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

Catalog Numbers 4441352 and 4441351

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *PrepFiler Express™ and PrepFiler Express™ BTA Forensic DNA Extraction Kits User Guide* (Pub. no. 4442699). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare sample lysate using the PrepFiler *Express*™ Forensic DNA Extraction Kit—Body fluid protocol

Sample types and inputs (body fluid)

Table 1 Example sample types and inputs for body fluid

Sample type	Example sample input[1]
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

[1] It is not necessary to use an entire sample punch or swab.

1 Perform lysis (body fluid)

a. If the 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 lysis solution. Each sample requires:
- 500 µL 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 LySep Column into a hingeless PrepFiler™ sample tube (together called the "column/tube assembly"), then carefully transfer the sample into the LySep column.

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1 Perform lysis (body fluid) *(continued)*

e. Add 500 µL of freshly prepared lysis solution to the column/tube assembly.

IMPORTANT! For effective DNA recovery, ensure that the entire sample is submerged in the lysis solution.

f. Tightly close the lid of the column/tube assembly.

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

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 can 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, see *PrepFiler Express™ and PrepFiler Express™ BTA Forensic DNA Extraction Kits User Guide* for suggestions for preventing and/or dissolving precipitated salts.

2 Remove the substrate from the sample lysis (body fluid)

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

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

b. Complete substrate removal as follows:

1. Carefully remove the LySep column from the sample tube. If there is clear lysate remaining in the LySep column, transfer the lysate to the sample tube.
2. Properly dispose of the 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. Precipitate can cause the instrument to crash, tips to clog, or filters becoming wet.

c. 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—Bone and tooth protocol

Sample types and inputs (bone and tooth)

Table 2 Example sample types and inputs for bone and tooth

Sample type	Example sample input
Bone	Up to 50 mg powdered bone
Tooth	Up to 50 mg powdered tooth

1 Perform lysis (bone and tooth)

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

b. Prepare a fresh lysis solution. Each sample requires:
- 220 µL 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.

c. Place the sample in a new PrepFiler™ Bone and Tooth Lysate Tube.

d. Add 230 µL of freshly prepared lysis solution to the Bone and Tooth Lysate Tube containing the bone or tooth sample.

e. Screw the cap on the Bone and Tooth Lysate Tube, vortex it for 5 seconds, then centrifuge it briefly.

Note: To avoid leaks, ensure 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 vortex again if needed to remove bubbles.

f. Place the Bone and Tooth Lysate Tube in a thermal shaker, then incubate it at 56°C and 1,100 rpm for at least 2 hours (sample can be incubated up to 18 hours).

2 Remove the substrate from the sample lysis (bone and tooth)

a. Centrifuge the Bone and Tooth Lysate Tube for 90 seconds at 10,000 × *g*.

b. Transfer the clear (no sediment) lysate to a new PrepFiler™ Sample Tube.

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

IMPORTANT! If the volume of sample lysate that is collected in the sample tube is less than 200 µL, see the *PrepFiler Express™ and PrepFiler Express™ BTA Forensic DNA Extraction Kits User Guide*. Lower lysate volume may cause liquid handling problems.

c. 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—Adhesive protocol

Sample types and inputs (adhesive substrate)

Table 3 Example sample types and inputs for adhesive substrates

Sample type	Example sample input
Chewing gum	Up to 50 mg (approximately 3×3×5-mm^3 piece)
Cigarette butt	Up to 25-mm^2 cutting of cigarette filter paper **IMPORTANT!** Remove all filter material from the filter paper.
Tape lifts	Up to 2 cm^2 cutting with saliva or blood

1 Perform lysis (adhesive substrate)

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

b. Prepare a fresh lysis solution. Each sample requires:
- 220 µL 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.

c. Insert a LySep Column into a hingeless PrepFiler™ sample tube (together called the "column/tube assembly"), then carefully transfer the sample into the LySep column.

d. Add 230 µL of freshly prepared lysis solution to the column/tube assembly.

IMPORTANT! For effective DNA recovery, ensure that the entire sample is submerged in the lysis solution.

e. Tightly close the lid of the column/tube assembly.

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

f. Place the column/tube assembly in a thermal shaker, then incubate it at 56°C and 750 rpm for 40 minutes.

2 Remove the substrate from the sample lysis (adhesive substrate)

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

IMPORTANT! If the volume of sample lysate that is collected in the sample tube is less than 200 µL, see the *PrepFiler Express™ and PrepFiler Express™ BTA Forensic DNA Extraction Kits User Guide*. Lower lysate volume may cause liquid handling problems.

b. Complete substrate removal as follows:

1. Carefully remove the LySep column from the sample tube. If there is clear lysate remaining in the LySep column, transfer the lysate to the sample tube.
2. Properly dispose of the 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.

2	Remove the substrate from the sample lysis (adhesive substrate) *(continued)*	c. 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

Workflow: Set up Automate Express

Set up and run automated DNA extraction

▼

Inspect the cartridges

▼

Insert a protocol card

▼

Load and insert the cartridge rack

▼

Load and insert the tip and tube rack

▼

Start an automated extraction run

▼

Store the extracted DNA

1	Inspect cartridges	Inspect the reagent 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. Heat only those cartridges that you plan to use that day.
2	Insert a protocol card	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 protocol card in the slot with the arrow pointing toward the instrument and the label facing left. d. Push the card completely into the card slot, then close the card slot. e. Power on the instrument.
3	Load and insert the cartridge rack	a. Open the instrument door (push up the door), then remove the tip and tube rack and the cartridge rack. b. Remove up to 13 cartridges from the kit box. **Note:** One cartridge is required per sample. Use only Thermo Fisher Scientific PrepFiler *Express*™ reagent 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.

3 Load and insert the cartridge rack *(continued)*

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. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

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

Note: Press ↵ after following each on-screen prompt.

a. Load the tip and tube rack in the following order:

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 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. See *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 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 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 an automated extraction run

a. Ensure that you have loaded and 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.
- 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 protocol run. To pause or cancel the run, see the *AutoMate Express™ Instrument User Guide*.

Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the digital display shows 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 positions 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"):

a. Press ↵ to return to the **Main** menu, then open the instrument door.

b. Remove the cartridge rack and tip and tube rack.

6 Store the extracted DNA *(continued)*

c. 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.

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

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.

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.

e. Close the instrument door. After each run, clean the tip and tube rack as needed.

Note: No cooling period is required between runs.

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

Maintenance schedule

See 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
Annually	Replace the D-rings
	Planned maintenance

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Revision history: Pub. No. 4443104

Revision	Date	Description
C	07 March 2017	Change minimum volume for PrepFiler *Express* BTA™ kit after bone-tooth or adhesive substrate removal from 150 µL to 200 µL.
B	June 2010	Baseline for this revision history.

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