QUICK REFERENCE

applied biosystems by life technologies™

PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide* (Part no. 4463348). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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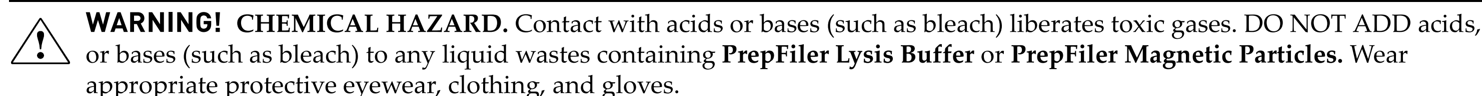
The PrepFiler® Forensic DNA Extraction Kit is designed for common forensic sample types, including body fluid stains and swabs of body fluids. ThePrepFiler® BTA Forensic DNA Extraction Kit is designed for challenging forensic sample types such as bone, tooth, and adhesive-containing substrates including cigarette butts, chewing gum, envelope flaps, and tape lifts. This Quick Reference provides a brief summary of the protocols for using these kits.

IMPORTANT! To select the correct protocol for your sample type, refer to the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide* (Part no. 4463348).

Prepare reagents



WARNING! CHEMICAL HAZARD. Contact with acids or bases (such as bleach) liberates toxic gases. DO NOT ADD acids, or bases (such as bleach) to any liquid wastes containing **PrepFiler Lysis Buffer** or **PrepFiler Magnetic Particles.** Wear appropriate protective eyewear, clothing, and gloves.

1. Before each use, incubate the Magnetic Particles at 37°C for 10 minutes, then vortex at medium speed until the particles are completely resuspended. Recap the tube immediately after each use.
2. If the PrepFiler® Lysis Buffer contains precipitate, heat to 37°C for 15 minutes, then vortex for 5 seconds.
3. Make sure you have a sufficient number of bottles of Wash Buffer A and B for your assay. If needed, fill fresh bottle(s) to the shoulder with freshly-opened 95% ethanol (93 mL for Wash Buffer A and 19.5 mL for Wash Buffer B) to prepare 1✕ solutions.
4. Thaw or prepare a fresh 1.0 M solution of DL-dithiothreitol (DTT) in molecular-biology grade DNA-free water.

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PrepFiler® Forensic DNA Extraction Kit protocol

1 Determine sample size or input amount

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

† To select the correct protocol and kit for your sample type, refer to the supplementary protocols in the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide*.

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

2 Perform lysis

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

b. Place a sample in a PrepFiler® Spin Tube or standard 1.5-mL microcentrifuge tube.

c. To the tube that contains the sample, add:
- **PrepFiler® Lysis Buffer**: 300 µL
- **DTT, 1.0 M**: 3 µL (use 5 µL for samples containing semen)

If the fluid does not cover the substrate, refer to the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide*.

Discard unused DTT after completing lysis.

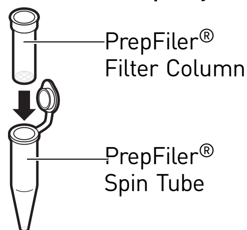
Note: You can pre-mix the lysis buffer and DTT (1.0 M) for all samples, then add 300 µL of the mixture to each tube. Prepare a fresh lysis buffer-DTT mixture for each experiment.

d. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.

e. Place the tube in a thermal shaker (or heat block), then incubate it at 70°C and 900 rpm for the time shown. Do not chill the sample.

Lysis times at 70°C incubation temperature	
Sample Type	**Lysis Time (Minutes)**
Liquid body fluids	20 minutes
Dried stains or samples on swabs	40 minutes
Neat semen samples	90 minutes

3 Remove substrate from sample lysate



If sample substrate is present perform the following steps. If no substrate is present, proceed to step 4.

a. Centrifuge the sample tube for 2 seconds.

b. Insert a PrepFiler® Filter Column into a new 1.5- mL PrepFiler® Spin Tube, then carefully transfer the sample tube contents into the filter column, using a pipette to transfer the liquid and the pipette tip or sterile tweezers to transfer the substrate.

c. Cap the filter column/spin tube, then centrifuge at maximum speed (2 minutes at 12,000-14,000rpm; 5 minutes at 3,000–4,000rpm).

d. If less than 180 µL sample lysate is collected in the spin tube, refer to the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide*.

e. Remove the filter column from the spin tube and dispose of it properly.

f. *(Optional)* If the spin tube cap does not securely close, transfer the lysate into a new, labeled 1.5-mL microcentrifuge tube (or keep the lysate in the spin tube during the remaining steps).

