

PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits



Note: For safety and biohazard guidelines, refer to the “Safety” section in the *PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide* (PN 4442699). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card covers:

- Prepare sample lysate using the PrepFiler Express™ Forensic DNA Extraction Kit. 1
- Prepare sample lysate using the PrepFiler Express BTA™ Forensic DNA Extraction Kit 2
- Set up and run automated DNA extraction 4
- Perform maintenance 6

Prepare sample lysate using the PrepFiler Express™ Forensic DNA Extraction Kit

1 Prepare sample

Sample type	Example sample input†
Liquid samples (blood, saliva)	Up to 40 µL
Blood (on FTA paper or fabric)	Up to 25-mm ² cutting or punch
Body fluids (saliva, semen) on fabric	Up to 25-mm ² cutting or punch
Body fluids on swabs (buccal and other body fluids)	Up to one swab
Hair root	Up to 5 mm cutting from root

† It is not necessary to use an entire sample punch or swab.

2 Perform lysis

- a. If the PrepFiler™ Lysis Buffer contains precipitate, heat the solution to 37 °C, then vortex the bottle for 5 seconds.
- b. Bring the thermal shaker temperature to 70 °C.
- c. Prepare a fresh PrepFiler™ lysis solution. Each sample requires:
 - 500 µL PrepFiler™ Lysis Buffer
 - 5 µL freshly-prepared 1 M DTT



WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

- d. Insert a PrepFiler LySep™ Column into a hingeless PrepFiler™ sample tube (together called the “column/tube assembly”), then carefully transfer the sample into the PrepFiler LySep™ Column.
- e. Add 500 µL of freshly-prepared PrepFiler lysis solution to the column/tube assembly.

IMPORTANT! For effective DNA recovery, make sure that the entire sample is submerged in the lysis solution.
- f. Tightly close the lid of the column/tube assembly.
- g. Place the column/tube assembly in a thermal shaker, then incubate it at 70 °C and 750 rpm for 40 minutes.



Note: Exceeding the recommended 40 minute incubation time may result in salt precipitation from the lysis buffer before or after centrifugation, potentially leading to instrument crash, tip clogging, or tip filter wetting. If precipitation occurs or the incubation time exceeds 40 minutes, refer to “Troubleshooting” in the *PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide*.

3 Remove the substrate from the sample lysate

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

 **IMPORTANT!** Refer to the *PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide* if the volume of sample lysate collected in the sample tube is less than 300 µL. Lower lysate volume may cause liquid handling problems.

b. Complete substrate removal as follows:

1. Carefully remove the PrepFiler LySep™ column from the sample tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube.
2. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially biohazardous.
3. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Sample Tube.

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

4. 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 sample lysate. Do not load any sample tube that contains precipitate on the AutoMate Express™ Instrument, or instrument crash, tip clogging, or filter wetting may occur.

4 Proceed directly to the automated extraction run

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

Prepare sample lysate using the PrepFiler Express BTA™ Forensic DNA Extraction Kit

1 Prepare sample

Example Sample Input	
Adhesive Substrates Protocol	Bone and Tooth Protocol
• Chewing gum – Up to 50 mg (approximately 3×3×5-mm³ piece) • Cigarette butt – Up to 25-mm² cutting of cigarette filter paper •  IMPORTANT! Remove all filter material from the filter paper. • Tape lifts – Up to 2 cm² cutting with saliva or blood	• Bone – Up to 50 mg powdered bone • Tooth – Up to 50 mg powdered tooth

2 Prepare for lysis

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

b. Prepare a fresh PrepFiler BTA™ lysis solution. Each sample requires:

- 220 µL PrepFiler BTA™ Lysis Buffer
- 3 µL freshly-prepared 1 M DTT
- 7 µL Proteinase K

