Applied Biosystems

MagMAX™-96 DNA Multi-Sample Kit

MagMAX™ Express-96 Magnetic Particle Processor

Protocol

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Contents

Safety information

Note: For general safety information, see this Preface and Appendix C, "Safety" on page 39. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 41.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

How to obtain support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

MagMAX™-96 DNA Multi-Sample Kit

Product information

Purpose of the product

The MagMAX™-96 DNA Multi-Sample Kit is designed for rapid, high-throughput purification of high-quality genomic DNA from a variety of sample types including: fresh or frozen animal tissue, blood or buffy coat, mammalian cell cultures, Whatman® FTA® or Schleicher and Schuell 903 cards, and buccal samples. DNA purified with this kit can be used in a broad range of molecular biology applications, such as sequencing, genotyping, PCR, Southern blotting, RAPD, AFLP, and RFLP.

This purification kit uses MagMAX™ magnetic bead-based nucleic acid isolation technology to produce high yields of purified DNA that are free of PCR inhibitors. The magnetic beads not only have a large available binding surface but they can also be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. Thus, this procedure consistently delivers maximal yields of high-quality DNA.

About the procedure

This protocol involves four processes:

1. Sample procurement

   Note: Techniques for surgically removing animal tissue are discretionary and are not discussed in this protocol.

2. Lysate generation through sample disruption by digestion or mechanical homogenization
3. Sample purification through sample binding and subsequent washing
4. DNA elution from the magnetic particles

The MagMAX™-96 DNA Multi-Sample Kit employs mechanical disruption to rapidly release nucleic acid into a guanidinium thiocyanate-based solution that provides protection by inactivating nucleases (Chirgwin, *et al.,* 1979; Chomczynski and Sacchi, 1987). For blood and tail samples, the kit employs an enzymatic proteinase K digestion followed by a treatment with guanidinium thiocyanate-based solution.

After lysis, samples are mixed with isopropanol, then combined with paramagnetic beads with a nucleic acid binding surface. The beads, with bound nucleic acid, are immobilized on magnets and washed to remove proteins and other contaminants. A second wash solution is used to remove residual binding solution, then the nucleic acid is eluted using low-salt buffers.

Reaction formats

The MagMAX™-96 DNA Multi-Sample Kit can accommodate a broad range of throughputs. The procedures in this protocol support 96-well reaction plate format in combination with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

About the MagMAX™ Express-96 Deep Well Magnetic Particle Processor

The MagMAX™ Express-96 Deep Well Magnetic Particle Processor is a plate-handling robot designed for automated, high-throughput processing of MagMAX™ nucleic acid isolation chemistries. The MagMAX™ instrument can prepare extracts of both genomic and viral nucleic acid from diverse sample matrices for several downstream applications. The instrument supports two 96-well consumables; however, this protocol requires a MagMAX™ Deep Well 96-Well Plate.

Time required

Using the MagMAX™-96 DNA Multi-Sample Kit, you can obtain samples of purified genomic DNA in approximately 1 hour.

Note: The estimate does not include the time for digestion or homogenization, which can vary widely depending on sample type.

Note: The MagMAX™ Express-96 Deep Well Magnetic Particle Processor significantly streamlines sample processing by eliminating the need for human participation during the washing and elution steps of the purification process.

For more information

To learn more about the MagMAX™-96 DNA Multi-Sample Kit and the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, go to:

www.appliedbiosystems.com/magmax

Materials and equipment required

Kit contents and storage

The MagMAX™-96 DNA Multi-Sample Kit arrives in two packages that contain the reagents and materials listed below. Upon receipt of the MagMAX™-96 Kit, open the packages and store the contents of the kit as shown below.

Table 1 MagMAX™-96 DNA Multi-Sample Kit (PN 4413021/PN 4413022)

Component	Quantity		Storage condition
	1 × 96-rxns (PN 4413021)	5 × 96-rxns (PN 4413022)	
DNA Binding Beads, 10 mg/mL‡	1 × 1.8 mL	1 × 8.5 mL	2 to 8 °C
DNA Elution Buffer 1	1 × 15 mL	1 × 72 mL	Room temperature
DNA Elution Buffer 2	1 × 15 mL	1 × 72 mL	
Elution Plate, 96-well	2	3	
Multi-Sample DNA Lysis Buffer	1 × 50 mL	1 × 250 mL	
MicroAmp® Clear Adhesive Film	1 × 4 films	5 × 4 films	
PK Buffer	1 × 11 mL	1 × 50 mL	
Processing Plate, 96-well, 1.2-mL	1	5	
Wash Solution 1 Concentrate§	1 × 15 mL	1 × 75 mL	
Wash Solution 2 Concentrate#	1 × 50 mL	1 × 250 mL	
Water, nuclease-free	1 × 50 mL	2 × 50 mL	
Proteinase K, 100 mg/mL	1 × 850 µL	1 × 4.3 mL	– 15 to – 25 °C
Ribonuclease A (RNase A), 1 mg/mL	1 × 530 µL	1 × 2.7 mL	

‡ The DNA Binding Beads are shipped at room temperature but must be refrigerated upon receipt.
§ Add the correct volume of Isopropanol written on the bottle before use.
# Add the correct volume of Ethanol written on the bottle before use.

