds in a microcentrifuge) to collect the contents at the bottom of each tube.
- Open and close all tubes carefully.

Perform lysis (spin/filter tubes, 300 µL)

1. Bring the thermal shaker temperature to 70°C.

2. Label a 1.5-mL PrepFiler™ Spin Tube or a standard 1.5-mL microfuge tube.

3. Carefully transfer the sample to the labeled 1.5-mL PrepFiler™ Spin Tube or standard 1.5-mL microfuge tube.

4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ Lysis Buffer	300 µL	30 mL
DTT, 1.0 M	3 µL (use 5 µL for samples that contain semen)	300 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.
[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 300 µL of the lysis buffer solution to each sample tube.
6. Cap each tube, vortex for 5 seconds, then briefly centrifuge.
7. Ensure that the tubes are well sealed.
8. Place the tubes in the thermal shaker, then incubate at 750–900 rpm for 40 minutes at 70°C.

Note: For most common sample and substrate types, we recommend a 40-minute incubation time. You can extend the incubation time for sample types that may be difficult to lyse, such as fixed stains or neat liquid semen. With the proper validation studies, the lysis time and temperature can be varied between 10–90 minutes and 50–80°C, respectively. We do not recommend times > 90 minutes or temperatures >80°C. We do not recommend overnight incubation because it may degrade DNA.

The lysis buffer can cause salt precipitation, especially with increased incubation times. Salt precipitation may lead to instrument crash, tip clogging, or tip filter wetting. See Appendix A, "Troubleshooting" for suggestions on preventing and/or dissolving precipitated salts.

If sample substrate is present, proceed to "(If needed) Remove substrate (spin/filter tubes, 300 µL) PrepFiler Auto Kit" on page 24. Otherwise proceed to Chapter 6, "Prepare for the automated purification run".

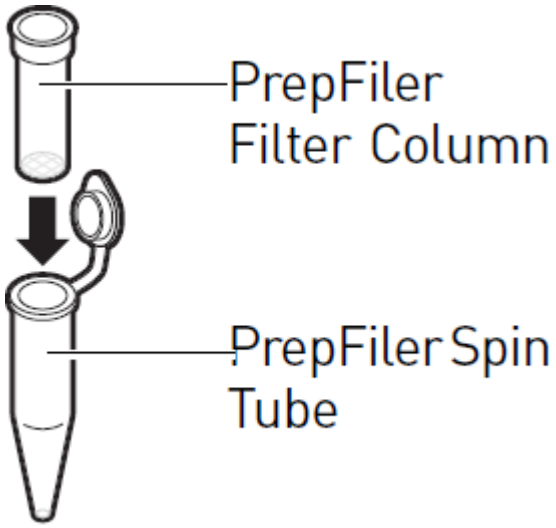
IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

(If needed) Remove substrate (spin/filter tubes, 300 µL)

1. Centrifuge the sample tube for 2 seconds to collect the contents at the bottom of the tube.
2. Label up to 96 new 1.5-mL PrepFiler™ Spin Tubes.

   Note: If you use barcodes to track samples, position the barcode on each tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ Filter Column into a new, labeled 1.5-mL PrepFiler™ Spin Tube (together called the "tube assembly").



4. Carefully transfer the sample tube contents into the filter column:
   - Use a pipette to transfer the liquid contents.
   - Use the pipette tip or sterile tweezers to transfer the substrate.
5. Cap the tube assembly, then centrifuge at 13,000–16,000 × *g* for 2 minutes.
6. Check the volume of sample lysate. If the volume is <180 µL, see “The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 300-µL protocols” on page 44.

   IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

7. Remove the filter column from the spin tube, then properly dispose of the filter column that contains the sample substrate.
8. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Spin Tube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

9. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

10. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

Note: The elongated neck of the PrepFiler™ Spin Tubes may not be compatible with some robotic workstations or carriers. *If needed,* you can do one of the following before performing the automated purification run:

- Transfer the lysate to a standard 1.5-mL microfuge tube.
- Cut the caps off of the PrepFiler™ Spin Tubes.

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

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5 PrepFiler™ BTA Automated Forensic DNA Extraction Kit: Prepare sample lysate

Standard 2-mL screw-cap protocol (230 µL)

Use the 2-mL screw-cap protocol for bone or tooth samples. Bone and tooth samples effervesce during lysis, and therefore require the 2-mL screw-cap tubes to prevent the tubes from popping open during the incubation step.

Perform lysis (screw-cap tubes, 230 µL)

1. Bring the thermal shaker temperature to 56°C.
2. Label a new 2-mL screw-cap tube.
3. Using the sample prepared in "Prepare samples for lysis: Bone or tooth" on page 15, carefully transfer ≤50 mg of powdered bone or tooth into the labeled tube.
4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ BTA Lysis Buffer	220 µL	22 mL
DTT, 1.0 M	3 µL	300 µL
Proteinase K	7 µL	700 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.

[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 230 µL of the lysis buffer solution to the labeled tube.
6. Cap the tube, vortex for 5 seconds, then briefly centrifuge.

   Note: To avoid leaks, ensure that the tube is tightly sealed. To avoid forming a pellet, do not centrifuge >5 seconds.

7. Place the tube in a thermal shaker, then incubate at 1,100 rpm for ≥2 hours at 56°C. (The sample can be incubated ≤18 hours.)

Proceed to "Separate lysate from sediment (screw-cap tubes, 230 µL)" on page 27.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Separate lysate from sediment (screw-cap tubes, 230 µL)

1. Label up to 96 new 1.5-mL microfuge tubes.

   Note: If you use barcodes to track samples, position the barcode on each tube according to your robotic workstation documentation.

2. Let the sample come to room temperature.
3. Centrifuge the 2-mL screw-cap tube at 10,000 × *g* for 90 seconds.
4. Check the volume of sample lysate. If the volume is <180 µL, see "The volume of collected lysate is low: PrepFiler™ BTA Automated Forensic DNA Extraction Kit, 230-µL protocols" on page 45.

   IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

5. Transfer the clear (no sediment) lysate to the labeled 1.5-mL microfuge tube.

   IMPORTANT! Sediment in the lysate can cause liquid handling problems during the automated purification run.

6. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.
7. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

   IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Standard LySep™ column protocol (230 µL)

Note: Do not use the LySep™ column protocol for bone or tooth samples. Bone and tooth samples effervesce during lysis, and therefore require the 2-mL screw-cap tubes to prevent the tubes from popping open during the incubation step.

Guidelines for handling LySep™ column/tube assemblies

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling the sample tubes. For example, change gloves after removing the column from the sample tube.
- Properly dispose of used PrepFiler™ LySep™ Columns. Used columns are potentially biohazardous.

Perform lysis (LySep™ columns, 230 µL)

1. Bring the thermal shaker temperature to 56°C.

   Note: For envelope flap samples, studies indicate that lysis at room temperature (instead of 56°C) may result in a higher quantity and quality of extracted DNA. Our validation studies were performed using a lysis temperature of 56°C. Your laboratory should perform appropriate internal validation studies.

2. Label a 1.5-mL PrepFiler™ Capless Microtube.

   Note: If you use barcodes to track samples, position the barcode on the tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ LySep™ Column into the labeled 1.5-mL PrepFiler™ Capless Microtube (together called the "column/tube assembly").

4. Using the sample prepared in Chapter 2, “Prepare BTA samples for lysis”, carefully transfer the sample into the column.

Sample Type	Transfer
Chewing gum	Cut and transfer ≤50 mg of gum (~3 mm^2) into the column.
Cigarette butt	Transfer all pieces into the column.

(continued)

Sample Type	Transfer
Tape lift	Transfer the tape into the column. **IMPORTANT!** Ensure that the side of the tape that contains the sample does not adhere to the side of the column.
Swab	Transfer into the column.

5. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ BTA Lysis Buffer	220 µL	22 mL
DTT, 1.0 M	3 µL	300 µL
Proteinase K	7 µL	700 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.
[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

6. Add 230 µL of the lysis buffer solution to each column/tube assembly.
7. Tightly cap the column/tube assembly.

Note: Do not place labels on tube caps; doing so can cause leakage.

