MagMAX™-96 DNA Multi-Sample Kit

Tissue and Whole Blood Preparation for 96-Well Reaction Plate Formats

Catalog Numbers 4413021 and 4413022

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *MagMAX™-96 DNA Multi-Sample Kit User Guide* (Pub. No. 4428201). 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™-96 DNA Multi-Sample Kits include the following:

Contents	Storage
DNA Binding Beads, 10 mg/mL	2°C to 8°C
DNA Elution Buffer 1	Room temperature (20°C to 25°C)
DNA Elution Buffer 2	
Elution Plate, 96-well	
MicroAmp™ Clear Adhesive Film	
Multi-Sample DNA Lysis Buffer	
PK Buffer	
Processing Plate, 96-well, 1.2-mL	
Wash Solution 1 Concentrate	
Wash Solution 2 Concentrate	
Water, nuclease-free	
Proteinase K, 100 mg/mL	–25°C to –15°C
Ribonuclease A (RNase A), 1 mg/mL	

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
Heated block, 96-well (or thermomixer)		MLS
Isopropanol, Molecular Biology Grade, Fisher BioReagents™, 4L		BP2618
Lab equipment		MLS
Titer plate shaker: Thermo Scientific™ Compact Digital Microplate Shaker		11-676-337
Magnetic stand for 96-well plates	Magnetic Stand-96	AM10027
	96-Well Magnetic-Ring Stand	AM10050
Microcentrifuge tubes, 2 -mL		AM18475

Procedural guidelines

- Perform the protocol at room temperature (20–25°C) except where noted.
- Avoid creating bubbles when pipetting up/down.
- Cover the plates during the binding, washing, and elution steps to prevent spill-over and cross-contamination.
- 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.
- The titer plate shaking speeds in this document are recommendations, as the speeds can vary depending on the model of shaker used. Ideal speeds mix the samples thoroughly without splashing.
- If you use a plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, verify that the:
  - Processing plates fit securely on your titer plate shaker.
  - Recommended speeds are compatible with your titer plate shaker.

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)	≤10 mg[1]
Tissue with a high DNA yield	5 mg
Blood samples	≤50 µL
Buccal swabs	1 swab
Buffy coat	≤50 µL
Cultured cells	≤1 × 10^6 cells
Whatman™ FTA™ or Schleicher and Schuell 903 cards	2 × 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" on page 2
- "Tail sample purification" on page 3
- "Cultured cell purification" on page 4
- "Buffy coat purification" on page 5
- "Buccal sample purification" on page 6
- "Whole blood purification" on page 7
- "Whatman™ FTA™ or SS 903 card purification" on page 8

Solid tissue purification

Use this procedure to purify genomic DNA from solid animal tissue (such as brain, kidney, blood vessel, heart, adipose, liver, lung, or muscle) using the MagMAX™-96 Kit.

Before you begin

- Preheat a thermomixer (or alternate 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

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	8 µL	808 µL
Water, nuclease-free	12 µL	1,212 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 4 mg/mL.

Disrupt the samples

1. Add 200 µL of Multi-Sample DNA Lysis Buffer to a 2-mL microcentrifuge tube, then add 10 mg of tissue. (For tissues that have large amounts of DNA, add 5 mg.)
2. Homogenize until no tissue is visible (10–20 seconds using speed 3 or 4 on a 10-speed homogenizer).
3. Add 200 µL of each homogenate to the processing plate, add 120 µL of 100% isopropanol, seal the plate, then shake for 3 minutes at speed 7.

Perform the DNA extraction

1. Add 20 µL of DNA Binding Bead mix (see Table 2) to each sample, reseal the plate, then shake for 3 minutes at speed 7.
2. Place on the magnetic stand for 3 minutes.
3. Wash the beads using 150 µL of Wash Solution 1:
   a. While on the magnet, remove the plate cover, then discard the supernatant from each well.
   b. Add 150 µL of Wash Solution 1 to each sample, reseal the plate, then shake for 1 minute at speed 7.
   c. Place on the magnetic stand for 1 minute.
4. Repeat step 3 once using 150 µL of Wash Solution 2.
5. While on the magnet, remove the plate cover, then discard the supernatant from each well. Shake uncovered for 2 minutes at speed 9.
6. While the plate dries, prepare the RNase A mix (see Table 3).