Materials required but not supplied

The following materials are required for the use of, but are not supplied with, the MagMAX™-96 DNA Multi-Sample Kit. Parts designated with a "AM" prefix are available from the Ambion website: **www.ambion.com/catalog/**

Table 2 Additional required materials

Item	Source‡
Ethanol	Major laboratory suppliers
Heated block, 96-well (or thermomixer)	
Isopropanol (ACS reagent grade or equivalent)	
Lab equipment (such as pipettors, pipet tips, vortexer, microcentrifuge)	
Titer plate shaker **Note:** The procedures in this document are optimized for use with the Thermo Scientific Barnstead/Lab-Line Titer Plate Shaker.	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	§
MagMAX™ Express-96 Deep Well Tip Combs	4388487
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Standard Plates	4388475
Microcentrifuge tubes, 2-mL	AM18475

‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

§ See the Applied Biosystems/Ambion website for pricing and availability.

Workflow

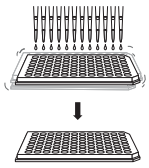
The following figure shows a simplified workflow for isolating DNA from a variety of tissues using the MagMAX™-96 DNA Multi-Sample Kit. Variations of this workflow, described in this protocol, can be used to purify isolates from a broader range of samples (see "Compatible sample types" on page 6).

Disrupt the samples

Prepare samples for lysis.

↓

Generate the lysates.



Perform the DNA extraction

Add DNA Binding Bead Mix and isopropanol.

↓

Wash once using Wash Solution 1.

↓

Wash once using Wash Solution 2.

↓

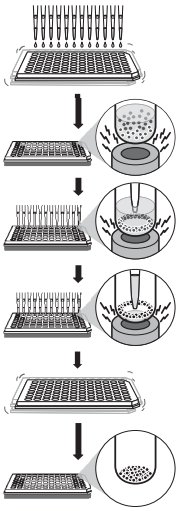
Perform the RNase A digestion.

↓

Wash twice using Wash Solution 2.

↓

Air dry the samples.



Perform the elution

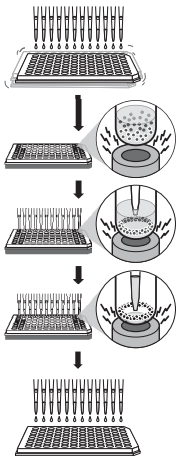
Elute samples using DNA Elution Buffer 1.

↓

Elute samples using DNA Elution Buffer 2.

↓

Transfer and store the eluant.



Sample preparation

Collection and storage recommendations

If you are isolating DNA from fresh samples using the MagMAX™-96 DNA Multi-Sample Kit, process the samples shortly after harvesting them. Soon after animal tissue is removed from an organism, the quality of nucleic acid in the tissue sample begins to degrade. Alternatively, samples that cannot be processed soon after they are collected should be frozen immediately and stored at – 20 to – 75 °C (depending on sample type). Avoid freezing and thawing samples repeatedly, since doing so can effect DNA integrity.

Compatible sample types

The MagMAX™-96 DNA Multi-Sample Kit can be used to process a variety of sample types, including fresh or frozen animal tissues, blood, and mammalian cell cultures. This document contains specific isolation protocols for the following tissues and sample types:

- Solid animal tissue (such as brain, kidney, blood vessel, heart, adipose, liver, lung, muscle, or tail)
- Cell cultures
- Buffy coat
- Buccal swabs
- Whole blood (fresh or frozen collected in EDTA, heparin, or citrate)
- Whatman® FTA® or Schleicher and Schuell 903 cards

Note: Protocol and reagents have not been tested for purifying plasmid preps or PCR reactions and are not recommended for these sample types.

Input quantity

The optimal starting sample quantity varies widely depending on tissue and sample type. The following table summarizes the recommended inputs for compatible samples.

Table 3 Starting sample input

Sample type	Recommended starting input‡
Tissue samples with small to moderate DNA yields (such as brain or liver)	≤ 10 mg§
Tissue samples with high DNA yields (such as spleen or thymus)	5 mg
Cultured cells	≤ 1×10^6 cells
Buffy coat	≤ 50 µL
Blood samples	≤ 50 µL
Whatman® FTA® or SS 903 cards	2 × 2-mm punches
Buccal swabs	1 swab

‡ Or MagMAX™ Express-96 Deep Well Magnetic Particle Processor.
§ You can increase the input quantity, but increasing it >50% can make the sample difficult to work with and reduce yield.

Typical yield

Purified DNA yields that are isolated using the MagMAX™-96 DNA Multi-Sample Kit vary depending on tissue and sample type. The ranges shown below were obtained empirically using the MagMAX™-96 DNA Multi-Sample Kit and the procedures in this document.

Table 4 Typical yield

Sample type	Input quantity	Typical yield
Mouse brain	10 mg	10 to 30 µg
Mouse liver	10 mg	15 to 60 µg
Mouse tail	0.5 cm	10 to 40 µg
Mouse spleen	10 mg	15 to 80 µg
Cultured cells	1×10^6 cells	15 to 30 µg
Buffy coat	50 µL	5 to 15 µg
Blood samples	50 µL	1 to 3 µg
Whatman® FTA® or SS 903 cards	2 punches	0.2 to 1 µg
Buccal swabs	1 swab	

Choose a purification protocol

Purification protocols in this document

This document describes how to perform both automated isolation of genomic DNA from compatible samples using a MagMAX™ Express-96 Deep Well Magnetic Particle Processor. Perform the procedure appropriate to your sample type.