8. Place the column/tube assembly in a thermal shaker, then incubate at 750–900 rpm for 40 minutes at 56°C.

Proceed to "Remove substrate (LySep™ columns, 230 µL) PrepFiler BTA Auto Kit" on page 30.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (LySep™ columns, 230 µL)

1. Centrifuge the column/tube assembly at 10,000 × *g* for 2 minutes to transfer the lysate to the sample tube.

2. Check the volume of sample lysate. If the volume is <180 µL, see "The volume of collected lysate is low: PrepFiler™ BTA Automated Forensic DNA Extraction Kit, 230-µL protocols" on page 45.

   IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

3. Carefully remove the column from the sample tube.
   - If there is clear lysate remaining in the column, use a pipette to transfer the lysate to the sample tube.
   - Properly dispose of the column that contains the sample substrate.

4. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Capless Microtube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

5. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

6. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

   IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Standard spin/filter tube protocol (230 µL)

Note: Do not use the spin/filter tube protocol for bone or tooth samples. Bone and tooth samples effervesce during lysis, and therefore require the 2-mL screw-cap tubes to prevent the tubes from popping open during the incubation step.

Guidelines for handling spin/filter tubes

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling tubes. For example, change gloves after removing the filter column from the spin tube.
- To avoid leaks, ensure that the tubes are tightly sealed before vortexing or incubation.

  IMPORTANT! Leaking tubes may result in DNA cross-contamination.

- Before opening, briefly centrifuge the tubes (~2 seconds in a microcentrifuge) to collect the contents at the bottom of each tube.
- Open and close all tubes carefully.

Perform lysis (spin/filter tubes, 230 µL)

1. Bring the thermal shaker temperature to 56°C.

   Note: For envelope flap samples, studies indicate that lysis at room temperature (instead of 56°C) may result in a higher quantity and quality of extracted DNA. Our validation studies were performed using a lysis temperature of 56°C. Your laboratory should perform appropriate internal validation studies.

2. Label a 1.5-mL PrepFiler™ Spin Tube or a standard 1.5-mL microfuge tube.

3. Using the sample prepared in Chapter 2, “Prepare BTA samples for lysis”, carefully transfer the sample to the labeled 1.5-mL PrepFiler™ Spin Tube or standard 1.5-mL microfuge tube.

Sample Type	Transfer
Chewing gum	Cut and transfer ≤50 mg of gum (~3 mm^2) into the spin tube.
Cigarette butt	Transfer all pieces into the spin tube.
Tape lift	Transfer the tape into the spin tube. **IMPORTANT!** Ensure that the side of the tape that contains the sample does not adhere to the side of the spin tube.
Swab	Transfer into the spin tube.

4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ BTA Lysis Buffer	220 µL	22 mL
DTT, 1.0 M	3 µL	300 µL
Proteinase K	7 µL	700 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.
[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 230 µL of the lysis buffer solution to each sample tube.
6. Cap each tube, vortex for 5 seconds, then briefly centrifuge.
7. Ensure that the tubes are well sealed.
8. Place the tubes in the thermal shaker, then incubate at 750–900 rpm for 40 minutes at 56°C.

Proceed to "Remove substrate (spin/filter tubes, 230 µL) PrepFiler BTA Auto Kit" on page 32.

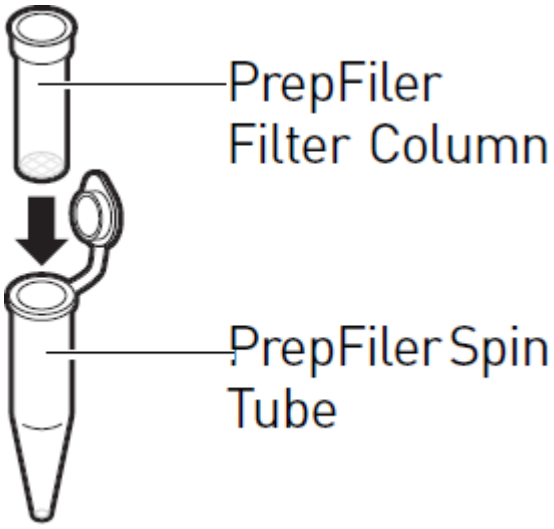
IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (spin/filter tubes, 230 µL)

1. Centrifuge the sample tube for 2 seconds to collect the contents at the bottom of the tube.
2. Label up to 96 new 1.5-mL PrepFiler™ Spin Tubes.

Note: If you use barcodes to track samples, position the barcode on each tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ Filter Column into a new, labeled 1.5-mL PrepFiler™ Spin Tube (together called the "tube assembly").



4. Carefully transfer the sample tube contents into the filter column:
   - Use a pipette to transfer the liquid contents.
   - Use the pipette tip or sterile tweezers to transfer the substrate.

5. Cap the tube assembly, then centrifuge at 13,000–16,000 × *g* for 2 minutes.

6. Check the volume of sample lysate. If the volume is <180 µL, see “The volume of collected lysate is low: PrepFiler™ BTA Automated Forensic DNA Extraction Kit, 230-µL protocols” on page 45.

IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

7. Remove the filter column from the spin tube, then properly dispose of the filter column that contains the sample substrate.

8. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Spin Tube.

IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

9. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

10. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, “Prepare for the automated purification run”.

Note: The elongated neck of the PrepFiler™ Spin Tubes may not be compatible with some robotic workstations or carriers. *If needed,* you can do one of the following before performing the automated purification run:

- Transfer the lysate to a standard 1.5-mL microfuge tube.
- Cut the caps off of the PrepFiler™ Spin Tubes.

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

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6 Prepare for the automated purification run

Before first use: Prepare the wash buffers

1. Mix 260 mL of PrepFiler™ Wash Buffer A Concentrate with 740 mL of freshly-opened 95% ethanol in a separate, clean container to prepare a 1X solution.
2. Mix 200 mL of PrepFiler™ Wash Buffer B Concentrate with 300 mL of freshly-opened 95% ethanol in a separate, clean container to prepare a 1X solution.

If the containers are kept closed when not in use, the prepared wash buffers have a shelf life of 6 months or the kit expiration date, whichever is earlier.

Before each use: Prepare the magnetic particles

1. Incubate the PrepFiler™ Magnetic Particles tubes at 37°C for 10 minutes.
2. Vortex at medium speed until the particles are completely resuspended and homogenous, then briefly centrifuge.
3. Use one of the following methods to remove any air bubbles:
   - Draw off bubbles with a disposable bulb pipette.
   - Use a clean pipette tip to break up the bubbles.
   - Use a lint-free wipe to absorb the bubbles.

IMPORTANT! Bubbles can interfere with automated liquid detection and aspiration.

Automation guidelines

The following are general guidelines for automating the binding, wash, and elution steps of the purification procedure on most standard liquid handling robotic workstations. Unless otherwise indicated, these guidelines apply to the PrepFiler™ Automated Forensic DNA Extraction Kit and the PrepFiler™ BTA Automated Forensic DNA Extraction Kit.

- Use a magnetic-ring stand, processing plates, and consumables that are compatible with your robotic workstation.

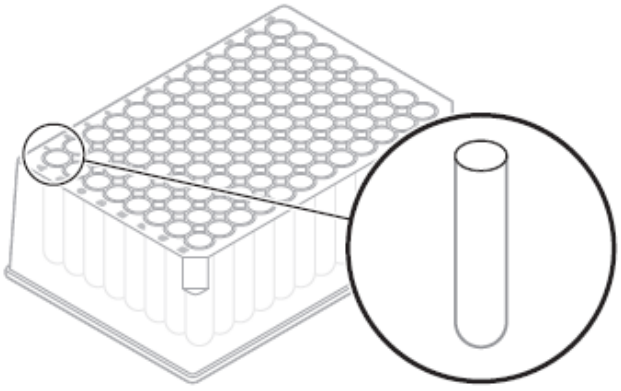
  Note: To ensure that the magnetic particles completely separate, the magnetic-ring stand and processing plate must be compatible. For example, the Magnetic-Ring Stand (96 well) (Cat. No. AM10050) and the PrepFiler™ 96-Well Processing Plates (Cat. No. A47010) are compatible with each other.