Table 3 RNase A mix

Component	Volume	
	1 well	**Plate[1]**
RNase A	5 µL	505 µL
Water, nuclease-free	95 µL	9,595 µL
Total (RNase A mix)	**100 µL**	**10,100 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

7. Add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7.
8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 3 twice using 150 µL of Wash Solution 2.
11. While on the magnet, remove the plate cover, then discard the supernatant from each well.
12. Dry the samples by shaking uncovered for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add DNA Elution Buffer 1 to each sample:
   - 150 µL to samples with large amounts of DNA
   - 50–100 µL to all other samples
2. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C 2. Shake for 5 minutes at speed 7 at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

3. Add DNA Elution Buffer 2 to each sample:
   - 150 µL to samples with large amounts of DNA
   - 50–100 µL to all other samples
4. Seal the plate, then shake for 2 minutes at speed 7.
5. Place on the magnetic stand for 3 minutes.
6. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

STOPPING POINT Use the purified samples immediately, or store the elution plate at 2–6°C for up to 24 hours or –80 to –20°C for prolonged storage.

Tail sample purification

Use this procedure to purify genomic DNA from mouse tail samples using the MagMAX™-96 Kit.

Before you begin

- Preheat a heated block (or alternate heat source) to 55°C before preparing the lysates. After the digestion, preheat the heated source to 70°C before the DNA 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	
	1 well	Plate[1]
DNA Binding Beads (10 mg/mL)	8 µL	808 µL
Water, nuclease-free	12 µL	1,212 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 4 mg/mL.

Disrupt the samples

1. Add 92 µL of PK buffer, 8 µL of Proteinase K (100 mg/mL), and ≤0.5 cm of mouse tail to a 1.5-mL tube.
2. Incubate overnight at 55°C.
3. Briefly centrifuge, then carefully transfer the liquid of each sample to the processing plate, leaving behind any tissue.
4. Add 100 µL of Multi-Sample DNA Lysis Buffer to each sample. After each transfer, mix by slowly pipetting up/down 3 or 4 times.
5. Add 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.

Perform the DNA extraction

1. Add 20 µL of DNA Binding Bead mix (see Table 4) to each sample, reseal the plate, then shake for 3 minutes at speed 7.
2. Place on the magnetic stand for 3 minutes.
3. Wash the beads using 150 µL of Wash Solution 1:
   a. While on the magnet, remove the plate cover, then discard the supernatant from each well.
   b. Add 150 µL of Wash Solution 1 to each sample, reseal the plate, then shake for 1 minute at speed 7.
   c. Place on the magnetic stand for 1 minute.
4. Repeat step 3 once using 150 µL of Wash Solution 2.
5. While on the magnet, remove the plate cover, then discard the supernatant from each well. Shake uncovered for 2 minutes at speed 9.
6. While the plate dries, prepare the RNase A mix (see Table 5).

Table 5 RNase A mix

Component	Volume	
	1 well	Plate[1]
RNase A	5 µL	505 µL
Water, nuclease-free	95 µL	9,595 µL
Total (RNase A mix)	**100 µL**	**10,100 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

7. Add 100 µL of RNase A mix to each sample, seal the plate, then shake for 2 minutes at speed 7.
8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 3 twice using 150 µL of Wash Solution 2.
11. While on the magnet, remove the plate cover, then discard the supernatant from each well.
12. Dry the samples by shaking uncovered for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add 100 µL of DNA Elution Buffer 1 to each sample.
2. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C 2. Shake for 5 minutes at speed 7 at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

3. Add 100 µL of DNA Elution Buffer 2 to each sample.
4. Seal the plate, then shake for 2 minutes at speed 7.
5. Place on the magnetic stand for 3 minutes.
6. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

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

STOPPING POINT Use the purified samples immediately, or store the elution plate at 2–6°C for up to 24 hours or –80 to –20°C for prolonged storage.

Cultured cell purification

Use this procedure to purify genomic DNA from cell cultures using the MagMAX™-96 Kit.

Before you begin

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

Table 6 DNA Binding Bead mix

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	16 µL	1,616 µL
Water, nuclease-free	4 µL	404 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 8 mg/mL.

Disrupt the samples

1. If necessary, thaw the cell pellets, then remove as much media or PBS as possible from each pellet.
2. To each cell pellet, add 200 µL of Multi-Sample DNA Lysis Buffer for every 1×10^6 cells.
3. Vortex and mix each sample by pipetting up/down until the cell pellet is completely dissolved.
4. Transfer 200 µL of each homogenate (1×10^6 cells) to the processing plate. Mix thoroughly prior to aliquotting.
5. Add 160 µL of 100% isopropanol, seal the plate, then shake for 3 minutes at speed 7.