Guidelines

- Perform the protocol at room temperature (20 to 25 °C) except where noted.
- Store and use the DNA Elution Buffers at room temperature.
- Avoid creating bubbles when mixing samples by pipetting up/down.
- Cover the plates during the binding steps to prevent spill-over and cross-contamination.
- The titer plate shaking speeds in this protocol 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 Barnstead/Lab-Line Titer Plate Shaker, verify that the:
  - MagMax™ Deep Well Processing Plate fits securely on your titer plate shaker.
  - Recommended speeds are compatible with your titer plate shaker.
- Prepare the plates for the instrument before you begin the protocol.
- During the run, add reagents to plates as indicated by the instrument.
- Carefully remove the elution plate during and after the run. The plates are uncovered and can spill if dropped.
- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4412021 DW blood** and/or **4413021 DW tissues** protocols. If not, download and install the appropriate protocol to your MagMAX™ instrument as follows:

  a. Go to **http://www.appliedbiosystems.com/magmax**

  b. In the Automation page of the Applied Biosystems web site, click **MagMAX™ Express-96 Deep Well Magnetic Particle Processor**.

  c. In the MagMAX™ Express-96 Deep Well Magnetic Particle Processor page, right-click **4412021 DW blood** or **4413021 DW tissues**, then select **Save Link As**.

d. In the Save As dialog box, save the protocol file (*.kf2) to your computer desktop.

e. Install the protocol to your MagMAX™ instrument as explained in the *MagMAX™ Express-96 Deep Well Magnetic Particle Processor User Guide*.

Isolation of genomic DNA from solid tissue

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	8	808
Water, nuclease-free	12	1212
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 4 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4413021 DW tissues** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Disrupt the samples

1. For each sample, add 200 µL of Multi-Sample DNA Lysis Buffer to a 2-mL microcentrifuge tube, then add 10 mg tissue. For tissues that have large amounts of DNA, such as thymus or spleen, add half the quantity (5 mg).

   Note: Use a maximum of 10 mg tissue per isolation. For larger quantities: add 200 µL of lysis buffer for every 10 mg of tissue (for example, 2 mL to 100 mg of tissue), homogenize, then divide the lysate into 200-µL aliquots.

2. Homogenize the tissue until no pieces are visible (10 to 20 seconds using speed 3 or 4 on a 10-speed mechanical homogenizer).

   IMPORTANT! Homogenization at high speeds can cause excessive splashing or foaming.

   Note: Applied Biosystems recommends mechanical homogenization to maximize the efficiency and yield of the DNA isolation. If a homogenizer is unavailable, disrupt the samples by performing an overnight incubation of 10 mg samples in PK buffer as described in the mouse tail preparation.

Perform the DNA extraction and elution

1. Transfer 200 µL of each homogenate (~10 mg of tissue such as brain; ~5 mg of tissue such as spleen) to a well of a MagMAX™ Deep Well 96-Well Plate (purchased separately), then add 120 µL of 100% isopropanol to each lysate.

2. Seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of prepared DNA Binding Bead Mix to each lysate on the plate. Seal the plate, then shake for 3 minutes at speed 8 on the titer plate shaker.

4. While the plate is shaking, prepare sufficient RNase A mix for the number of samples you are preparing.

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

Component	Volume (µL)	
	1 well	96-well plate‡
RNase A	5	505
Water, nuclease-free	95	9595
Total (RNase A mix)	**100**	**10100**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

5. Prepare the plates for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor:

Plate		Reagent	Volume per well (µL)	Plate Type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 3)	180 to 380	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
RNase	4	RNase A mixture (5 µL RNase + 95 µL water per well)	100	
Wash 3	5	Wash Buffer 2	150	
Wash 4	6	Wash Buffer 2	150	
Elution	7	DNA Elution Buffer 1 for initial heated and elution	75 to 150	Standard

6. Load the MagMAX™ instrument, then start the protocol.

   a. Power on the MagMAX™ instrument.

   b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and the MagMAX™ Express 96-Well Plate.

   c. Using the keypad or MagMAX™ Express Software, select the **4413021 DW tissues** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates into the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, load the following reagents into the plates at the loading stations listed in the following table when prompted by the instrument.

Plate		Reagent	Volume per well (µL)
ID	Position		
RNase	4	Multi-Sample DNA Lysis Buffer and isopropanol **Note:** The instrument prompts you to add 100 µL lysis buffer and 120 µL isopropanol after the RNase digestion is complete.	220
Elution‡	7	DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the samples after the elution with DNA Elution Buffer 1 is complete.	75 to 150§

‡ Use equal volumes of Elution Buffer 1 and Elution Buffer 2.
§ Final volume is approximately 130 to 275 µL. Use greater volumes for tissues with large amounts of DNA.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from mouse tails

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Preheat a heated block (or alternate heat source) to 55 °C before beginning the proteinase K digestion.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	8	808
Water, nuclease-free	12	1212
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 4 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4413021 DW tissues** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Disrupt the samples

1. For each sample, transfer the following to a 1.5-mL microcentrifuge tube:
   - 92 µL of PK buffer
   - 8 µL of Proteinase K (100 mg/mL)
   - ≤ 0.5 cm of mouse tail
2. Incubate the samples in a heated block overnight at 55 °C.
3. Remove the samples from the heated block, then carefully transfer the liquid of each sample to a well on a MagMAX™ Deep Well 96-Well Plate (purchased separately), leaving behind any remaining tissue.

