MagMAX™ DNA Multi-Sample Kit

Tissue and Whole Blood Preparation for Reaction Tube Formats

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *MagMAX™ DNA Multi-Sample Kit User Guide* (Pub. No. 4425070). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Contents and storage

The Applied Biosystems™ MagMAX™ DNA Multi-Sample Kit includes the following:

Contents	Storage
DNA Binding Beads, 10 mg/mL[1]	2°C to 8°C
DNA Elution Buffer 1	Room temperature (20°C to 25°C)
DNA Elution Buffer 2	
Multi-Sample DNA Lysis Buffer	
PK Buffer	
Processing Tubes, 2-mL	
Wash Solution 1 Concentrate [2]	
Wash Solution 2 Concentrate [3]	
Water, nuclease-free	
Proteinase K, 100 mg/mL	–25°C to –15°C
Ribonuclease A (RNase A), 1 mg/mL	

[1] Shipped at room temperature but must be refrigerated upon receipt.
[2] Add the correct volume of isopropanol before use.
[3] Add the correct volume of ethanol before use.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item		Source
Ethanol		MLS
Isopropanol, Molecular Biology Grade, Fisher BioReagents™, 4L		BP2618
Lab equipment		MLS
Magnetic stand for single tubes	Single Place Magnetic Stand	AM10026
	6-Tube Magnetic Stand	AM10055
Microcentrifuge tubes, 2-mL		AM18475
Vortex adaptor		AM10014

Procedural guidelines

- Perform the protocol at room temperature (20–25°C) except where noted.
- Use 2-mL tubes for all samples.
- Avoid creating bubbles when pipetting up/down.
- Close the tubes during the binding, washing, and elution steps to prevent spill-over and cross-contamination.
- If the DNA Binding Beads are being removed with the supernatant, use smaller pipette tips.
- When aspirating, be careful not to dislodge the DNA Binding Beads from the magnet.
- When capturing beads, you can remove the supernatant once the solution is clear. The bead collection times can vary depending on sample type and nucleic acid quantity.
- During the washing and elution steps, vortex the samples in short pulses to prevent the beads from sticking to the sides of the tubes.

Sample preparation and input quantity

Process samples shortly after harvesting or freeze immediately and store them at –75°C to –20°C (depending on sample type).

Table 1 Sample input quantity

Sample type	Input
Tissue (most samples)	≤50 mg[1]
Tissue with a high DNA yield	25 mg
Blood samples	≤200 µL
Buccal swabs	1 swab
Buffy coat	≤200 µL
Cultured cells	≤5 × 10^6 cells
Whatman™ FTA™ or Schleicher and Schuell 903 cards	≤4 × 2-mm punches

[1] You can increase the input quantity, but increasing it >50% can make the sample difficult to work with and reduce the yield.

Choose a purification protocol

See the appropriate method for isolating genomic DNA:

Solid tissue purification

Use this procedure to purify genomic DNA from solid animal tissue (such as brain, kidney, blood vessel, heart, adipose, liver, lung, muscle, or tail) using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or other heat source) to 70°C.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 2) for the number of samples that you are processing.

Table 2 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	16 µL
Water, nuclease-free	24 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 4 mg/mL.

Prepare the lysates

1. Add 500 µL of Multi-Sample DNA Lysis Buffer and 50 mg of tissue to a 2-mL microcentrifuge tube. (For tissues that have large amounts of DNA, add 25 mg.)
2. Homogenize until no tissue is visible (10–20 seconds using speed 3 or 4 on a 10-speed homogenizer).
3. Add 300 µL of 100% isopropanol.