- After you select the lysate and eluate labware and configure the automated workstation for that labware, use the same labware going forward. For example, do not go back and forth between capless tubes, microfuge tubes, and spin tubes. If you do change the labware, you will have to reconfigure the workstation and software.
- For validated and supported procedures for the HID EVOlution™ systems, see the *PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin* (Pub. No. MAN0019298).
- For validated and supported procedures for the ID NIMBUS® Presto, see the *PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the ID NIMBUS® Presto Workstation User Bulletin* (Pub. No. MAN0019368).

Binding step

1. Place the sample lysate container on the robot.

   Note: The lysate may be contained in a deep-well plate or in individual tubes, depending on your robotic workstation.

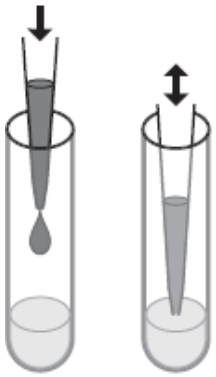


2. *(PrepFiler™ BTA Automated Forensic DNA Extraction Kit only)* Add 70–300 µL of PrepFiler™ Lysis Buffer to each well or tube that contains lysate.

   IMPORTANT! The addition of PrepFiler™ Lysis Buffer at this step is essential for proper DNA binding to the magnetic particles. Do not use the PrepFiler™ BTA Lysis Buffer at this step.

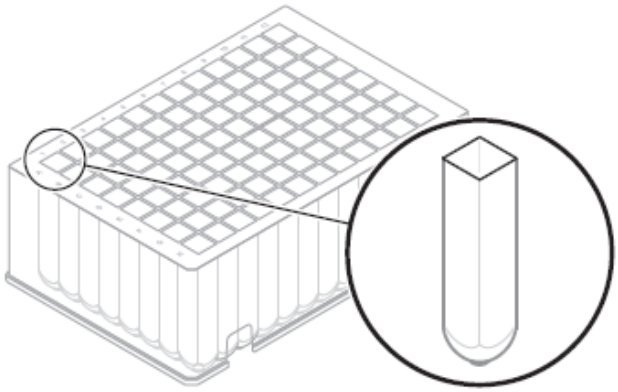
   Note: To reduce the overall reagent volume in the well, and therefore reduce the potential for cross-contamination on different robotic platforms, you can adjust the PrepFiler™ Lysis Buffer between 70–300 µL, as needed. To determine the appropriate volume for your laboratory and robotic platform, perform internal validation studies.

3. Add 16.5 µL of PrepFiler™ Magnetic Particles to each well or tube that contains lysate. With the robot at the default mixing speed, pipet the well contents up and down until the particles are completely resuspended.



   Note: Mixing speed does not affect magnetic particle performance.

4. Transfer the contents of each well or tube to a separate well in a processing plate that is compatible with your robotic workstation.



   Note: In the remaining steps, only one well of the processing plate is shown.

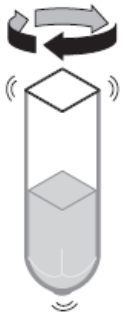
5. To each well of the processing plate, add the required amount of isopropanol, depending on your kit and lysis volume.

Kit	Lysis volume	Isopropanol
PrepFiler™ Automated Forensic DNA Extraction Kit	300 µL	180 µL
	500 µL	300 µL
PrepFiler™ BTA Automated Forensic DNA Extraction Kit	230 µL	180–300 µL[1]

[1] To reduce the overall reagent volume in the well, and therefore reduce the potential for cross-contamination on different robotic platforms, you can adjust the isopropanol between 180–300 µL, as needed. To determine the appropriate volume for your laboratory and robotic platform, perform internal validation studies.

6. Shake the processing plate at 500–1,000 rpm for 10 minutes to bind the DNA to the magnetic particles.

Note: *(PrepFiler™ BTA Automated Forensic DNA Extraction Kit protocols only)* Because of the large volume of reagents, there is a high risk of splash-over if the shaking speed is too high. To ensure complete mixing without splash-over, determine the optimal shaking speed within the 500–1,000 rpm range.



7. Place the processing plate on a magnetic-ring stand for 5 minutes to separate the magnetic particles.

8. Remove all the liquid phase without disturbing the magnetic particles.

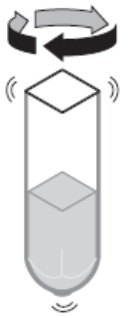
Wash step (repeat three times)

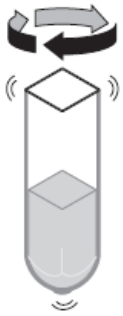
1. Place the processing plate on the shaker.
2. To each well, add 600 µL of prepared Wash Buffer A (first wash), 300 µL of prepared Wash Buffer A (second wash), or 300 µL of prepared Wash Buffer B (third wash).

Note: In the remaining steps, only one well of the processing plate is shown.

3. Shake the processing plate at 500–1,000 rpm for 1 minute.

Note: Because of the large volume of reagents, there is a high risk of splash-over if the shaking speed is too high. To ensure complete mixing without splash-over, determine the optimal shaking speed within the 500–1,000 rpm range.





4. Place the processing plate on a magnetic-ring stand for 1 minute.

5. Remove all the liquid phase without disturbing the magnetic particles.

Elution step

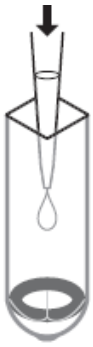
1. Keeping the processing plate on the magnetic-ring stand, uncover the plate and allow the magnetic particles-bound DNA to air-dry for 7–10 minutes.

   IMPORTANT! Air-drying for >10 minutes may reduce DNA yield. If the room temperature is > 25°C, reduce the drying time to 5 minutes.

2. Place the processing plate on the shaker.

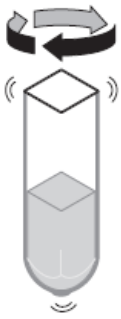
3. To each well, add the preferred amount of PrepFiler™ Elution Buffer, as determined by your laboratory protocol.

   Note: In the remaining steps, only one well of the processing plate is shown.



4. Incubate and shake the processing plate at 500–1,000 rpm for 5–10 minutes at 70°C.

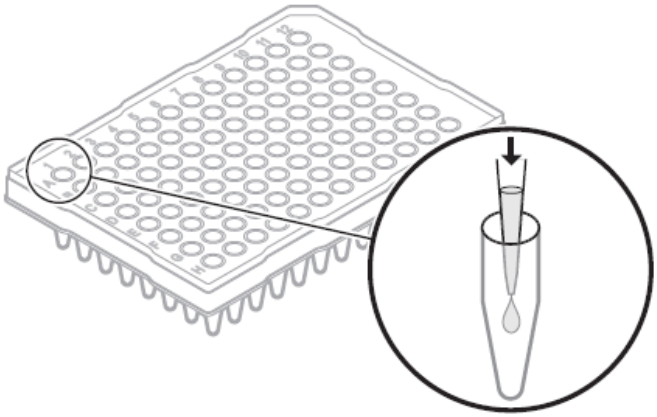
   Note: Because of the large volume of reagents, there is a high risk of splash-over if the shaking speed is too high. To ensure complete mixing without splash-over, determine the optimal shaking speed within the 500–1,000 rpm range.



5. Place the processing plate on a magnetic-ring stand for 7 minutes.

6. Remove all the DNA-containing liquid phase without disturbing the magnetic particles.

7. Pipet the DNA-containing liquid phase into a new 96-well microplate or new 1.5-mL microfuge tubes.



A

Troubleshooting

Troubleshooting reagent preparation

Observation	Possible cause	Recommended action
There is precipitate in the PrepFiler™ Magnetic Particles tube	Magnetic particles were exposed to low temperatures during shipping or storage.	Before each use, incubate the magnetic particles for 10 minutes at 37°C, then vortex at medium speed until the particles are completely resuspended.
There is precipitate in the PrepFiler™ Lysis Buffer or PrepFiler™ BTA Lysis Buffer	The lysis buffer was exposed to low temperatures during shipping or storage.	Before each use, incubate the lysis buffer for 10 minutes at 37°C, then vortex at medium speed until the solution is completely resuspended.