Perform the DNA extraction and elution

1. Add 100 µL of Multi-Sample DNA Lysis Buffer to each mouse tail sample on the MagMAX™ Deep Well 96-Well Plate (purchased separately), gently pipetting up/down 3 to 4 times after each transfer, then add 120 µL of 100% isopropanol to each sample on the plate.

2. Seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of prepared DNA Binding Bead Mix to each lysate on the plate. Seal the plate, then shake for 3 minutes at speed 8 on the titer plate shaker.

4. While the plate is shaking, prepare sufficient RNase A mix for the number of samples you are preparing.

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

Component	Volume (µL)	
	1 well	96-well plate‡
RNase A	5	505
Water, nuclease-free	95	9595
Total (RNase A mix)	**100**	**10100**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

5. Prepare the plates for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor:

Plate		Reagent	Volume per well (µL)	Plate Type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 3)	180 to 380	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
RNase	4	RNase A mixture (5 µL RNase + 95 µL water per well)	100	
Wash 3	5	Wash Buffer 2	150	
Wash 4	6	Wash Buffer 2	150	
Elution	7	DNA Elution Buffer 1 for initial heated and elution	150	Standard

6. Load the MagMAX™ instrument, then start the protocol.

   a. Power on the MagMAX™ instrument.

   b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and the MagMAX™ Express 96-Well Plate.

   c. Using the keypad or MagMAX™ Express Software, select the **4413021 DW tissues** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates into the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, load the following reagents into the plates at the loading stations listed in the following table when prompted by the instrument.

Plate		Reagent	Volume per well (µL)
ID	Position		
RNase	4	Multi-Sample DNA Lysis Buffer and isopropanol **Note:** The instrument prompts you to add 100 µL lysis buffer and 120 µL isopropanol after the RNase digestion is complete.	220
Elution	7	DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the samples after the elution with DNA Elution Buffer 1 is complete.	150‡

‡ Final volume is approximately 275 µL.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from cultured cells

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	16	1616
Water, nuclease-free	4	404
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 8 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4413021 DW tissues** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Disrupt the samples

1. If necessary, thaw the cell pellets at room temperature, 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, then vortex and mix each sample by pipetting up/down until the cell pellet is completely dissolved.

Perform the DNA extraction and elution

1. Transfer 200 µL of each cultured cell lysate (1×10^6 cells) to a well of a MagMAX™ Deep Well 96-Well Plate (purchased separately), then add 160 µL of 100% isopropanol to each sample.

   IMPORTANT! Mix each sample thoroughly before aliquotting.

2. Seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of prepared DNA Binding Bead Mix to each lysate on the plate. Seal the plate, then shake for 3 minutes at speed 8 on the titer plate shaker.

4. While the plate is shaking, prepare sufficient RNase A mix for the number of samples you are preparing.

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

Component	Volume (µL)	
	1 well	96-well plate‡
RNase A	5	505
Water, nuclease-free	95	9595
Total (RNase A mix)	**100**	**10100**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

5. Prepare the plates for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor:

Plate		Reagent	Volume per well (µL)	Plate Type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 3)	360	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
RNase	4	RNase A mixture (5 µL RNase + 95 µL water per well)	100	
Wash 3	5	Wash Buffer 2	150	
Wash 4	6	Wash Buffer 2	150	
Elution	7	DNA Elution Buffer 1 for initial heated and elution	150	Standard

6. Load the MagMAX™ instrument, then start the protocol.

   a. Power on the MagMAX™ instrument.

   b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and the MagMAX™ Express 96-Well Plate.

c. Using the keypad or MagMAX™ Express Software, select the **4413021 DW tissues** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates into the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, load the following reagents into the plates at the loading stations listed in the following table when prompted by the instrument.

Plate		Reagent	Volume per well (µL)
ID	Position		
RNase	4	Multi-Sample DNA Lysis Buffer and isopropanol **Note:** The instrument prompts you to add 100 µL lysis buffer and 120 µL isopropanol after the RNase digestion is complete.	220
Elution	7	DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the samples after the elution with DNA Elution Buffer 1 is complete.	150‡

‡ Final volume is approximately 275 µL.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from buffy coat samples

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	16	1616
Water, nuclease-free	4	404
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 8 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4413021 DW tissues** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Disrupt the samples

1. Centrifuge 10 mL of whole blood (in EDTA or other anticoagulant) at 3200 rpm for 15 minutes at room temperature.
2. For each sample, transfer the buffy coat layer to a new 2-mL tube, add 150 µL of Multi-Sample DNA Lysis Buffer for every 50 µL of buffy coat layer, then vortex and mix by pipetting up/down until the sample is homogenous.

Perform the DNA extraction and elution

1. Transfer 200 µL of each buffy coat lysate to a well of a MagMAX™ Deep Well 96-Well Plate (purchased separately), then add 160 µL of 100% isopropanol to each sample on the plate.

2. Seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of prepared DNA Binding Bead Mix to each lysate on the plate. Seal the plate, then shake for 3 minutes at speed 8 on the titer plate shaker.

4. While the plate is shaking, prepare sufficient RNase A mix for the number of samples you are preparing.

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

Component	Volume (µL)	
	1 well	96-well plate‡
RNase A	5	505
Water, nuclease-free	95	9595
Total (RNase A mix)	**100**	**10100**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

5. Prepare the plates for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor:

Plate		Reagent	Volume per well (µL)	Plate Type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 3)	180 to 380	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
RNase	4	RNase A mixture (5 µL RNase + 95 µL water per well)	100	
Wash 3	5	Wash Buffer 2	150	
Wash 4	6	Wash Buffer 2	150	
Elution	7	DNA Elution Buffer 1 for initial heated and elution	75	Standard

6. Load the MagMAX™ instrument, then start the protocol.

   a. Power on the MagMAX™ instrument.

   b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and the MagMAX™ Express 96-Well Plate.

   c. Using the keypad or MagMAX™ Express Software, select the **4413021 DW tissues** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates into the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, load the following reagents into the plates at the loading stations listed in the following table when prompted by the instrument.

Plate		Reagent	Volume per well (µL)
ID	Position		
RNase	4	Multi-Sample DNA Lysis Buffer and isopropanol **Note:** The instrument prompts you to add 100 µL lysis buffer and 120 µL isopropanol after the RNase digestion is complete.	220
Elution‡	7	DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the samples after the elution with DNA Elution Buffer 1 is complete.	75§

‡ Use equal volumes of Elution Buffer 1 and Elution Buffer 2.
§ Final volume is approximately 130 µL. Use greater volumes for tissues with large amounts of DNA.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from buccal swabs

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	16	1616
Water, nuclease-free	4	404
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 8 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4413021 DW tissues** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Disrupt the samples

1. For each buccal swab sample:
   a. Add 400 µL of Multi-Sample DNA Lysis Buffer to a well of a MagMAX™ Deep Well 96-Well Plate (purchased separately).
   b. Cut and remove the stick portion of the swab (to make shaking easier), then place it into a well containing lysis buffer.
2. Shake the unsealed plate on a titer plate shaker for 3 minutes at speed 8.
3. Remove the plate from the shaker, then carefully remove the swabs, leaving behind as much lysis buffer as possible (approximately 200 µL).

Perform the DNA extraction and elution

1. Add 160 µL of 100% isopropanol to each buccal swab sample on the MagMAX™ Deep Well 96-Well Plate (purchased separately).
2. Seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 8 on a titer plate shaker.
3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of prepared DNA Binding Bead Mix to each lysate on the plate. Seal the plate, then shake for 3 minutes at speed 8 on the titer plate shaker.
4. While the plate is shaking, prepare sufficient RNase A mix for the number of samples you are preparing.

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

Component	Volume (µL)	
	1 well	96-well plate‡
RNase A	5	505
Water, nuclease-free	95	9595
Total (RNase A mix)	**100**	**10100**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

5. Prepare the plates for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor:

Plate		Reagent	Volume per well (µL)	Plate Type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 3)	180 to 380	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
RNase	4	RNase A mixture (5 µL RNase + 95 µL water per well)	100	
Wash 3	5	Wash Buffer 2	150	
Wash 4	6	Wash Buffer 2	150	
Elution	7	DNA Elution Buffer 1 for initial heated and elution	75	Standard

6. Load the MagMAX™ instrument, then start the protocol.
   a. Power on the MagMAX™ instrument.
   b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and the MagMAX™ Express 96-Well Plate.
   c. Using the keypad or MagMAX™ Express Software, select the **4413021 DW tissues** protocol.
   d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates into the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, load the following reagents into the plates at the loading stations listed in the following table when prompted by the instrument.

Plate		Reagent	Volume per well (µL)
ID	Position		
RNase	4	Multi-Sample DNA Lysis Buffer and isopropanol **Note:** The instrument prompts you to add 100 µL lysis buffer and 120 µL isopropanol after the RNase digestion is complete.	220
Elution‡	7	DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the samples after the elution with DNA Elution Buffer 1 is complete.	75§

‡ Use equal volumes of Elution Buffer 1 and Elution Buffer 2.
§ Final volume is approximately 130 µL. Use greater volumes for tissues with large amounts of DNA.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from whole blood

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Preheat a heated block (or alternate heat source) to 60 to 65 °C before preparing the Proteinase K mixture.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	16	1616
Water, nuclease-free	4	404
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 8 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4412021 DW blood** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Perform the DNA extraction and elution

1. Perform the proteinase K digestion:

   a. Prepare the PK buffer/enzyme mix:

Component	Volume (µL)	
	1 well	96-well plate‡
Proteinase K Solution (100 mg/mL)	8	808
PK Digestion Buffer	42	4242
Total (PK buffer/enzyme mix)	**50**	**5050**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

   b. For each sample, add 50 µL of PK buffer/enzyme mix to a well on a MagMAX™-96 Deep Well Plate or processing plate (included in the MagMAX™-96 Kit) followed by 50 µL of blood sample. After each sample transfer, mix the solution by pipetting up/down 5 to 7 times.