Perform the DNA extraction

1. Add 20 µL of DNA Binding Bead mix (see Table 6) to each sample, reseal the plate, then shake for 3 minutes at speed 7.
2. Place on the magnetic stand for 3 minutes.
3. Wash the beads using 150 µL of Wash Solution 1:
   a. While on the magnet, remove the plate cover, then discard the supernatant from each well.
   b. Add 150 µL of Wash Solution 1 to each sample, reseal the plate, then shake for 1 minute at speed 7.
   c. Place on the magnetic stand for 1 minute.
4. Repeat step 3 once using 150 µL of Wash Solution 2.
5. While on the magnet, remove the plate cover, then discard the supernatant from each well. Shake uncovered for 2 minutes at speed 9.
6. While the plate dries, prepare the RNase A mix (see Table 7).

Table 7 RNase A mix

Component	Volume	
	1 well	**Plate[1]**
RNase A	5 µL	505 µL
Water, nuclease-free	95 µL	9,595 µL
Total (RNase A mix)	**100 µL**	**10,100 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

7. Add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7.
8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 3 twice using 150 µL of Wash Solution 2.
11. While on the magnet, remove the plate cover, then discard the supernatant from each well.
12. Dry the samples by shaking uncovered for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add 100 µL of DNA Elution Buffer 1 to each sample.
2. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C 2. Shake for 5 minutes at speed 7 at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

3. Add 100 µL of DNA Elution Buffer 2 to each sample.
4. Seal the plate, then shake for 2 minutes at speed 7.
5. Place on the magnetic stand for 3 minutes.
6. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

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

STOPPING POINT Use the purified samples immediately, or store the elution plate at 2–6°C for up to 24 hours or –80 to –20°C for prolonged storage.

Buffy coat purification

Use this procedure to purify genomic DNA from buffy coat samples using the MagMAX™-96 Kit.

Before you begin

- Preheat a heated block (or alternate 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

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	16 µL	1,616 µL
Water, nuclease-free	4 µL	404 µL
Total (DNA Binding Bead mix)[2]	20 µL	2,020 µL

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 8 mg/mL.

Disrupt the samples

1. Centrifuge 10 mL of whole blood at 3200 rpm for 15 minutes at room temperature (or preferred protocol).
2. Add 50 µL of each buffy coat to the processing plate.
3. Add 50 µL of Multi-Sample DNA Lysis Buffer to each sample and mix by pipetting up and down until homogeneous.
4. Add 80 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.

Perform the DNA extraction

1. Add 20 µL of DNA Binding Bead mix (see Table 8) to each sample, reseal the plate, then shake for 3 minutes at speed 7.
2. Place on the magnetic stand for 3 minutes.
3. Wash the beads using 150 µL of Wash Solution 1:
   a. While on the magnet, remove the plate cover, then discard the supernatant from each well.
   b. Add 150 µL of Wash Solution 1 to each sample, reseal the plate, then shake for 1 minute at speed 7.
   c. Place on the magnetic stand for 1 minute.
4. Repeat step 3 once using 150 µL of Wash Solution 2.
5. While on the magnet, remove the plate cover, then discard the supernatant from each well. Shake uncovered for 2 minutes at speed 9.
6. While the plate dries, prepare the RNase A mix (see Table 9).

Table 9 RNase A mix

Component	Volume	
	1 well	**Plate[1]**
RNase A	5 µL	505 µL
Water, nuclease-free	95 µL	9,595 µL
Total (RNase A mix)	**100 µL**	**10,100 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

7. Add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7.
8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 3 twice using 150 µL of Wash Solution 2.
11. While on the magnet, remove the plate cover, then discard the supernatant from each well.
12. Dry the samples by shaking uncovered for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add 50 µL of DNA Elution Buffer 1 to each sample.
2. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C 2. Shake for 5 minutes at speed 7 at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

3. Add 50 µL of DNA Elution Buffer 2 to each sample.
4. Seal the plate, then shake for 2 minutes at speed 7.
5. Place on the magnetic stand for 3 minutes.
6. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

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

STOPPING POINT Use the purified samples immediately, or store the elution plate at 2–6°C for up to 24 hours or –80 to –20°C for prolonged storage.