Troubleshooting after lysis incubation

Observation	Possible cause	Recommended action
The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 300-µL protocols	• Some lysate remained in the sample substrate after centrifugation. • The labware was not properly sealed during incubation or vortexing, resulting in volume loss through leakage or evaporation. • The spin/filter tubes were not briefly centrifuged after incubation or vortexing, and droplets inside the tube lid leaked when the tube was opened.	**If the lysate volume is ≥180 µL:** Proceed to the next step in the protocol.
		If the lysate volume is <180 µL and the sample required substrate removal: 1. Centrifuge the spin tube or plate that contains the substrate for an additional 5 minutes. 2. If the resulting lysate volume is: • ≥180 µL, proceed to the next step in the protocol. • <180 µL, add PrepFiler™ Lysis Buffer to bring the lysate volume to 180 µL, then proceed to the next step in the protocol.
		If the lysate volume is <180 µL and the sample *did not* require substrate removal: Add PrepFiler™ Lysis Buffer to bring the lysate volume to 180 µL, then proceed to the next step in the protocol.
The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 500-µL protocols	• Some lysate remained in the sample substrate after centrifugation. • The labware was not properly sealed during incubation or vortexing, resulting in volume loss through leakage or evaporation. • The spin/filter tubes were not briefly centrifuged after incubation or vortexing, and droplets inside the tube lid leaked when the tube was opened.	**If the lysate volume is ≥300 µL:** Proceed to the next step in the protocol.
		If the lysate volume is <300 µL and the sample required substrate removal: 1. Centrifuge the spin tube or plate that contains the substrate for an additional 5 minutes. 2. If the resulting lysate volume is: • ≥300 µL, proceed to the next step in the protocol. • <300 µL, add PrepFiler™ Lysis Bufferto bring the lysate volume to 300 µL, then proceed to the next step in the protocol.
		If the lysate volume is <300 µL and the sample *did not* require substrate removal: Add PrepFiler™ Lysis Buffer to bring the lysate volume to 300 µL, then proceed to the next step in the protocol.

Observation	Possible cause	Recommended action
The volume of collected lysate is low: PrepFiler™ BTA Automated Forensic DNA Extraction Kit, 230-µL protocols	• Some lysate remained in the sample substrate after centrifugation. • The labware was not properly sealed during incubation or vortexing, resulting in volume loss through leakage or evaporation. • The spin/filter tubes were not briefly centrifuged after incubation or vortexing, and droplets inside the tube lid leaked when the tube was opened.	**If the lysate volume is ≥180 µL:** Proceed to the next step in the protocol.

		If the lysate volume is <180 µL and the sample required substrate removal:
		1. Centrifuge the spin tube or plate that contains the substrate for an additional 5 minutes.
		2. If the resulting lysate volume is:
		  • ≥180 µL, proceed to the next step in the protocol.
		  • <180 µL, add PrepFiler™ BTA Lysis Buffer to bring the lysate volume to 180 µL, then proceed to the next step in the protocol.

		If the lysate volume is <180 µL and the sample *did not* require substrate removal: Add PrepFiler™ BTA Lysis Buffer to bring the lysate volume to 180 µL, then proceed to the next step in the protocol.

Precipitate is observed in a column before centrifugation	• The tube was incubated for >40 minutes.
	• The lysate was chilled.
	• The temperature in the laboratory is low.
		• Do one of the following:
		  – Use a pipette to mix the sample lysate and dissolve the salt precipitate.
		  – Before centrifuging, vortex the column/sample tube assembly at high speed.
		In future runs:
		• Do not incubate the tubes for >40 minutes.
		• Do not chill the sample lysate in a refrigerator or centrifuge.

Precipitate is observed in a sample tube after centrifugation	• The tube was incubated for >40 minutes. • The lysate was chilled. • The temperature in the laboratory is low.	Heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. In future runs: • Do not incubate the tubes for >40 minutes. • Do not chill the sample lysate in a refrigerator or centrifuge.

B

PrepFiler™ Automated Forensic DNA Extraction Kit: Supplementary lysis protocols

This appendix is only for the PrepFiler™ Automated Forensic DNA Extraction Kit. We have not developed large-sample protocols for the PrepFiler™ BTA Automated Forensic DNA Extraction Kit. Large-sample protocols for the PrepFiler™ BTA Automated kit may promote cross-contamination on robotic workstations because of the higher liquid volumes required. In addition, our studies showed no significant difference in yield between a 230-µL protocol and a 460-µL protocol for the PrepFiler™ BTA Automated kit.

Note: The ID NIMBUS® Presto workstation does not currently accommodate a 500-µL lysis volume.

Large-sample LySep™ column protocol (500 µL)

You may choose to perform the large-sample protocol if you require >300 µL of lysis buffer to submerge your sample. If you are unsure about using the large-sample protocol, start with the standard protocol, add the recommended amount of lysis buffer, then consider the large-sample protocol if needed.

IMPORTANT! The large-sample (500-µL) protocols were evaluated for sensitivity and cross-contamination using LySep™ columns for lysis and the ID NIMBUS® Presto workstation for automated purification; however, a full validation has not been completed. The large-sample protocols are intended only for samples that are not submerged by the 300 µL of lysis buffer that is used in the standard protocols. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large-sample protocols may be lower than yields that are obtained using the standard protocols with comparable sample input amounts.

Guidelines and required materials (LySep™ columns)

See:

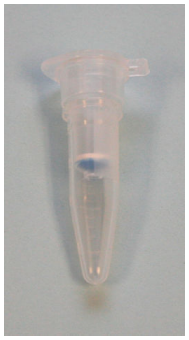
- "Guidelines for handling LySep™ column/tube assemblies" on page 20
- Table 7 under "Required materials not supplied" on page 11

Perform lysis (LySep™ columns, 500 µL)

1. Bring the thermal shaker temperature to 70°C.
2. Label a 1.5-mL PrepFiler™ Capless Microtube.

Note: If you use barcodes to track samples, position the barcode on the tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ LySep™ Column into the labeled 1.5-mL PrepFiler™ Capless Microtube (together called the "column/tube assembly"), then carefully transfer the sample into the column.



4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ Lysis Buffer	500 µL	50 mL
DTT, 1.0 M	5 µL (use 8 µL for samples that contain semen)	500 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See “Before each use: Prepare the lysis reagents” on page 18.
[2] Includes ~4% excess volume to compensate for pipetting losses.

IMPORTANT! We do not recommend using >500 µL of lysis buffer per reaction.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 500 µL of the lysis buffer solution to each column/tube assembly.
6. Tightly cap the column/tube assembly.

Note: Do not place labels on tube caps; doing so can cause leakage.

7. Place the column/tube assembly in a thermal shaker, then incubate at 750–900 rpm for 40 minutes at 70°C.

   Note: For most common sample and substrate types, we recommend a 40-minute incubation time. You can extend the incubation time for sample types that may be difficult to lyse, such as fixed stains or neat liquid semen. With the proper validation studies, the lysis time and temperature can be varied between 10–90 minutes and 50–80°C, respectively. We do not recommend times > 90 minutes or temperatures >80°C. We do not recommend overnight incubation because it may degrade DNA.

   The lysis buffer can cause salt precipitation, especially with increased incubation times. Salt precipitation may lead to instrument crash, tip clogging, or tip filter wetting. See Appendix A, "Troubleshooting" for suggestions on preventing and/or dissolving precipitated salts.

Proceed to "Remove substrate (LySep™ columns, 500 µL)" on page 48.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (LySep™ columns, 500 µL)

1. Centrifuge the column/tube assembly at 10,000 × *g* for 2 minutes to transfer the lysate to the sample tube.

2. Check the volume of sample lysate. If the volume is <300 µL, see "The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 500-µL protocols" on page 44.