4 Bind genomic DNA to magnetic particles

a. Allow the sample lysate to come to room temperature (approximately 5 minutes). Do not chill it.

b. Vortex the PrepFiler® Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly. For multiple samples, vortex the magnetic particles tube every 5 minutes during the next step.

c. Pipet 15 µL of magnetic particles into the tube containing the sample lysate.

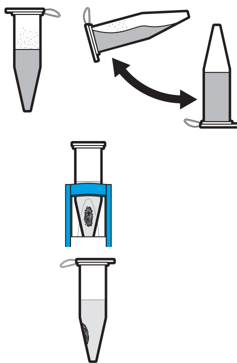
d. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 – 1200 rpm) for 10 seconds, then centrifuge it briefly.

e. After centrifuging, add 180 µL of isopropanol to the sample lysate tube.

f. Cap the sample lysate tube, vortex it at *low* speed (approximately 500 – 1200 rpm) for 5 seconds, then centrifuge it briefly.

g. Put the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1000 rpm for 10 minutes.

5 Wash bound DNA



After binding the DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors.

a. Vortex the sample DNA tube:

1. If magnetic particles are present on the sides of the sample DNA tube above the meniscus, invert the tube to resuspend the particles.
2. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) for 10 seconds, then centrifuge briefly.

   Note: It is acceptable to have magnetic particle aggregates suspended in the solution or on the side of the tube below the meniscus.

b. Place the sample DNA tube in the magnetic stand and wait until the size of the pellet of magnetic particles on the back of the tube stops increasing (approximately 1 – 2 minutes).

c. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard *all* visible liquid phase. Do not aspirate magnetic particles or disturb the magnetic particle pellet. You can use a size P200 or P1000 pipettor to remove most of the liquid, then use a size P20 pipettor to remove the remaining liquid.

d. Perform wash steps 1 through 5 *three* times:

1. Add prepared wash buffer to the sample DNA tubes:
   - First wash: 600 µL Wash Buffer **A**
   - Second wash: 300 µL Wash Buffer **A**
   - Third wash: 300 µL Wash Buffer **B**
2. Cap the sample DNA tube and remove the tube from the magnetic stand.
3. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge briefly.

   Note: It is acceptable to have magnetic particle aggregates suspended in the solution.
4. Place the sample DNA tube in the magnetic stand for 30 – 60 seconds.
5. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard *all* visible liquid phase. Do not aspirate magnetic particles or disturb the magnetic particle pellet.

e. With the sample DNA tube remaining in the magnetic stand, open the tube, then allow the magnetic particles-bound DNA to air-dry for 7–10 minutes (air-drying for more than 10 minutes may reduce DNA yield). If the room temperature is >25°C, reduce drying time to 5 minutes.

6 Elute the DNA

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

b. Add 50 µL of PrepFiler® Elution Buffer to the sample DNA tube. Do not use water. You can use low-TE buffer.

c. Cap the sample DNA tube, vortex it at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge it briefly.

d. Place the sample DNA tube in a thermal shaker (or heat block), then incubate at 70°C and 900 rpm for 5 minutes. If you use a heat block, briefly vortex and centrifuge the tube every 2 – 3 minutes.

e. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 2 seconds), then centrifuge briefly.

f. Place the sample DNA tube in the magnetic stand, then wait until the size of the pellet at the side of the tube stops increasing (at least 1 minute).

g. Pipet the liquid in the sample DNA tube (which contains the isolated genomic DNA) to a new spin tube or 1.5-mL microcentrifuge tube for storage. Do not aspirate magnetic particles or disturb the magnetic particle pellet.

h. If the eluted DNA extract is turbid, centrifuge the tube for 5 – 7 minutes at maximum speed (approximately 10,000 rpm), then transfer the clear supernatant to a new 1.5-mL microcentrifuge tube.

The isolated DNA can be stored at 4°C for up to one week, or at –20°C for longer storage.

PrepFiler® BTA Forensic DNA Extraction Kit protocol

1 Determine sample size or input amount

Sample type	Example sample input
Bone	Up to 50 mg powdered bone
Tooth	Up to 50 mg powdered tooth
Envelope flap	Up to 1×1.5-cm cutting
Chewing gum	Up to 50 mg (approximately 3×3×5-mm 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 5-cm^2 cutting with saliva or blood

2 Perform lysis

a. Bring the thermal shaker to 56°C.

b. Place each sample in a tube:

Sample type	Tube
Bone, tooth, or tape	2.0-mL screw-cap tube
All other PrepFiler® BTA Forensic DNA Extraction Kit sample types	1.5-mL microcentrifuge tube

c. Prepare a fresh lysis buffer solution immediately before each experiment, including up to 5% excess for pipetting loss. Combine the components and mix gently.