 **WARNING!** Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.

3 Perform lysis

Adhesive substrates protocol	Bone and tooth protocol
1. Insert a PrepFiler LySep™ Column into a hingeless PrepFiler™ sample tube (together called the “column/tube assembly”), then carefully transfer the sample into the PrepFiler LySep™ Column. 2. Add 230 µL of freshly-prepared PrepFiler BTA lysis solution to the column/tube assembly.  IMPORTANT! For effective DNA recovery, make sure that the entire sample is submerged in the lysis solution. 3. Tightly close the lid of the column/tube assembly. 4. Place the column/tube assembly in a thermal shaker, then incubate it at 56 °C and 750 rpm for 40 minutes.	1. Place the sample in a PrepFiler™ Bone and Tooth Lysate Tube. 2. Add 230 µL of freshly-prepared PrepFiler BTA lysis solution to the PrepFiler™ Bone and Tooth Lysate Tube containing sample. 3. Screw the cap on the PrepFiler Bone and Tooth Lysate Tube, vortex it for 5 seconds, then centrifuge it briefly.  Note: To avoid leaks, make sure that tubes are tightly sealed before vortexing and incubating the tubes. To avoid forming a pellet, do not centrifuge longer than 5 seconds. After vortexing a tube, check the tube for air bubbles, then re-vortex if necessary to remove bubbles. 4. Place the PrepFiler Bone and Tooth Lysate Tube in a thermal shaker, then incubate it at 56 °C and 1100 rpm for at least 2 hours (sample can be incubated up to 18 hours).

4 Remove the substrate from the sample lysate

Adhesive substrates protocol	Bone and tooth protocol
1. Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube.  IMPORTANT! Refer to the <i>PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide</i> if the volume of sample lysate collected in the sample tube is less than 150 µL. Lower lysate volume may cause liquid handling problems. 2. Complete substrate removal as follows: a. Carefully remove the PrepFiler LySep™ column from the sample tube. If there is clear lysate remaining in the PrepFiler LySep column, transfer the lysate to the sample tube. b. Properly dispose of the PrepFiler LySep column. Used LySep columns are potentially biohazardous. c. If a pellet is visible in the sample tube, transfer the clear (no sediment) lysate to a new PrepFiler™ Sample Tube.  IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated extraction run.	1. Centrifuge the PrepFiler Bone and Tooth Lysate Tube for 90 seconds at 10,000 × g. 2. Transfer the clear (no sediment) lysate to a new PrepFiler™ Sample Tube.  IMPORTANT! Sediment in the lysate may cause liquid handling problems during the automated extraction run.  IMPORTANT! Refer to the <i>PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide</i> if the volume of sample lysate collected in the sample tube is less than 150 µL. Lower lysate volume may cause liquid handling problems.

-
- | | | | |
|---|--|---|---|
| 5 | Proceed directly to the automated extraction run | ! | IMPORTANT! To avoid precipitation of lysis buffer components, do not chill the sample lysate after performing lysis. |
|---|--|---|---|

Set up and run automated DNA extraction

-
- | | | | |
|---|---|--|--|
| 1 | Inspect the PrepFiler Express™ cartridges | | If precipitate forms in compartments 1 or 2 (lysis buffer and magnetic particle suspension), heat the cartridge in an incubator at 37 °C for 30 minutes or until the precipitate is no longer visible. |
|---|---|--|--|

-
- | | | | |
|---|--|---|--|
| 2 | Insert the protocol card and power on the instrument | ! | IMPORTANT! Do not remove the protocol card while the instrument is on. Removing the card stops the run, and it may cause instrument data file loss. If you accidentally remove the protocol card during a run, power off the instrument immediately to minimize potential for instrument data loss. |
|---|--|---|--|

- a. Confirm that the power switch is in the OFF position.



Note: If you insert the card while the instrument is on, the instrument does not recognize the card.

- b. Open the card slot.

- c. Insert the PrepFiler Express™ & PrepFiler Express BTA™ Protocol Card in the slot with the arrow pointing toward the instrument and the card label facing left.

- d. Push the card completely into the card slot, then close the card slot.

- e. Power on the instrument.

When the card is fully inserted in the correct orientation, the display briefly shows information including the instrument version, then displays the Main menu.

- f. Press **Start**.



Note: Press  after following each on-screen prompt.

-
- | | | | |
|---|------------------------------------|--|---|
| 3 | Load and insert the cartridge rack | | <p>a. Open the instrument door (push up the door), then remove the cartridge rack and tip and tube rack.</p> <p>b. Remove up to 13 cartridges from the kit box.</p> |
|---|------------------------------------|--|---|



Note: One cartridge is required per sample. Use only Applied Biosystems PrepFiler Express™ cartridges.