Perform DNA extraction

1. Shake the prepared samples for 3 minutes at speed 1 or 2.
2. Add 40 µL of prepared DNA Binding Bead mix (see Table 2), then shake for 3 minutes at speed 1 or 2.
3. Place on the magnetic stand for 5 minutes.
4. Wash using 300 µL of prepared Wash Solution 1:
   a. While on the magnetic stand, discard the supernatant.
   b. Add 300 µL of Wash Solution 1, then vortex in pulses for 20 to 30 seconds.
   c. Place on the magnetic stand for 1 minute.
5. Repeat step 4 using 300 µL of Wash Solution 2.
6. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.
7. Add 100 µL of RNase A mix (see Table 3), then shake for 2 minutes at speed 1 or 2.

   Note: Prepare the RNase A mix just before use. Prolonged storage at room temperature can reduce its efficiency.

Table 3 RNase A mix

Component	Volume per tube/sample
RNase A	5 µL
Water, nuclease-free	95 µL
Total (RNase A mix)	**100 µL**

8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol, then shake for 3 minutes at speed 1 or 2.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 4 twice using 300 µL of Wash Solution 2.
11. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.

Perform the elution

1. Add a volume of DNA Elution Buffer 1:
   - 300 µL to samples with large amounts of DNA
   - 200 µL to all other samples
2. Briefly vortex or gently pipet up/down.
3. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

4. Add a volume of DNA Elution Buffer 2:
   - 300 µL to samples with large amounts of DNA
   - 200 µL to all other samples
5. Gently vortex for 20–30 seconds until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
6. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Tail tissue purification

Use this procedure to purify genomic DNA from mouse tail tissue using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or equivalent) to 55°C before preparing the lysates. After the digestion, preheat the heated source to 70°C before the extraction.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 4) for the number of samples that you are processing.

Table 4 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	16 µL
Water, nuclease-free	24 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 4 mg/mL.

Prepare the lysates

1. Add 184 µL of PK buffer, 16 µL of Proteinase K (100 mg/mL), and ~1.0 cm of mouse tail to a 2-mL microcentrifuge tube.
2. Incubate overnight at 55°C.
3. Transfer the liquid to a 2-mL processing tube.
4. Add 200 µL of Multi-Sample DNA Lysis Buffer. After each transfer, pipet up/down 3 or 4 times.
5. Add 240 µL of 100% isopropanol.

Perform DNA extraction

1. Shake the prepared samples for 3 minutes at speed 1 or 2.
2. Add 40 µL of prepared DNA Binding Bead mix (see Table 4), then shake for 3 minutes at speed 1 or 2.
3. Place on the magnetic stand for 5 minutes.
4. Wash using 300 µL of prepared Wash Solution 1:
   a. While on the magnetic stand, discard the supernatant.
   b. Add 300 µL of Wash Solution 1, then vortex in pulses for 20–30 seconds.
   c. Place on the magnetic stand for 1 minute.
5. Repeat step 4 using 300 µL of Wash Solution 2.
6. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.
7. Add 100 µL of RNase A mix (see Table 5), then shake for 2 minutes at speed 1 or 2.

   Note: Prepare the RNase A mix just before use. Prolonged storage at room temperature can reduce its efficiency.

   Table 5 RNase A mix

Component	Volume per tube/sample
RNase A	5 µL
Water, nuclease-free	95 µL
Total (RNase A mix)	**100 µL**

8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol, then shake for 3 minutes at speed 1 or 2.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 4 twice using 300 µL of Wash Solution 2.
11. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.

Perform the elution

1. Add 200 µL of DNA Elution Buffer 1.
2. Briefly vortex or gently pipet up/down.
3. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

4. Add 200 µL of DNA Elution Buffer 2.
5. Gently vortex for 20–30 seconds until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
6. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Cultured cell purification

Use this procedure to purify genomic DNA from cell cultures using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or other heat source) to 70°C.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 6) for the number of samples that you are processing.

Table 6 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	32 µL
Water, nuclease-free	8 µL
Total (DNA Binding Bead mix)[1]	40 µL

[1] Final concentration is 8 mg/mL.

Prepare the lysates

1. Thaw the cell pellets, then remove any media or PBS.
2. Add 1000 µL of Multi-Sample DNA Lysis Buffer for every 5×10^6 cells.
3. Vortex, then mix by pipetting up/down.
4. Add 800 µL of 100% isopropanol.