   IMPORTANT! A 300-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

3. Carefully remove the column from the sample tube.
   - If there is clear lysate remaining in the column, use a pipette to transfer the lysate to the sample tube.
   - Properly dispose of the column that contains the sample substrate.

4. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Capless Microtube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

5. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

6. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, “Prepare for the automated purification run”.

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Large-sample spin/filter tube protocol (500 µL)

You may choose to perform the large-sample protocol if you require >300 µL of lysis buffer to submerge your sample. If you are unsure about using the large-sample protocol, start with the standard protocol, add the recommended amount of lysis buffer, then consider the large-sample protocol if needed.

IMPORTANT! The large-sample (500-µL) protocols were evaluated for sensitivity and cross-contamination using LySep™ columns for lysis and the ID NIMBUS® Presto workstation for automated purification; however, a full validation has not been completed. The large-sample protocols are intended only for samples that are not submerged by the 300 µL of lysis buffer that is used in the standard protocols. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large-sample protocols may be lower than yields that are obtained using the standard protocols with comparable sample input amounts.

Guidelines and required materials (spin/filter tubes)

See:

- "Guidelines for handling spin/filter tubes" on page 22
- Table 8 under "Required materials not supplied" on page 11

Perform lysis (spin/filter tubes, 500 µL)

1. Bring the thermal shaker temperature to 70°C.
2. Label a 1.5-mL PrepFiler™ Spin Tube or a standard 1.5-mL microfuge tube.
3. Carefully transfer the sample to the labeled 1.5-mL PrepFiler™ Spin Tube or standard 1.5-mL microfuge tube.
4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ Lysis Buffer	500 µL	50 mL
DTT, 1.0 M	5 µL (use 8 µL for samples that contain semen)	500 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.

[2] Includes ~4% excess volume to compensate for pipetting losses.

IMPORTANT! We do not recommend using >500 µL of lysis buffer per reaction.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add 500 µL of the lysis buffer solution to each sample tube.
6. Cap each tube, vortex for 5 seconds, then briefly centrifuge.
7. Ensure that the tubes are well sealed.
8. Place the tubes in the thermal shaker, then incubate at 750–900 rpm for 40 minutes at 70°C.

Note: For most common sample and substrate types, we recommend a 40-minute incubation time. You can extend the incubation time for sample types that may be difficult to lyse, such as fixed stains or neat liquid semen. With the proper validation studies, the lysis time and temperature can be varied between 10–90 minutes and 50–80°C, respectively. We do not recommend times > 90 minutes or temperatures >80°C. We do not recommend overnight incubation because it may degrade DNA.

The lysis buffer can cause salt precipitation, especially with increased incubation times. Salt precipitation may lead to instrument crash, tip clogging, or tip filter wetting. See Appendix A, "Troubleshooting" for suggestions on preventing and/or dissolving precipitated salts.

Proceed to "Remove substrate (spin/filter tubes, 500 µL)" on page 51.

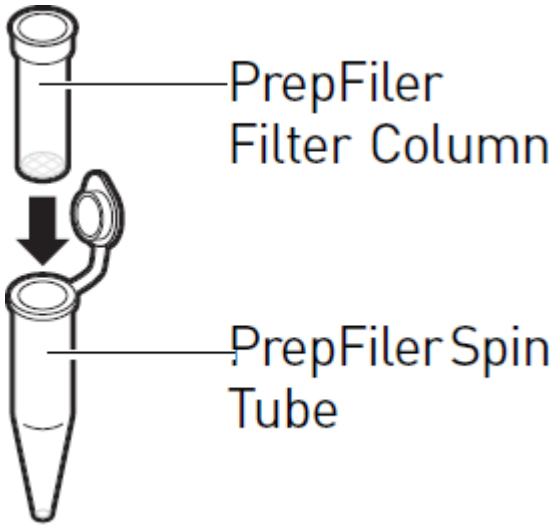
IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (spin/filter tubes, 500 µL)

1. Centrifuge the sample tube for 2 seconds to collect the contents at the bottom of the tube.
2. Label up to 96 new 1.5-mL PrepFiler™ Spin Tubes.

Note: If you use barcodes to track samples, position the barcode on each tube according to your robotic workstation documentation.

3. Insert a PrepFiler™ Filter Column into a new, labeled 1.5-mL PrepFiler™ Spin Tube (together called the "tube assembly").



4. Carefully transfer the sample tube contents into the filter column:
   - Use a pipette to transfer the liquid contents.
   - Use the pipette tip or sterile tweezers to transfer the substrate.

5. Cap the tube assembly, then centrifuge at 13,000–16,000 × *g* for 2 minutes.

6. Check the volume of sample lysate. If the volume is <300 µL, see “The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 500-µL protocols” on page 44.

   IMPORTANT! A 300-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

7. Remove the filter column from the spin tube, then properly dispose of the filter column that contains the sample substrate.

8. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Spin Tube.

   IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

9. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample tube that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

10. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, “Prepare for the automated purification run”.

Note: The elongated neck of the PrepFiler™ Spin Tubes may not be compatible with some robotic workstations or carriers. *If needed,* you can do one of the following before performing the automated purification run:

- Transfer the lysate to a standard 1.5-mL microfuge tube.
- Cut the caps off of the PrepFiler™ Spin Tubes.

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Spin/filter plate protocols (300 µL and 500 µL)

The procedures in this section cover the following protocols:

- Standard spin/filter plate protocol (300 µL)
- Standard spin/filter plate protocol for liquid samples (300 µL)
- Large-sample spin/filter plate protocol (500 µL)

  You may choose to perform the large-sample protocol if you require >300 µL of lysis buffer to submerge your sample. If you are unsure about using the large-sample protocol, start with the standard protocol, add the recommended amount of lysis buffer, then consider the large-sample protocol if needed.

IMPORTANT! The large-sample (500-µL) protocols were not tested as part of our full validation studies. The large-sample protocols are intended only for samples that are not submerged by the 300 µL of lysis buffer that is used in the standard protocols. If your laboratory intends to use the large-sample protocols, perform the appropriate validation studies. DNA yields from the large-sample protocols may be lower than yields that are obtained using the standard protocols with comparable sample input amounts.

Required materials not supplied (spin/filter plates)

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Table 15 Required materials for standard (300-µL) and large-sample (500-µL) protocols

Item	Source[1]
DL-dithiothreitol [DTT; molecular biology grade; ≥98% (TLC), ≥99% (titration)]	MLS
General purpose thermal shaker or shaking incubator that is compatible with deep-well plates, capable of temperatures ≤70°C and speeds ≤150 rpm	MLS
Vortexer, variable-speed	MLS

Table 15 Required materials for standard (300-µL) and large-sample (500-µL) protocols *(continued)*

Item	Source[1]
Benchtop centrifuge with rotor for 96-deep-well plates	MLS
Deep-well plate (for balancing the centrifuge rotor during centrifugation)	MLS
PrepFiler™ 96-Well Spin Plates and Filter Plates, ethylene oxide-treated (10 sets)	4476031
MicroAmp™ Multi Removal Tool	4313950
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Adhesive Film Applicator	4333183

[1] Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

Table 16 Additional required materials for the standard (300-µL) liquid sample protocol

Item	Source
Nonstick RNase-free Microfuge Tubes (1.5-mL), certified DNase- and RNase-free (250 tubes), or equivalent **Note:** If you use an equivalent tube, select tubes that allow you to observe the tube contents.	AM12450
Laboratory microcentrifuge capable of 13,000–16,000 × *g*	MLS

Guidelines for handling spin/filter plates

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling the spin/filter plates. For example, change gloves after separating the filter plate from the spin plate.
- To avoid leaks, ensure that the spin/filter plate unit is tightly assembled before shaking and incubation.

  IMPORTANT! Leaking plates may result in DNA cross-contamination.

- Use caution when separating the filter plate (top of the unit) from the spin plate (bottom of the unit) with the MicroAmp™ Multi Removal Tool. If the contents of the spin plate are shaken during plate separation, place an adhesive cover on the spin plate, place the spin plate in the deep-well centrifuge, then briefly centrifuge to collect the contents at the bottom of the wells.
- Use caution when placing each sample in a separate well in the filter plate (top of the unit). Consider covering unfilled wells to prevent depositing two samples in the same well.
- Cover and uncover all plates carefully.

