Applied Biosystems

MagMAX™-96 DNA Multi-Sample Kit

96-Well Reaction Plates

Protocol

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

NOTICE TO PURCHASER:

This product or portions thereof is manufactured and sold under license from GE Healthcare under U.S. Patent numbers 5,523,231 and 5,681,946 and other foreign patents.

End User Terms and Conditions

Acceptance. These terms and conditions shall govern the purchase, use, and transfer and acceptance of the products described in the purchase order, quotation or invoice, which products are sold and distributed by Applera Corporation to the Buyer/transferee of such products (the “End User”). The transfer/sale of products to the End User is expressly conditional upon End User’s acceptance of these terms and conditions.

Restrictions on Use. End Users are specifically not authorized to and are forbidden from reselling, transferring or distributing any products either as a stand alone product or as a component of another product. The right to use the products does not, in and of itself, include or carry any right of the End User to any GE Healthcare Bio-Sciences Corporation’s technology or intellectual property other than expressly provided herein. End Users may not attempt to reverse engineer parameters of any of GE Healthcare Bio-Sciences Corporation proprietary products or services.

DISCLAIMER OF WARRANTIES. GE HEALTHCARE BIO-SCIENCES CORPORATION PROVIDES NO WARRANTIES TO END USER (STATUTORY OR IMPLIED), INCLUDING WITHOUT LIMITATION, AS TO PRODUCT QUALITY, CONDITION, DESCRIPTION, MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, AND ALL SUCH WARRANTIES ARE HEREBY EXPRESSLY DISCLAIMED. GE HEALTHCARE BIO-SCIENCES CORPORATION HEREBY EXPRESSLY DISCLAIMS ANY WARRANTY REGARDING RESULTS OBTAINED THROUGH THE USE OF THE PRODUCTS, INCLUDING WITHOUT LIMITATION ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS.

Exclusion of Liability. GE Healthcare Bio-Sciences Corporation and its affiliates shall have no liability to an End User, including, without limitation, for any loss of use or profits, business interruption or any consequential, incidental, special or other indirect damages of any kind, regardless of how caused and regardless of whether an action in contract, tort, strict product liability or otherwise.

TRADEMARKS:

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

Part Number 4428201 Rev. B
08/2009

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 the 96-well reaction plate format without the use of a MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

Note: If you are using a MagMAX™ Processor to run the MagMAX™-96 Kit, see *MagMAX™-96 DNA Multi-Sample Kit Protocol for MagMAX™ Express-96 Deep Well Magnetic Particle Processor* (PN 4428202) for the automation protocol.

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.

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.	
Magnetic stand for 96-well plates: • Magnetic Stand-96 (24-magnet block) *or* • 96-Well Magnetic-Ring Stand (96-ring magnet block)	 AM10027 AM10050
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.

Workflow

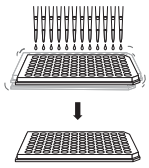
The following figure shows a simplified workflow for isolating DNA from a variety of tissues using the MagMAX™-96 DNA Multi-Sample Kit. Variations of this workflow, described in this protocol, can be used to purify isolates from a broader range of samples (see "Compatible sample types" on page 6).

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.

↓

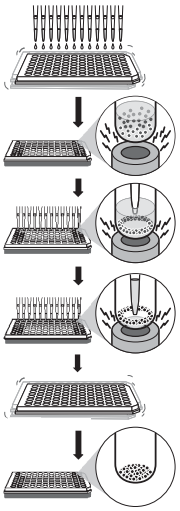
Perform the RNase A digestion.

↓

Wash twice using Wash Solution 2.

↓

Air dry the samples.



Perform the elution

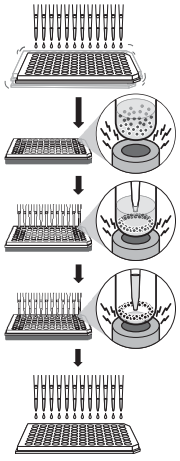
Elute samples using DNA Elution Buffer 1.