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

   c. Seal the plate using a MicroAmp® Clear Adhesive Film, then incubate the sealed plate on a 96-well heated block for 20 minutes at 60 to 65 °C.

d. Remove the plate from the heat source, then carefully remove the cover.

2. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample on the plate, seal the plate, then shake for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of DNA binding bead mix to each sample on the plate. Seal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.

4. Remove the plate from the shaker, carefully remove the cover, then add 240 µL of 100% isopropanol to each sample on the plate. Seal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.

5. Prepare the plates for the MagMAX™ Express-96 Processor:

Plate		Reagent	Volume per well (µL)	Plate type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 4).	580	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
Wash 3	4	Wash Buffer 2	150	
Elution	5	DNA Elution Buffer 1 for initial heated and elution	75	Standard
		DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the sample after heating and mixing is complete with DNA Elution Buffer 1.	75‡	

‡ Final volume is approximately 130 µL.

6. Load the MagMAX™ instrument, then start the protocol:

a. Power on the MagMAX™ instrument.

b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and MagMAX™ Express 96-Well Plate.

c. Using the keypad or MagMAX™ Express Software, select the **4412021 DW blood** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates onto the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, add DNA Elution Buffer 2 to the elution plate when prompted by the instrument.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Isolation of genomic DNA from Whatman® FTA® of SS 906 cards

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Preheat a heated block (or alternate heat source) to 60 to 65 °C before preparing the Proteinase K mixture.
- Add isopropanol and ethanol to Wash Solution 1 Concentrate and Wash Solution 2 Concentrate respectively. See the reagent bottles for preparation instructions. Store the solutions at room temperature.
- Prepare sufficient DNA Binding Bead Mix for your sample extraction and store at room temperature. If you are preparing multiple samples, prepare 5% excess to account for error.

Component	Volume (µL)	
	1 well	96-well plate‡
DNA Binding Beads (10 mg/mL)	16	1616
Water, nuclease-free	4	404
Total (DNA binding bead mix)§	**20**	**2020**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.
§ Final concentration is 8 mg/mL.

- Confirm that your MagMAX™ Express-96 Deep Well Magnetic Particle Processor has installed the **4412021 DW blood** protocol. If not, download and install the protocol as explained in "Guidelines" on page 8.

Perform the DNA extraction and elution

1. Perform the proteinase K digestion:

   a. Prepare the PK buffer/enzyme mix:

Component	Volume (µL)	
	1 well	96-well plate‡
Proteinase K Solution (100 mg/mL)	8	808
PK Digestion Buffer	42	4242
Total (PK buffer/enzyme mix)	**50**	**5050**

‡ Includes volume sufficient for one 96-well plate plus 5% excess.

   b. For each sample, add 50 µL of PK buffer/enzyme mix to a well on a MagMAX™-96 Deep Well Plate or processing plate (included in the MagMAX™-96 Kit) followed by 2 × 2-mm punches of the FTA or SS 903 card. After each sample transfer, mix the solution by pipetting up/down 5 to 7 times.

   c. Seal the plate using a MicroAmp® Clear Adhesive Film, then incubate the sealed plate on a 96-well heated block for 20 minutes at 60 to 65 °C.

   d. Remove the plate from the heat source, then carefully remove the cover.

e. Carefully transfer the liquid from each well of the plate to another well of the same plate or to a new deep-well plate, leaving behind the card material.

2. Add 100 µL of Multi-Sample DNA Lysis Buffer to each sample on the plate, seal the plate, then shake for 3 minutes at speed 8 on a titer plate shaker.

3. Remove the plate from the shaker, carefully remove the cover, then add 20 µL of DNA binding bead mix to each sample on the plate. Seal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.

4. Remove the plate from the shaker, carefully remove the cover, then add 120 µL of 100% isopropanol to each sample on the plate. Seal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.

5. Prepare the plates for the MagMAX™ Express-96 Processor:

Plate		Reagent	Volume per well (µL)	Plate type
ID	Position			
Binding	1	Lysate, isopropanol, and beads (from steps 1 to 4).	580	Deep-well
Wash 1	2	Wash Buffer 1	150	
Wash 2	3	Wash Buffer 2	150	
Wash 3	4	Wash Buffer 2	150	
Elution	5	DNA Elution Buffer 1 for initial heated and elution	75	Standard
		DNA Elution Buffer 2 for equilibration **Note:** The instrument prompts you to add DNA Elution Buffer 2 to the sample after heating and mixing is complete with DNA Elution Buffer 1.	75‡	

‡ Final volume is approximately 130 µL.

6. Load the MagMAX™ instrument, then start the protocol:

a. Power on the MagMAX™ instrument.

b. Combine the MagMAX™ Express-96 Deep Well Tip Comb and MagMAX™ Express 96-Well Plate.

c. Using the keypad or MagMAX™ Express Software, select the **4412021 DW blood** protocol.

d. If using the keypad, press **start**. Alternatively, begin the run as instructed by the MagMAX™ Express Software.

e. If the lid is in place, open the sliding door.

f. Load the plates onto the loading station as prompted by the instrument. Press **start** after loading each plate.

g. After the run is started, add DNA Elution Buffer 2 to the elution plate when prompted by the instrument.