Perform lysis (spin/filter plates, 300 µL and 500 µL)

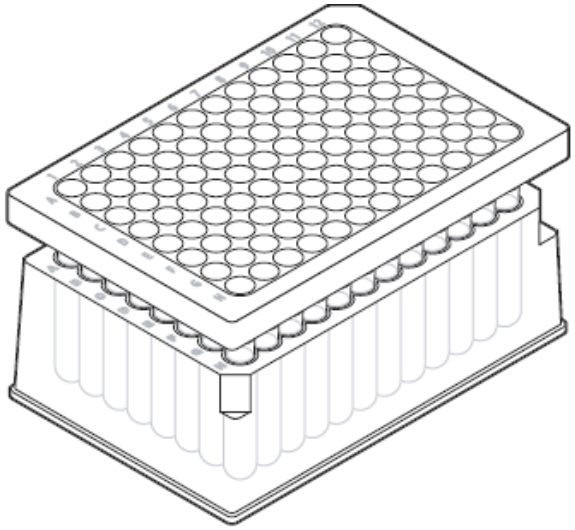
1. Bring the thermal shaker temperature to 70°C.

2. Label the PrepFiler™ Spin Plate.

   Note: If you use barcodes to track samples, position the barcode on the plate according to your robotic workstation documentation.

3. Tightly assemble the PrepFiler™ 96-Well Spin Plate and Filter Plate (together called the "plate assembly").

   a. Ensure that the filter plate is placed firmly on top of the spin plate, as shown in the following figure.



   b. Weigh the plate assembly, then place it in a deep-well centrifuge.

   c. Create a counterweight to ensure that the centrifuge rotor is well-balanced:

      1. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the plate assembly.
      2. Seal the deep-well plate, then place it in the deep-well centrifuge as a counterweight.

   d. Spin the plates at 650 × *g* for 2 minutes.

4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]			
	Standard protocol (300 µL)		Large-sample protocol (500 µL)	
	1 reaction	96 reactions	1 reaction	96 reactions
PrepFiler™ Lysis Buffer	300 µL	30 mL	500 µL	50 mL
DTT, 1.0 M	3 µL (use 5 µL for samples that contain semen)	300 µL	5 µL (use 8 µL for samples that contain semen)	500 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See "Before each use: Prepare the lysis reagents" on page 18.
[2] Includes ~4% excess volume to compensate for pipetting losses.

IMPORTANT! We do not recommend using >500 µL of lysis buffer per reaction.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Add sample and lysis buffer solution to the plate wells.

Note: If you are using <96 samples, see your robotic workstation documentation for plate layouts.

Protocol	Action
Standard protocol (300 µL)	1. Place each sample in a separate well in the filter plate (top plate). 2. Add 300 µL of the lysis buffer solution to each sample well.
Standard protocol (300 µL, liquid samples)	1. Add 300 µL of the lysis buffer solution to a separate 1.5-mL microfuge tube that contains ≤30 µL of liquid sample (such as blood or saliva). If adding liquid blood, invert the tube containing blood several times to mix before adding the blood to the 1.5-mL tube. 2. Cap the 1.5-mL microfuge tube, vortex at high speed for ~15 seconds, then centrifuge at maximum speed for 20 seconds. 3. Aspirate the entire liquid sample with a pipette, then dispense the sample in a separate well in the filter plate (top plate).
Large-sample protocol (500 µL)	1. Place each sample in a separate well in the filter plate (top plate). 2. Add 500 µL of the lysis buffer solution to each sample well.

Note: You may observe that some liquid drips through to the filter plate during lysis. Lysis is still occurring.

6. Immediately seal the plate assembly with a new sheet of MicroAmp™ Clear Adhesive Film and an applicator. See “Guidelines for sealing the plate assembly” on page 57.

7. Place the plate assembly in the thermal shaker, then incubate at 150 rpm for 60 minutes at 70°C.

   IMPORTANT! Keep the plate assembly horizontal during transport to the thermal shaker and throughout incubation to prevent possible cross-contamination.

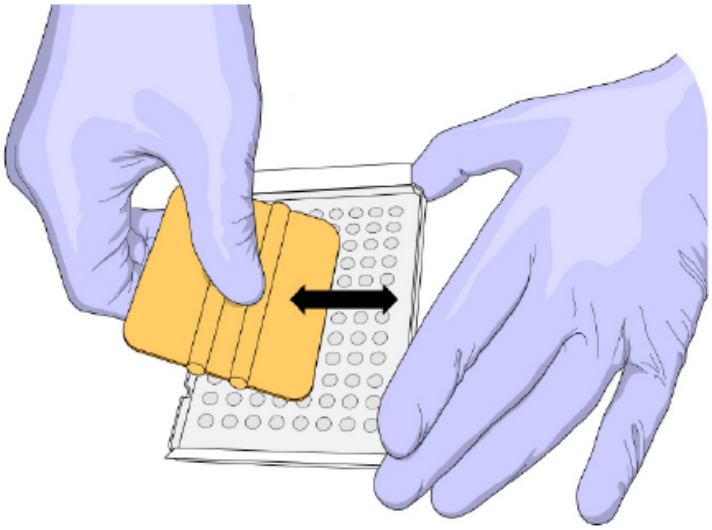
Proceed to “Remove substrate (spin/filter plates, 300 µL and 500 µL)” on page 58.

Guidelines for sealing the plate assembly

IMPORTANT! When using the applicator, apply significant downward pressure to ensure that the plate assembly is well sealed.

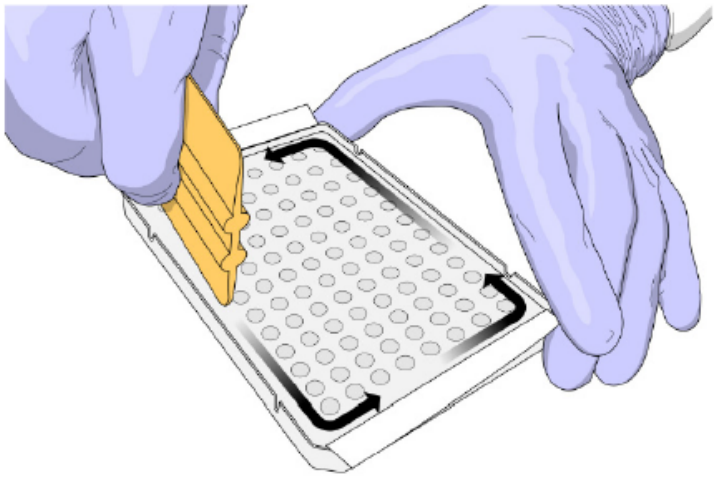
1. Place the adhesive film on the filter plate, then rub the flat edge of the applicator back and forth along the long edge of the filter plate.

2. Rub the flat edge of the applicator back and forth along the short edge of the filter plate.



3. Rub the end of the applicator horizontally and vertically between all wells (not shown).

4. Rub the end of the applicator around all outside edges of the filter plate using small back and forth motions to form a complete seal around the outside wells.



Remove substrate (spin/filter plates, 300 µL and 500 µL)

1. Transfer the sample lysate into the PrepFiler™ Spin Plate:
   a. Place the PrepFiler™ 96-Well Spin Plate and Filter Plate (together called the "plate assembly") in a deep-well centrifuge.

   b. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the plate assembly. Seal the deep-well plate, then place it in the deep-well centrifuge as a counterweight to ensure that the rotor is well balanced.

c. Centrifuge the plates at 650 × *g* for 1 minute. If all lysate does not pass into the spin plate after 1 minute, centrifuge for an additional 1 minute.

Note: The sample substrate remains in the filter plate.