Buccal sample purification

Use this procedure to purify genomic DNA from buccal swab samples using the MagMAX™-96 Kit.

Before you begin

- Preheat a thermomixer (or alternate 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

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	16 µL	1,616 µL
Water, nuclease-free	4 µL	404 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 8 mg/mL.

Disrupt the samples

1. For each sample, add 400 µL of Multi-Sample DNA Lysis Buffer to a well of the processing plate.
2. Remove the stick, then place the swab into the lysis buffer.
3. Shake the unsealed plate for 3 minutes at speed 7.
4. Remove the swabs, leaving behind as much lysis buffer as possible (approximately 200 µL).
5. Add 160 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.

Perform the DNA extraction

1. Add 20 µL of DNA Binding Bead mix (see Table 10) to each sample, reseal the plate, then shake for 3 minutes at speed 7.
2. Place on the magnetic stand for 3 minutes.
3. Wash the beads using 150 µL of Wash Solution 1:
   a. While on the magnet, remove the plate cover, then discard the supernatant from each well.
   b. Add 150 µL of Wash Solution 1 to each sample, reseal the plate, then shake for 1 minute at speed 7.
   c. Place on the magnetic stand for 1 minute.
4. Repeat step 3 once using 150 µL of Wash Solution 2.
5. While on the magnet, remove the plate cover, then discard the supernatant from each well. Shake uncovered for 2 minutes at speed 9.
6. While the plate dries, prepare the RNase A mix (see Table 11).

Table 11 RNase A mix

Component	Volume	
	1 well	**Plate[1]**
RNase A	5 µL	505 µL
Water, nuclease-free	95 µL	9,595 µL
Total (RNase A mix)	**100 µL**	**10,100 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

7. Add 100 µL of RNase A mix to each sample, seal the plate, then shake for 2 minutes at speed 7.
8. Add 100 µL of Multi-Sample DNA Lysis Buffer and 120 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 7.
9. Place on the magnetic stand for 1 minute.
10. Repeat step 3 twice using 150 µL of Wash Solution 2.
11. While on the magnet, remove the plate cover, then discard the supernatant from each well.
12. Dry the samples by shaking uncovered for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add 50 µL of DNA Elution Buffer 1 to each sample.
2. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C 2. Shake for 5 minutes at speed 7 at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

3. Add 50 µL of DNA Elution Buffer 2 to each sample, seal the plate, then shake for 2 minutes at speed 7.
4. Place on the magnetic stand for 3 minutes.
5. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

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

STOPPING POINT Use the purified samples immediately, or store the elution plate at 2–6°C for up to 24 hours or –80 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™-96 Kit.

Before you begin

1. Preheat a heated block (or alternate heat source) to 60–65°C before preparing the Proteinase K mixture, then 70°C after the Proteinase K digestion.
2. Prepare sufficient volumes of Wash Solution 1 and 2 according to the instructions on the reagent bottles.
3. Prepare sufficient DNA Binding Bead mix (see Table 12) for the number of samples that you are processing.

Table 12 DNA Binding Bead mix

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	16 µL	1,616 µL
Water, nuclease-free	4 µL	404 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 8 mg/mL.

Perform the DNA extraction

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

Table 13 PK buffer/enzyme mix

Component	Volume	
	1 well	**Plate[1]**
Proteinase K Solution	8 µL	808 µL
PK Digestion Buffer	42 µL	4,242 µL
Total (PK buffer/enzyme mix)	50 µL	5,050 µL

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

   b. Add 50 µL of PK buffer/enzyme mix to a well on the processing plate followed by 50 µL of whole blood. Mix by pipetting up/down 5–7 times or by gentle vortexing.