Perform DNA extraction

1. Shake the prepared samples for 3 minutes at speed 1 or 2.
2. Add 40 µL of prepared DNA Binding Bead mix (see Table 6), then shake for 3 minutes at speed 1 or 2.
3. Place on the magnetic stand for 5 minutes.
4. Wash using 300 µL of prepared Wash Solution 1:
   a. While on the magnetic stand, discard the supernatant.
   b. Add 300 µL of Wash Solution 1, then vortex in pulses for 20–30 seconds.
   c. Place on the magnetic stand for 1 minute.
5. Repeat step 4 using 300 µL of Wash Solution 2.
6. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.
7. Add 100 µL of RNase A mix (see Table 7), then shake for 2 minutes at speed 1 or 2.

   Note: Prepare the RNase A mix just before use. Prolonged storage at room temperature can reduce its efficiency.

   Table 7 RNase A mix

Component	Volume per tube/sample
RNase A	5 µL
Water, nuclease-free	95 µL
Total (RNase A mix)	**100 µL**

8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol, then shake for 3 minutes at speed 1 or 2.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 4 twice using 300 µL of Wash Solution 2.
11. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.

Perform the elution

1. Add 300 µL of DNA Elution Buffer 1.
2. Briefly vortex or gently pipet up/down.
3. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

4. Add 300 µL of DNA Elution Buffer 2.
5. Gently vortex for 20–30 seconds until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
6. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Buffy coat purification

Use this procedure to purify genomic DNA from buffy coat samples using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or other heat source) to 70°C.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 8) for the number of samples that you are processing.

Table 8 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	32 µL
Water, nuclease-free	8 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 8 mg/mL.

Prepare the lysates

1. Centrifuge 10 mL of whole blood at 3200 rpm for 15 minutes.
2. Transfer 200 µL of buffy coat to a 2-mL processing tube.
3. Add 200 µL of Multi-Sample DNA Lysis Buffer to the sample, then mix by pipetting up/down until homogenous.
4. Add 320 µL of 100% isopropanol.

Perform DNA extraction

1. Shake the prepared samples for 3 minutes at speed 1 or 2.
2. Add 40 µL of prepared DNA Binding Bead mix (see Table 8), then shake for 3 minutes at speed 1 or 2.
3. Place on the magnetic stand for 5 minutes.
4. Wash using 300 µL of prepared Wash Solution 1:
   a. While on the magnetic stand, discard the supernatant.
   b. Add 300 µL of Wash Solution 1, then vortex in pulses for 20–30 seconds.
   c. Place on the magnetic stand for 1 minute.
5. Repeat step 4 using 300 µL of Wash Solution 2.
6. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.
7. Add 100 µL of RNase A mix (see Table 9), then shake for 2 minutes at speed 1 or 2.

   Note: Prepare the RNase A mix just before use. Prolonged storage at room temperature can reduce its efficiency.

   Table 9 RNase A mix

Component	Volume per tube/sample
RNase A	5 µL
Water, nuclease-free	95 µL
Total (RNase A mix)	**100 µL**

8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol, then shake for 3 minutes at speed 1 or 2.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 4 twice using 300 µL of Wash Solution 2.
11. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.

Perform the elution

1. Add 100 µL of DNA Elution Buffer 1.
2. Briefly vortex or gently pipet up/down.
3. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

4. Add 100 µL of DNA Elution Buffer 2.
5. Gently vortex for 20–30 seconds until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
6. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Buccal sample purification

Use this procedure to purify genomic DNA from buccal swab samples using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or other heat source) to 70°C.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 10) for the number of samples that you are processing.

Table 10 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	16 µL
Water, nuclease-free	24 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 4 mg/mL.