2. Check the volume of sample lysate:

Protocol	Action
Standard protocol (300 µL)	If the volume is <180 µL, see "The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 300-µL protocols" on page 44. **IMPORTANT!** A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.
Large-sample protocol (500 µL)	If the volume is <300 µL, see "The volume of collected lysate is low: PrepFiler™ Automated Forensic DNA Extraction Kit, 500-µL protocols" on page 44. **IMPORTANT!** A 300-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

3. Use the MicroAmp™ Multi Removal Tool to separate the filter plate (top plate) from the spin plate (bottom plate). See "Guidelines for using the MicroAmp™ Multi Removal Tool" on page 60.

IMPORTANT! If the contents of the spin plate are shaken during plate separation, place an adhesive cover on the spin plate, place the spin plate in the deep-well centrifuge, then briefly centrifuge to collect the contents at the bottom of the wells.

4. Properly dispose of the filter plate that contains the sample substrate.

5. If pellets are visible in the spin plate, transfer the clear (no sediment) lysate to a new PrepFiler™ Spin Plate

IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

6. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample plate that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

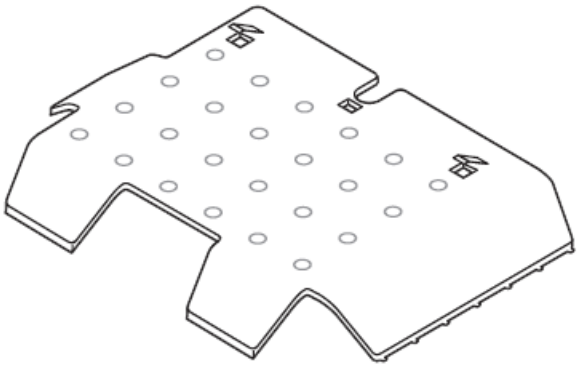
7. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

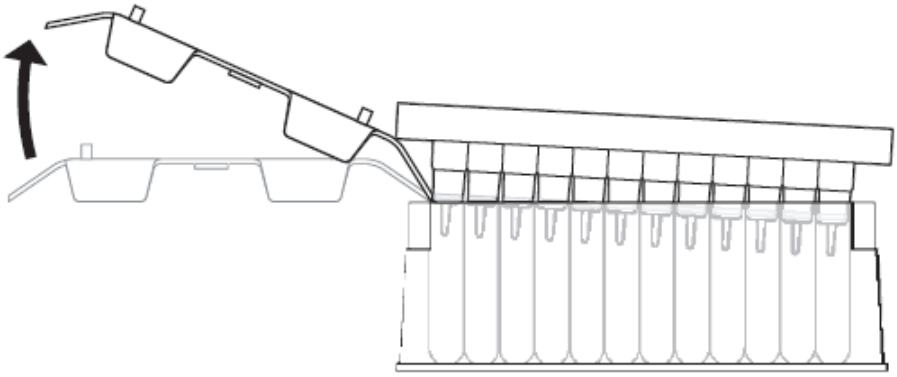
Guidelines for using the MicroAmp™ Multi Removal Tool

Before using the MicroAmp™ Multi Removal Tool for the first time, use the tool to practice separating an empty filter plate from the spin plate.

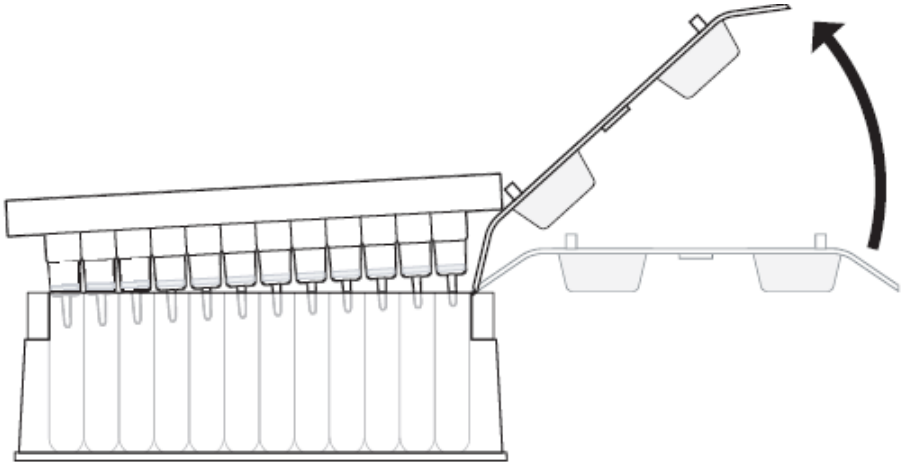
Note: When you use the tool on plate assemblies that have been processed through lysis, the seal may be tighter than the seal on the empty plates.



1. Insert the tool between the two plates, then gently lift up to partially separate the plates.



2. Repeat the process on the opposite side. Continue using the tool on opposite sides of the plate assembly until the filter plate can be easily lifted off the spin plate.



C

PrepFiler™ BTA Automated Forensic DNA Extraction Kit: Supplementary lysis protocols

Spin/filter plate protocol (230 µL)

Note: Do not use the spin/filter plate protocol for bone or tooth samples. Bone and tooth samples effervesce during lysis, and therefore require the 2-mL screw-cap tubes to prevent the tubes from popping open during the incubation step.

IMPORTANT! The spin/filter plate protocol was not optimized or tested as part of our validation studies for the PrepFiler™ BTA Automated Forensic DNA Extraction Kit.

Required materials not supplied (spin/filter plates)

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Table 17 Required materials for the standard (230-µL) protocol

Item	Source[1]
DL-dithiothreitol [DTT; molecular biology grade; ≥98% (TLC), ≥99% (titration)]	MLS
General purpose thermal shaker or shaking incubator that is compatible with deep-well plates, capable of temperatures ≤70°C and speeds ≤150 rpm	MLS
Vortexer, variable-speed	MLS
Benchtop centrifuge with rotor for 96-deep-well plates	MLS
Deep-well plate (for balancing the centrifuge rotor during centrifugation)	MLS
PrepFiler™ 96-Well Spin Plates and Filter Plates, ethylene oxide-treated (10 sets)	4476031
MicroAmp™ Multi Removal Tool	4313950

Table 17 Required materials for the standard (230-µL) protocol *(continued)*

Item	Source[1]
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Adhesive Film Applicator	4333183

[1] Recommended sources. Equivalent materials from other suppliers can be used after appropriate validation studies by the user laboratory.

Guidelines for handling spin/filter plates

Avoid cross-contamination by observing the following guidelines.

- Change gloves frequently when handling the spin/filter plates. For example, change gloves after separating the filter plate from the spin plate.
- To avoid leaks, ensure that the spin/filter plate unit is tightly assembled before shaking and incubation.

  IMPORTANT! Leaking plates may result in DNA cross-contamination.

- Use caution when separating the filter plate (top of the unit) from the spin plate (bottom of the unit) with the MicroAmp™ Multi Removal Tool. If the contents of the spin plate are shaken during plate separation, place an adhesive cover on the spin plate, place the spin plate in the deep-well centrifuge, then briefly centrifuge to collect the contents at the bottom of the wells.
- Use caution when placing each sample in a separate well in the filter plate (top of the unit). Consider covering unfilled wells to prevent depositing two samples in the same well.
- Cover and uncover all plates carefully.

Perform lysis (spin/filter plates, 230 µL)

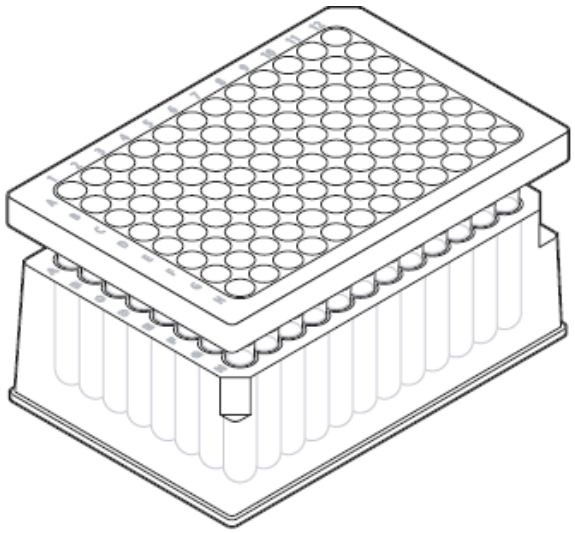
1. Bring the thermal shaker temperature to 56°C.