IMPORTANT! Add PK buffer/enzyme mix to the plate before the sample.

   c. Seal the plate, then incubate for 20 minutes at 60–65°C.
2. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample, seal the plate, then shake for 3 minutes at speed 7.
3. Add 20 µL of prepared DNA Binding Bead mix (see Table 12) to each sample, seal the plate, then shake for 3 minutes at speed 6.
4. Add 240 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 3 or 4.
5. Place on the magnetic stand for 5 minutes.
6. While on the magnet, remove the plate cover, then discard the supernatant from each well.
7. Wash the samples using 150 µL of Wash Solution 1:
   a. Add 150 µL of Wash Solution 1 to each sample, seal the plate, then shake for 1 minute at speed 7.
   b. Place on the magnetic stand for 1 minute.
   c. While on the magnet, carefully remove the plate cover, then discard the supernatant from each well.
8. Repeat step 7 twice using 150 µL of Wash Solution 2.
9. Shake uncovered for 2 minutes at speed 9.
10. Add 50 µL of DNA Elution Buffer 1 to each sample.
11. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate on a 96-well heated block for 5 minutes at 70°C. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

12. Add 50 µL of DNA Elution Buffer 2 to each sample. After each transfer, mix gently by pipetting up/down 10 times. Seal the plate, then shake for 2 minutes at speed 7.
13. Place on the magnetic stand for 5 minutes.
14. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

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

STOPPING POINT Use the purified samples immediately, or store the elution plate for later use at 2–6°C for up to 24 hours or −80 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.

Before you begin

- Preheat a heated block (or alternate heat source) to 60–65°C before preparing the Proteinase K mixture, and 70°C after the Proteinase K digestion.
- Prepare sufficient Wash Solution 1 and Wash Solution 2.
- Prepare sufficient DNA Binding Bead mix (see Table 14) for the number of samples that you are processing.

Table 14 DNA Binding Bead mix

	Volume	
Component	**1 well**	**Plate[1]**
DNA Binding Beads (10 mg/mL)	16 µL	1,616 µL
Water, nuclease-free	4 µL	404 µL
Total (DNA Binding Bead mix)[2]	**20 µL**	**2,020 µL**

[1] Includes volume sufficient for one 96-well plate plus 5% excess.
[2] Final concentration is 8 mg/mL.

Perform the DNA extraction

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

Table 15 PK buffer/enzyme mix

Component	Volume	
	1 well	**Plate[1]**
Proteinase K Solution	8 µL	808 µL
PK Digestion Buffer	42 µL	4,242 µL
Total (PK buffer/enzyme mix)	50 µL	5,050 µL

[1] Includes volume sufficient for one 96-well plate plus 5% excess.

   b. Add 50 µL of PK buffer/enzyme mix to a well on the processing plate followed by 2 × 2-mm punches of an FTA™ or SS 903 card. Mix by pipetting up/down 5–7 times or by gentle vortexing.
   c. Seal the plate, then incubate for 20 minutes at 60–65°C.
2. Transfer the liquid from each well to a new well of the same plate or a well of a new 1.2 mL plate, leaving behind the card material. Add 100 µL of Multi-Sample DNA Lysis Buffer to each sample.
3. Seal the plate, then shake for 3 minutes at speed 7.
4. Add 20 µL of DNA Binding Bead mix (see Table 14) to each sample, seal the plate, then shake for 3 minutes at speed 6.
5. Add 160 µL of 100% isopropanol to each sample, seal the plate, then shake for 3 minutes at speed 4.
6. Place on the magnetic stand for 5 minutes.
7. While on the magnet, remove the plate cover, then discard the supernatant from each well.
8. Wash the samples using 150 µL of Wash Solution 1:
   a. Add 150 µL of Wash Solution 1 to each sample, seal the plate, then shake for 1 minute at speed 7.
   b. Place on the magnetic stand for 1 minute.
   c. While on the magnet, carefully remove the plate cover, then discard the supernatant from each well.
9. Repeat step 8 twice using 150 µL of Wash Solution 2.
10. Shake uncovered for 2 minutes at speed 9.
11. Add 50 µL of DNA Elution Buffer 1 to each sample.
12. Incubate the plate using one of the following methods.

Incubation method	Description
Non-heated shaking using a 96-well heated block	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.
Heated shaking using a thermomixer (or equivalent)	1. Seal the plate, then incubate for 5 minutes at 70°C. 2. Shake at 900 rpm for 5 minutes at 70°C.

13. Add 50 µL of DNA Elution Buffer 2 to each sample. After each transfer, mix gently by pipetting up/down 10 times. Seal the plate, then shake for 2 minutes at speed 7.
14. Place on the magnetic stand for 5 minutes.
15. While on the magnet, remove the plate cover, transfer the eluates to the elution plate, then seal the plate.

STOPPING POINT Use the purified samples immediately, or store the elution plate for later use at 2–6°C for up to 24 hours or −80 to −20°C for prolonged storage.

Limited product warranty

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