Prepare the lysates

1. Add 400 µL of Multi-Sample DNA Lysis Buffer to a 2-mL processing tube.
2. Remove the stick, then place the buccal swab into the buffer.
3. Shake for 3 minutes at speed 1 or 2.
4. Remove the swab, leaving behind the buffer (~200 µL).
5. Add 160 µL of 100% isopropanol.

Perform DNA extraction

1. Shake the prepared samples for 3 minutes at speed 1 or 2.
2. Add 40 µL of prepared DNA Binding Bead mix (see Table 10), then shake for 3 minutes at speed 1 or 2.
3. Place on the magnetic stand for 5 minutes.
4. Wash using 300 µL of prepared Wash Solution 1:
   a. While on the magnetic stand, discard the supernatant.
   b. Add 300 µL of Wash Solution 1, then vortex in pulses for 20–30 seconds.
   c. Place on the magnetic stand for 1 minute.
5. Repeat step 4 using 300 µL of Wash Solution 2.
6. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.
7. Add 100 µL of RNase A mix (see Table 11), then shake for 2 minutes at speed 1 or 2.

   Note: Prepare the RNase A mix just before use. Prolonged storage at room temperature can reduce its efficiency.

   Table 11 RNase A mix

Component	Volume per tube/sample
RNase A	5 µL
Water, nuclease-free	95 µL
Total (RNase A mix)	**100 µL**

8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol, then shake for 3 minutes at speed 1 or 2.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 4 twice using 300 µL of Wash Solution 2.
11. While on the magnetic stand, discard the supernatant, then air-dry uncapped for 3 minutes.

Perform the elution

1. Add 50 µL of DNA Elution Buffer 1.
2. Briefly vortex or gently pipet up/down.
3. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

4. Add 50 µL of DNA Elution Buffer 2.
5. Gently vortex for 20–30 seconds until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
6. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Whole blood purification

Use this procedure to purify genomic DNA from whole blood samples (fresh or frozen collected in EDTA, heparin, or citrate) using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or equivalent) to 60°C before preparing the Proteinase K mixture, then 70°C after the Proteinase K digestion.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 12) for the number of samples that you are processing and store the mix at room temperature.

Table 12 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	32 µL
Water, nuclease-free	8 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 8 mg/mL.

Perform DNA extraction

1. Perform the Proteinase K digestion:
   a. Prepare the PK buffer/enzyme mix (see Table 13).

      Note: Prepare the PK buffer/enzyme mix just before use.

      Table 13 PK buffer/enzyme mix

Component	Volume per tube/sample
Proteinase K Solution (100 mg/mL)	16 µL
PK Digestion Buffer	184 µL
Total (PK buffer/enzyme mix)	**200 µL**

   b. Add 200 µL of PK buffer/enzyme mix to a 2-mL processing tube followed by 200 µL of blood sample, then mix by pipetting up/down 5–7 times or by gentle vortexing.

      IMPORTANT! Add the PK buffer/enzyme mix to the tube before adding the blood sample.

   c. Incubate for 20 minutes at 60°C on a heated block or thermomixer without shaking.
2. Add 600 µL of Multi-Sample DNA Lysis Buffer, then vortex to mix.
3. Shake for 3 minutes at speed 1 or 2.
4. Add 40 µL of prepared DNA Binding Bead mix (see Table 12), then shake for 3 minutes at speed 1 or 2.
5. Add 800 µL of 100% isopropanol.
6. Vortex to mix, then shake for 3 minutes at speed 1 or 2.
7. Place on the magnetic stand for 5 minutes.
8. While on the magnet, discard the supernatant.
9. Wash the sample using 300 µL of Wash Solution 1:
   a. Remove from the magnetic stand, add 300 µL of Wash Solution 1, then vortex in pulses for 10–20 seconds.
   b. Place on the magnetic stand for 1 minute.
   c. While on the magnet, discard the supernatant.
10. Repeat step 9 twice using 300 µL of Wash Solution 2.
11. Air-dry uncapped on the magnetic stand for 3 minutes.