   Note: For envelope flap samples, studies indicate that lysis at room temperature (instead of 56°C) may result in a higher quantity and quality of extracted DNA. Our validation studies were performed using a lysis temperature of 56°C. Your laboratory should perform appropriate internal validation studies.

2. Label a PrepFiler™ Spin Plate.

   Note: If you use barcodes to track samples, position the barcode on the plate according to your robotic workstation documentation.

3. Tightly assemble the PrepFiler™ 96-Well Spin Plate and Filter Plate (together called the "plate assembly").
   a. Ensure that the filter plate is placed firmly on top of the spin plate, as shown in the following figure.



   b. Weigh the plate assembly, then place it in a deep-well centrifuge.

   c. Create a counterweight to ensure that the centrifuge rotor is well-balanced:
      1. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the plate assembly.
      2. Seal the deep-well plate, then place it in the deep-well centrifuge as a counterweight.

   d. Spin the plates at 650 × *g* for 2 minutes.

4. Prepare a fresh lysis buffer solution immediately before each assay.
   a. Calculate the volume required based on the number of reactions. Include up to 5% excess volume to compensate for pipetting losses.

Component[1]	Volume[2]	
	1 reaction	96 reactions
PrepFiler™ BTA Lysis Buffer	220 µL	22 mL
DTT, 1.0 M	3 µL	300 µL
Proteinase K	7 µL	700 µL

[1] Prepare the lysis buffer and DTT before preparing the lysis buffer solution. See “Before each use: Prepare the lysis reagents” on page 18.

[2] Includes ~4% excess volume to compensate for pipetting losses.

   b. Combine the required volumes of components, then gently mix.

Note: After completing the lysis step, discard unused lysis solution and DTT.

5. Using the sample prepared in "Prepare samples for lysis: All other sample types" on page 15, place each sample in a separate well in the filter plate (top plate).

Note: If you are using <96 samples, see your robotic workstation documentation for plate layouts.

Sample Type	Transfer
Chewing gum	Cut and transfer ≤50 mg of gum (~3 mm^2) into a well of the filter plate.
Cigarette butt	Transfer all pieces into a well of the filter plate.
Tape lift	Transfer the tape into a well of the filter plate. **IMPORTANT!** Ensure that the side of the tape that contains the sample does not adhere to the well.
Swab	Transfer into a well of the filter plate.

6. Add 230 µL of the lysis buffer solution to each sample well.

Note: You may observe that some lysis buffer drips through to the filter plate during lysis. Lysis is still occurring.

7. Immediately seal the plate assembly with a new sheet of MicroAmp™ Clear Adhesive Film and an applicator. See "Guidelines for sealing the plate assembly" on page 57.

8. Place the plate assembly in the thermal shaker. *As a starting point,* incubate the samples at 150 rpm for 60 minutes at 56°C.

Note: Longer incubation times may be required because of less efficient heat transfer with spin/filter plates. Your laboratory should perform appropriate internal validation studies to optimize the shaking speed and time.

IMPORTANT! Keep the plate assembly horizontal during transport to the thermal shaker and throughout incubation to prevent possible cross-contamination.

Proceed to "Remove substrate (spin/filter plates, 230 µL)" on page 64.

IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate.

Remove substrate (spin/filter plates, 230 µL)

1. Transfer the sample lysate into the PrepFiler™ Spin Plate:
   a. Place the PrepFiler™ 96-Well Spin Plate and Filter Plate (together called the "plate assembly") in a deep-well centrifuge.
   b. Fill a deep-well plate with water so that the weight of the deep-well plate is equal to the weight of the plate assembly. Seal the deep-well plate, then place it in the deep-well centrifuge as a counterweight to ensure that the rotor is well balanced.

c. Centrifuge the plates at 650 × *g* for 1 minute. If all lysate does not pass into the spin plate after 1 minute, centrifuge for an additional 1 minute.

Note: The sample substrate remains in the filter plate.

2. Check the volume of sample lysate. If the volume is <180 µL, see "The volume of collected lysate is low: PrepFiler™ BTA Automated Forensic DNA Extraction Kit, 230-µL protocols" on page 45.

IMPORTANT! A 180-µL lysate volume is required for effective binding of DNA to the magnetic particles, proper mixing, and to prevent formation of air bubbles in the tip during the automated purification run.

3. Use the MicroAmp™ Multi Removal Tool to separate the filter plate (top plate) from the spin plate (bottom plate). See "Guidelines for using the MicroAmp™ Multi Removal Tool" on page 60.

IMPORTANT! If the contents of the spin plate are shaken during plate separation, place an adhesive cover on the spin plate, place the spin plate in the deep-well centrifuge, then briefly centrifuge to collect the contents at the bottom of the wells.

4. Properly dispose of the filter plate that contains the sample substrate.

5. If pellets are visible in the spin plate, transfer the clear (no sediment) lysate to a new PrepFiler™ Spin Plate

IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated purification run.

6. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the lysate. Do not load any sample plate that contains precipitate on your robotic workstation. Precipitate can cause the instrument to crash, tips to clog, or filters to become wet.

7. Equilibrate the lysate to room temperature, then proceed directly to Chapter 6, "Prepare for the automated purification run".

IMPORTANT! To avoid precipitation of the lysis buffer components, do not chill the sample lysate after performing lysis.

Procedural guidelines

Guidelines for reagent preparation

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves frequently to prevent contamination of reagents. For example, change gloves after opening tubes that contain DNA standards or control DNA to avoid contaminating other kit reagents.
- Label each tube and vial cap to ensure that the caps are replaced on the correct tube or vial.
- Before opening, briefly centrifuge each reagent tube or vial to remove liquid from the cap and sides of the tube. To avoid forming air bubbles, do not vortex reagent tubes or vials unless directed to do so.
- When uncapping tubes and vials, place the caps in a clean area to minimize the potential for contamination.
- If air bubbles form on the surface of the reagents in the tubes or vials, remove the air bubbles by pipetting. Surface bubbles may interfere with liquid-level detection during the automated purification run.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Laboratory practices to minimize DNA contamination

DNA extraction and PCR assays require special laboratory practices to avoid cross-contamination. The high sensitivity of these assays may result in the amplification of a single DNA molecule.

To minimize DNA contamination because of amplifiable material in your work area, follow these recommended laboratory practices:

- When possible, maintain separate work areas and dedicated equipment and supplies for:
  - Sample preparation
  - DNA extraction
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Prepare kit reagents in a clean, dedicated area free of amplified PCR products.
- During sample preparation and extraction, separate samples containing high quantities of DNA from those containing low quantities of DNA.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or during sample preparation) when performing DNA extractions and preparing samples for PCR amplification.
- Change gloves whenever you suspect they are contaminated and before leaving the work area.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Never bring amplified PCR products into the DNA extraction or PCR setup areas.
- Try not to splash or spray PCR samples.
- When pipetting from a kit component tube, hold the cap of the tube in your gloved hand, or be sure to set it down on a clean, decontaminated surface.
- Keep reactions and components sealed when possible.
- Clean lab benches and equipment periodically with deionized water, then wipe with a lint-free lab wipe dampened with laboratory-grade 70% ethanol. Do not use acids, or bases (such as bleach) to clean the worktable.

  Consult safety data sheets (SDS) and product labeling of cleaning agents and reagents or chemicals used on the instrument for compatibility before cleaning or decontaminating the instrument.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits User Guide	4463349
PrepFiler™ and PrepFiler™ BTA Forensic DNA Extraction Kits User Guide	4463348
PrepFiler™ Automated Forensic DNA Extraction Kit: Automated DNA Purification on the HID EVOlution™ Systems User Bulletin	MAN0019298
PrepFiler™ and PrepFiler™ BTA Automated Forensic DNA Extraction Kits: Automated DNA Purification on the ID NIMBUS® Presto Workstation User Bulletin	MAN0019368

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.