Lysis buffer solution component	Volume	
	One reaction	96 reactions
PrepFiler® BTA Lysis Buffer	220 µL	22 mL
1.0 M DTT	3 µL	300 µL
Proteinase K	7 µL	700 µL

Discard unused DTT after completing lysis.

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

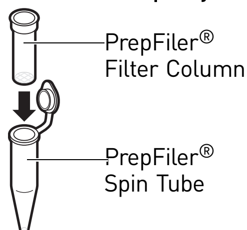
e. Cap the tubes, vortex for 5 seconds, centrifuge briefly, then gently flick to resuspend any powder or substrate.

f. Make sure the tubes are well sealed, then place them in a thermal shaker and incubate:

Sample type	Incubation
Bone or tooth	1100 rpm and 56°C for 2 hours
All other PrepFiler® BTA Forensic DNA Extraction Kit sample types	900 rpm and 56°C for 40 minutes

Do not chill the sample lysate.

3 Remove substrate from sample lysate



Bone, tooth, and chewing gum samples

a. Label up to 96 new 1.5-mL microcentrifuge tubes.
b. Allow the sample to equilibrate to room temperature.
c. Centrifuge the sample tubes at 10,000 × g for 90 seconds, then carefully transfer the clear lysate to a new, labeled 1.5-mL microcentrifuge tube. Do not disturb the sediment.
d. If needed, add PrepFiler® BTA Lysis Buffer to bring the total sample lysate volume to 180 µL.

All other sample types

a. Label up to 96 new 1.5-mL microcentrifuge tubes.
b. Allow the sample to equilibrate to room temperature.
c. Centrifuge the sample tubes for 2 seconds to collect any residual tube contents from the sides and cap.
d. Insert a PrepFiler® Filter Column into a new 1.5- mL PrepFiler® Spin Tube, then carefully transfer the sample tube contents into the filter column, using a pipette to transfer the liquid and the pipette tip or sterile tweezers to transfer the substrate.
e. Cap the filter column/spin tube, then centrifuge for 90 seconds at 8000 rpm.
f. Remove the filter column from the spin tube, then properly dispose of the filter column.
g. If needed, add PrepFiler® BTA Lysis Buffer to bring the total sample lysate volume to 180 µL.
h. Transfer the lysate to a new, labeled, 1.5 mL microcentrifuge tube.

4 Supplement lysate volume with PrepFiler® Lysis Buffer

Add 300 µL of PrepFiler® Lysis Buffer to the sample lysate tube, vortex it briefly to mix, then centrifuge it briefly. Make sure to add Lysis Buffer in this step, *not* BTA Lysis Buffer

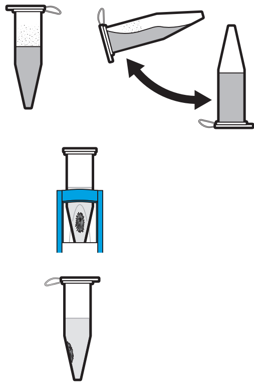
Note: The unprocessed lysate is stable for up to 24 hours at room temperature (20°C) in a sealed tube. Do not chill the sample lysate.

5 Bind genomic DNA to magnetic particles

a. Make sure the sample has equilibrated to room temperature.
b. Vortex the PrepFiler® Magnetic Particles tube for approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge it briefly.

   Note: If you are pipetting multiple samples, vortex the magnetic particles tube every 5 minutes until you complete the next step.
c. Pipet 15 µL of thoroughly resuspended magnetic particles into the sample lysate tube.
d. After adding the particles, recap the PrepFiler® Magnetic Particles tube to prevent evaporation.
e. Cap the sample lysate tube, vortex it at *low* speed (approximately 500–1,200 rpm) for 10 seconds, then centrifuge it briefly to collect any residual tube contents from the sides and cap of the tube.
f. After centrifuging, add isopropanol and mix one sample at a time to promote binding:
   1. Add 300 µL isopropanol (99.5% molecular biology grade) to a sample lysate tube.
   2. Immediately after adding isopropanol, cap the sample lysate tube, vortex it at *low* speed (approximately 500–1,200 rpm) for 5 seconds, then centrifuge it briefly to collect any residual tube contents from the sides and cap of the tube.
g. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at 700 rpm at room temperature for 10 minutes.

6 Wash bound DNA



a. Vortex the sample DNA tube:
   1. If magnetic particles are present on the sides of the sample DNA tube above the meniscus, invert the tube to resuspend the particles.
   2. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) for 10 seconds, then centrifuge briefly to collect any residual tube contents from the sides and cap of the tube.