Perform the elution

1. Add 100 µL of DNA Elution Buffer 1.
2. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

3. Add 100 µL of DNA Elution Buffer 2.
4. Vortex for 10–20 seconds or until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
5. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

Whatman™ FTA™ or SS 903 card purification

Use this procedure to purify genomic DNA from Whatman™ FTA™ or Schleicher/Schuell 903 cards using the MagMAX™ Kit.

Before you begin

- Preheat a heated block (or equivalent) to 60°C before preparing the Proteinase K mixture, then 70°C after the Proteinase K digestion.
- Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
- Prepare sufficient DNA Binding Bead mix (see Table 14) for the number of samples that you are processing and store the mix at room temperature.

Table 14 DNA Binding Bead mix

Component	Volume per tube/sample
DNA Binding Beads (10 mg/mL)	32 µL
Water, nuclease-free	8 µL
Total (DNA Binding Bead mix)[1]	**40 µL**

[1] Final concentration is 8 mg/mL.

Perform DNA extraction

1. Perform the Proteinase K digestion:
   a. Prepare the PK buffer/enzyme mix (see Table 15).

      Note: Prepare the PK buffer/enzyme mix just before use.

      Table 15 PK buffer/enzyme mix

Component	Volume per tube/sample
Proteinase K Solution (100 mg/mL)	16 µL
PK Digestion Buffer	184 µL
Total (PK buffer/enzyme mix)	**200 µL**

   b. Add 200 µL of PK buffer/enzyme mix to a 2-mL processing tube followed by ≤4 × 2-mm punches of an FTA™ or SS 903 card, then mix by pipetting up/down 5–7 times or by gentle vortexing.
   c. Incubate for 20 minutes at 60°C without shaking.
2. Remove the tube from the heat source, transfer the liquid to a new 2-mL reaction tube followed by 400 µL of Multi-Sample DNA Lysis Buffer, then vortex to mix.
3. Shake for 3 minutes at speed 1 or 2.
4. Add 40 µL of prepared DNA Binding Bead mix (see Table 14), then shake for 3 minutes at speed 1 or 2.
5. Add 480 µL of 100% isopropanol to the sample.
6. Vortex to mix, then shake for 3 minutes at speed 1 or 2.
7. Place on the magnetic stand for 5 minutes.
8. While on the magnet, discard the supernatant.
9. Wash the sample using 300 µL of Wash Solution 1:
   a. Remove from the magnetic stand, add 300 µL of Wash Solution 1, then vortex in pulses for 10–20 seconds.
   b. Place on the magnetic stand for 1 minute.
   c. While on the magnet, discard the supernatant.
10. Repeat step 9 twice using 300 µL of Wash Solution 2.
11. Air-dry uncapped on the magnetic stand for 3 minutes.

Perform the elution

1. Add 50 µL of DNA Elution Buffer 1.
2. Incubate accordingly using one of the following methods.

Incubation method	Description
Non-heated shaking	1. Incubate for 5 minutes at 70°C on a heated block. 2. Shake for 5 minutes at speed 1 or 2.
Heated shaking using a thermomixer	1. Incubate for 5 minutes at 70°C. 2. Shake at 750 rpm for 5 minutes at 70°C.

3. Add 50 µL of DNA Elution Buffer 2.
4. Vortex for 10–20 seconds or until resuspended, then place on the magnetic stand for 5 minutes. If necessary, resuspend by gently pipetting up/down.
5. While on the magnet, transfer the eluate (which contains the purified DNA) to a new tube, then close immediately.

IMPORTANT! To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

STOPPING POINT Use the purified DNA immediately or store the eluate in a tightly-capped tube at 2–6°C for up to 2 weeks or at –80°C to –20°C for prolonged storage.

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 found on Life Technologies' website 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**.

Revision history: Pub. No. 4425071

Revision	Date	Description
C	27 April 2016	Format, style, and legal updates
B	August 2009	Baseline for this revision history