Unload the instrument

1. When the MagMAX™ instrument has completed the protocol, remove all plates from the loading station as prompted by the instrument. Press **start** after removing each plate.

   IMPORTANT! After removing the elution plate (the first plate removed) from the MagMAX™ instrument, which contains the purified DNA, cover the plate immediately. To prevent evaporation, do not allow the sample to sit uncovered at room temperature for an extended time.

   If precipitated DNA is visible in the samples, pipet up/down 5 to 10 times before covering the plate to ensure complete resuspension. Precipitate is common when preparing tissues that have a large amount of DNA (such as spleen or thymus).

2. When the MagMAX™ instrument displays END_OF_RUN, press **stop**.

3. Power off the MagMAX™ instrument.

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

Troubleshooting

Table 5 Troubleshooting MagMAX™-96 DNA Multi-Sample Kit experiments

Observation	Possible cause	Solution
Tissue samples: Low yield	DNA Binding Bead Mix stored incorrectly	Store the DNA Binding Bead Mix at room temperature for the short-term, or at 2 to 8 °C for the long-term. **IMPORTANT!** *Do not* freeze the DNA Binding Bead Mix.
	Insufficient amount of beads added	• Vortex the tube containing the DNA Binding Beads thoroughly immediately before making the mix. • Vortex the DNA Binding Bead Mix before adding the mix to the sample.
	Bead pellet lost during binding or washing steps	• When removing supernatant, angle the pipet tip(s) away from beads. • Place the plate on the magnetic stand for the recommended times.
	Beads added before isopropanol	Add the DNA Binding Bead Mix to the sample *after* adding the isopropanol.
	Incorrect volume of isopropanol used	Use the recommended volumes stated in the protocol.
	DNA is still bound to the beads or beads remain clumped after elution	• Equally increase the volumes of the DNA Elution Buffers. • Increase the speed of the thermomixer or shaker used during the first elution step. • After adding DNA Elution Buffer 2, pipet up/down to break apart the beads.
	DNA is not fully resuspended	
	Samples did not go through rebinding step	Verify that the samples were mixed with Multi-Sample DNA Lysis Buffer and isopropanol after the RNase digestion and before collection on the magnetic stand.
	Incomplete homogenization or lysis	*Tissue samples*: Verify that all tissue pieces are completely pulverized during homogenization. *Cultured cells or buffy coat*: Ensure that the cell pellet or buffy coat is completely dissolved and that the lysate is homogeneous. If necessary, pipet up/down before removing each aliquot.
	Incorrect elution conditions	• Add only one DNA Elution Buffer at a time to the sample. Use only DNA Elution Buffer 1 during the heating/mixing step. • Always add DNA Elution Buffer 2 *before* performing the collection on the magnetic stand. • Do not mix DNA Elution Buffer 1 and DNA Elution Buffer 2. • Do not use water or TE buffer to elute the DNA. • Heat the samples to 65 to 75 °C before and/or during shaking in DNA Elution Buffer 1. • Always preheat the heat source before performing the incubation.
	Poor sample quality	• Do not thaw frozen tissue prior to homogenization. • Do not allow frozen tissue pieces to sit in lysis buffer; homogenize the pieces immediately. • Remove all excess media from the cell pellets. • Be careful not to remove an excess of red blood cells or plasma with the buffy coat.

Table 5 Troubleshooting MagMAX™-96 DNA Multi-Sample Kit experiments

Observation	Possible cause	Solution
Blood samples: Low yield	Proteinase K stored incorrectly	Store the Proteinase K at 2 to 6 °C for the short-term, or -15 to -25 °C for the long-term.
	Incorrect volume of Proteinase K added	Add the specified volume listed in the protocol.
	Sample not heated sufficiently during protienase K digestion	• Always preheat the heat source before performing the incubation. • Always heat the samples at 60 to 65 °C so that the samples are in contact with the heat source. **Note:** If you use a hybridization oven or incubator to perform the incubation, verify that the plate or tube is heated completely and at a constant temperature.
	Beads stored incorrectly	Store the DNA Binding Bead Mix at room temperature for the short-term, or at 2 to 6 °C for the long-term. **IMPORTANT!** *Do not* freeze the DNA binding beads.
	Insufficient volume of beads added	• Vortex the tube containing the DNA binding beads thoroughly immediately before making the mix. • Vortex the DNA Binding Bead Mix before adding the mix to the sample.
	Beads added after isopropanol	Add the DNA binding beads to the sample *before* adding the isopropanol.
	Bead pellet lost during binding or washing steps	• When removing supernatant, angle the pipet tip(s) away from beads. **IMPORTANT!** The blood lysate is reddish-brown and opaque, so you must know where the beads are in relation to the magnet while you remove the supernatant. • Place the plate on magnetic stand for the recommended times.
	Incorrect volume of isopropanol used	Use the recommended volumes stated in the protocol.
	DNA is still bound to the beads or beads remain clumped after elution	• Equally increase the volumes of the DNA Elution Buffers. • Increase the speed of the thermomixer or shaker during the first elution step. • Pipet beads up/down (after adding DNA Elution Buffer 2) to break them apart.
	Incorrect elution conditions	• Add only one DNA Elution Buffer at a time to the sample. Use only DNA Elution Buffer 1 during the heating/mixing step. • Always add DNA Elution Buffer 2 *before* performing the collection on the magnetic stand. • Do not mix DNA Elution Buffer 1 and DNA Elution Buffer 2. • Do not use water or TE buffer to elute the DNA. • Heat the samples to 65 to 75 °C before and/or during shaking in DNA Elution Buffer 1. • Always preheat the heat source before performing the incubation.
	Poor sample quality	• Do not use blood stored at 2 to 6 °C for longer than 1 week. • Do not use frozen blood that has been thawed and then refrozen. • Verify the cell count using a hemocytometer (confirm that the blood has a low white cell count).