      Note: It is acceptable to have magnetic particle aggregates suspended in the solution or on the side of the tube below the meniscus.

b. Place the sample DNA tube in the magnetic stand and observe that the magnetic particles form a pellet against the back of the tube. Wait until the size of the pellet stops increasing (approximately 10 minutes).

   Note: Samples containing high levels of proteins or other impurities may require more time.

   Note: For some sample types, the solution may remain colored after the magnetic particles are separated.

c. With the sample DNA tube remaining in the magnetic stand, carefully aspirate and discard *all* visible liquid phase. Do not aspirate or disturb the magnetic particle pellet.

d. Perform steps 1 through 5 *three* times:
   1. Add prepared wash buffer to the sample DNA tubes:
      - First wash: 600 µL Wash Buffer **A**
      - Second wash: 300 µL Wash Buffer **A**
      - Third wash: 300 µL Wash Buffer **B**
   2. Cap the sample DNA tubes and remove them from the magnetic stand.
   3. Vortex the sample DNA tubes for 15 seconds, then centrifuge briefly to collect any residual tube contents from the sides and cap.
   4. Place the sample DNA tubes in the magnetic stand for 1 minute.
   5. With the sample DNA tubes remaining in the magnetic stand, carefully aspirate and discard *all* visible liquid phase. Do not aspirate or disturb the magnetic particle pellet.

e. Centrifuge the tubes briefly, place the tubes back on the magnetic stand for 30-60 seconds, then collect any residual liquid using a medium- or low-volume pipettor (200-µL or 20-µL tip).

f. If residual liquid is still present, with the sample DNA tubes remaining in the magnetic stand, open the tubes, then allow the magnetic particles-bound DNA to air-dry for up to 5 minutes. Air-drying for more than 10 minutes may reduce DNA yield.

7 Elute the DNA

a. Bring the thermal shaker to 70°C.

b. Add 50 µL of PrepFiler® Elution Buffer to the sample DNA tube, then vortex at maximum speed until the pellet is resuspended.

c. Place the sample DNA tube in a thermal shaker (or heat block), then incubate at 70°C and 900 rpm for 10 minutes. If you use a heat block, briefly vortex and centrifuge the tube every 2–3 minutes.

d. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 2 seconds), then centrifuge briefly to collect any residual tube contents from the sides and cap.

e. Place the sample DNA tube in the magnetic stand, then wait until the size of the pellet at the side of the tube stops increasing (approximately 5 minutes).

f. Carefully aspirate *all* visible liquid phase in the sample DNA tube (this is the isolated genomic DNA). Do not aspirate or disturb the magnetic particle pellet. Transfer the eluate to a new, labeled 1.5-mL microcentrifuge tube for storage.

The isolated DNA can be stored at 4°C for up to one week, or at –20°C for longer storage.

Kit contents

The kits contain materials sufficient to perform 100 extractions using the standard protocols. Plastics are sold separately. For other required materials, refer to the *PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits User Guide*.

Materials provided with the PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits			
Reagent	**Description**		**Storage conditions**
	PrepFiler® Forensic DNA Extraction Kit (Part no. 4463351)	**PrepFiler® BTA Forensic DNA Extraction Kit (Part no. 4463352)**	
PrepFiler® Lysis Buffer	One 50-mL bottle	One 35-mL bottle	Store all kit components at room temperature.
PrepFiler® BTA Lysis Buffer	–	One 25-mL bottle	
PrepFiler® Magnetic Particles	One 1.5-mL tube	One 1.5-mL tube	
PrepFiler® Wash Buffer A Concentrate	Two 125-mL bottles	Two 125-mL bottles	
PrepFiler® Wash Buffer B Concentrate	Two 30-mL bottles	Two 30-mL bottles	
PrepFiler® Elution Buffer	One 12.5-mL bottle	One 12.5-mL bottle	
Proteinase K	–	One 0.85-mL tube	

Plastics for use with the PrepFiler® and PrepFiler® BTA Forensic DNA Extraction Kits		
Plasticware	**Description**	**Part Number**
PrepFiler® Spin Tubes and Filter Columns	300 Spin Tubes and 100 Filter Columns	4392342
1.5-mL Non-stick RNase-free Microfuge Tubes	500 tubes	AM12450
2-mL Microcentrifuge Tubes and Caps	500 tubes with caps	AM12425

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 PREPFILER AND PREPFILER BTA FORENSIC DNA EXTRACTION KITS PRODUCT INSERTS AND USER GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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