- c. Shake and tap the reagent cartridges to resuspend the magnetic particles and to deposit any particles or liquid droplets underneath the foil seal into the compartments.

- d. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Make sure that the notches in the cartridge align with the notches in the cartridge rack.



Note: An incorrectly loaded cartridge rack may cause the instrument to stop during a protocol run.

- e. Insert the loaded cartridge rack into the instrument.



WARNING! Do not touch the surface of the heat block. The temperature of the heat block can reach 95 °C. Touching the block can cause burns.

4 Load and insert the tip and tube rack

a. Load the tip and tube rack:



Note: If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack.

1. **Row S** (fourth row): Load with the PrepFiler™ sample tubes containing the lysate.



IMPORTANT! Before loading each sample tube in the rack, make sure that no salt precipitation is visible in the sample tube. Precipitate in sample tubes may cause instrument crash, tip clogging, or filter wetting. Refer to “Troubleshooting” in the *PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide* for suggestions on preventing and/or dissolving precipitated salts.



Note: Make sure that the PrepFiler LySep™ columns have been removed from the sample tubes.

2. **Row T2** (third row): Load with AutoMate Express™ Tips inserted into tip holders.



Note: One tip and tip holder set is required per sample.

3. **Row T1** (second row): Leave empty.

4. **Row E** (first row): Load with labeled PrepFiler™ elution tubes, with the caps open and secured.

b. Insert the loaded tip and tube rack into the instrument with row E in the front.

5 Start the automated extraction run

a. Confirm that you have inserted the cartridge rack and tip and tube rack correctly, then close the instrument door.

b. Press , then, if you are using the:

- **PrepFiler Express™ kit** – Press **1** to select the PF Express option.

or

- **PrepFiler Express BTA™ kit** – Press **2** to select the PF Express BTA option.



IMPORTANT! For correct operation, make sure that the option matches the kit you are using.

c. Press **Start**.

The screen shows the steps and the approximate run time remaining.



IMPORTANT! Do not open the door during a run.



Note: To pause the run, press **Stop**. To restart the run, press **Start**.

To cancel the run, Press **Stop** two times, press **1** to go to the Manual screen, then return the tips to the original position as described in the *AutoMate Express™ Instrument User Guide*.

If you lose power or the power cord is unplugged, the run stops. When the power resumes, the screen displays the Main menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original position as described in the *AutoMate Express™ Instrument User Guide*.

See the *AutoMate Express™ Instrument User Guide* if necessary to troubleshoot issues during the run.

- 6 Store the extracted DNA** At the end of the run (the instrument beeps briefly and the digital display shows “Finished Protocol”):
- Press  to return to the Main menu, then open the instrument door.
 - Remove the cartridge rack and tip and tube rack.
 - Remove and cap the elution tubes containing the purified DNA.



Note: The isolated DNA can be stored at 4 °C for up to two weeks, or at –20 °C for longer storage.

- Properly dispose of the used reagent cartridges, tips, and tubes.



WARNING! Do not add acids, or bases (such as bleach) to any wastes containing lysis buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the lysis buffer and generate toxic gas.



WARNING! The used reagent cartridges may contain the following: guanidine thiocyanate, isopropanol, and ethanol. Refer to Safety Data Sheets and local, state, and national regulations for proper labeling, handling, and disposal.

- Close the instrument door.



Note: No cooling period is required between runs.

To perform a new run using a different AutoMate Express™ Instrument protocol card, power off the instrument, then change the protocol card.

Perform maintenance

Refer to the *AutoMate Express™ Instrument User Guide* for details.

Schedule	Procedure
Daily	Clean the piercing unit
Daily or as needed	- Clean the platform surface (racks and bottom tray) - Clean the tip and tube rack - Clean the magnets
Every 2 weeks	Maintain the D-rings
Monthly	Perform axis and temperature tests
Annual	Replace the D-rings
	Planned maintenance

For Research, Forensic, or Paternity Use Only. Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE PREPFLER EXPRESS™ AND PREPFLER EXPRESS BTA™ FORENSIC DNA EXTRACTION KITS USER GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

© 2010 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

Part Number 4443104 Rev. B 06/2010