Table 5 Troubleshooting MagMAX™-96 DNA Multi-Sample Kit experiments

Observation	Possible cause	Solution
Bead carryover	Large amount of DNA in elution	• Increase the volumes of DNA Elution Buffers added to the sample. • Thoroughly pipet the sample up/down before collection on the magnetic stand. • Use smaller amounts of starting material. • Perform an additional collection on the magnetic stand, then transfer the sample to a new plate.
	Loose beads inadvertently transferred with eluate	After transferring the eluate to the elution plate/tube, perform another collection on the magnetic stand, then transfer the sample to a new plate/tube.
DNA does not perform in downstream applications	Salt carryover	• Verify that the correct type and volume of alcohol is added to each wash concentrate. • Remove the supernatant completely after each binding or wash step.
	Alcohol carryover	Allow the sample to dry completely before adding DNA Elution Buffer 1.
	Presence of inhibitors (eluate is a green or reddish color)	• Ensure that the collected blood samples have greater than half the expected volume of blood mixed with anticoagulant. **Note:** Excess anticoagulant, especially heparin, can cause inhibition. • Use a quantitative PCR or genotyping master mix that functions in the presence of inhibitors (for example, the TaqMan® GTXpress™ Master Mix [PN 4403311]). • Briefly centrifuge the samples and remove the clean eluate fraction to a new tube or plate.
DNA is sheared or degraded	Poor sample quality	• Do not thaw frozen tissue prior to homogenization. • Do not allow frozen tissue pieces to sit in lysis buffer; homogenize the pieces immediately. • Do not use blood stored at 2 to 6 °C for longer than 1 week. • Do not use frozen blood that has been thawed and then re-frozen.
	DNA repeatedly frozen and thawed	• Aliquot the DNA for storage. • Limit the freezing and thawing of the samples.
	DNA stored at elevated temperatures for extended periods (such as 37 °C)	• Do not store the purified DNA at elevated temperatures for more than 16 hours (overnight). • Store the purified DNA at 2 to 6 °C for the short-term, or -15 to -25 °C for the long-term.
Sample evaporation	DNA stored at 2 to 6 °C or room temperature for too long	• Aliquot and store the purified DNA at -15 to -25 °C. • Store the purified DNA at 2 to 6 °C for the short-term, or -15 to -25 °C for the long-term.
	Purified DNA eluate left uncovered	If evaporation occurs, resuspend the samples using water to correct the volume.
		Cover the plate immediately after transferring the eluate.

Ordering Information

Kits, consumables, and additional materials

MagMAX™ DNA Multi-Sample Kits

Kit	Quantity	Part number
MagMAX™ DNA Multi-Sample Kit	50-rxns	4413020
MagMAX™-96 DNA Multi-Sample Kit	1 × 96-rxns	4413021
MagMAX™-96 DNA Multi-Sample Kit	5 × 96-rxns	4413022

MagMAX™ instrumentation and consumables

Item	Source
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	‡
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Deep Well Tip Combs	4388487
MagMAX™ Express-96 Standard Plates	4388475

‡ See the Applied Biosystems/Ambion website for pricing and availability.

Magnetic stands and additional materials

Item	Source
6-Tube Magnetic Stand	AM10055
96-Well Magnetic-Ring Stand (96-ring magnet block)	AM10050
Magnetic Stand-96 (24-magnet block)	AM10027
Single Place Magnetic Stand	AM10026
Vortex adaptor	AM10014

PCR Good Laboratory Practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Appendix C

Safety

This appendix covers:

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 41.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page v.

General alerts for all chemicals

Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Chirgwin J, Przybyla A, MacDonald A, and Rutter W (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochem.* 18:5294.

Chomczynski P and Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.* 162:156–159.

Related documentation

The following related documents are shipped with the system:

Document	Part number
MagMAX™ DNA Multi-Sample Kit Protocol: 2-mL Reaction Tube	4425070
MagMAX™ DNA Multi-Sample Kit Protocol: 96-Well Reaction Plate	4428201
MagMAX™ DNA Multi-Sample Kit Protocol: MagMAX™ Express-96 Deep Well Magnetic Particle Processor	4428202
MagMAX™ DNA Multi-Sample Kit Quick Reference Card	4425071
MagMAX™-96 DNA Multi-Sample Kit Quick Reference Card	4425072

Portable document format (PDF) versions of this and other protocols, and the quick reference card are also available on the MagMAX™/MagMAX™-96 DNA Multi-Sample Kit CD.

Note: To open the user documentation included on the MagMAX™/MagMAX™-96 DNA Multi-Sample Kit CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "How to obtain support" on page vi.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page vi.

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Applied Biosystems

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

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