↓

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 manual isolation of genomic DNA from compatible samples using the 96-well plate reaction format. 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.
- When aspirating, be careful not to dislodge the DNA binding beads from the magnet.
- When capturing beads on the magnetic stand, you can remove the supernatant once the solution is clear. The bead collection times can vary depending on sample type and the quantity of nucleic acid used.
- Cover the plates during the binding, washing, and elution 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:
  - Processing plates fit securely on your titer plate shaker.
  - Recommended speeds are compatible with your titer plate shaker.

Isolation of genomic DNA from tissue

Before you begin

- Review the guidelines described in "Guidelines" on page 8.
- Preheat a thermomixer (or alternate heat source) to 70 °C.
- 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 the mix 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.

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 of buffer to 100 mg of tissue), homogenize, then divide the lysate into 200-µL aliquots.

2. Homogenize the tissue until no pieces of tissue 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 your samples by performing an overnight proteinase K digestion as described in the mouse tail preparation protocol.

Perform the DNA extraction

1. Add 200 µL of each homogenate to a well of the processing plate, add 120 µL of 100% isopropanol to each sample, seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 7 on a titer plate shaker.

2. Remove the plate from the shaker, then carefully remove the cover. Add 20 µL of prepared DNA Binding Bead Mix to each sample, reseal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.

3. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

4. Wash the beads using 150 µL of Wash Solution 1:

   IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

   b. Remove the plate from the magnetic stand, add 150 µL of Wash Solution 1 to each sample on the plate, reseal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

   c. Place the plate on the magnetic stand for approximately 1 minute to collect the beads.

5. Repeat step 4 once using 150 µL of Wash Solution 2.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads. Shake the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

7. While the plate is drying, prepare sufficient RNase A mix.

   IMPORTANT! Prepare the RNase A mix up to 20 minutes before use. Prolonged storage of the mix 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.

8. Remove the plate from the shaker, then add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

9. Remove the sealed plate from the shaker, carefully remove the cover, then 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 on the titer plate shaker.

   Note: Do not mix the DNA Lysis Buffer and isopropanol before adding them to the sample.

10. Place the sealed plate on the magnetic stand for 1 minute or until the beads are pelleted against the magnets.

11. Repeat step 4 twice using 150 µL of Wash Solution 2.

12. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

13. Dry the samples by shaking the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Add the appropriate volume of DNA Elution Buffer 1 to each sample:
   - 150 µL – Samples with large DNA content (such as spleen and thymus)
   - 50 to 100 µL – All other samples

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate for 5 minutes at 70 °C in a thermomixer (or another heated shaking device). 2. Shake the sealed plate at 900 rpm for 5 minutes at 70 °C on the thermomixer.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the sealed plate from the thermomixer or shaker, then carefully remove the cover. Add the appropriate volume of DNA Elution Buffer 2 to each sample:
   - 150 µL – Samples with large DNA content (such as spleen and thymus)
   - 50 to 100 µL – All other samples

   IMPORTANT! Add equal volumes of DNA Elution Buffer 1 and DNA Elution Buffer 2 to the samples.

4. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

5. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

   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 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 preparing the lysates. After the digestion, preheat the heated source to 70 °C before beginning the DNA extraction.
- 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 the mix 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.

Disrupt the samples

1. For each sample, add 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 mouse tail
2. Incubate the samples in a heated block overnight at 55 °C.
3. Remove the samples from the heated block, briefly centrifuge the plate, then carefully transfer the liquid of each sample to a well on the processing plate, leaving behind any remaining 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.

Perform the DNA extraction

1. Add 120 µL of 100% isopropanol to each sample, seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 7 on a titer plate shaker.
2. Remove the plate from the shaker, then carefully remove the cover. Add 20 µL of prepared DNA Binding Bead Mix to each sample, reseal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.
3. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

4. Wash the beads using 150 µL of Wash Solution 1:

   IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

   b. Remove the plate from the magnetic stand, add 150 µL of Wash Solution 1 to each sample on the plate, reseal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

   c. Place the plate on the magnetic stand for approximately 1 minute to collect the beads.

5. Repeat step 4 once using 150 µL of Wash Solution 2.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads. Shake the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

7. While the plate is drying, prepare sufficient RNase A mix.

   IMPORTANT! Prepare the RNase A mix up to 20 minutes before use. Prolonged storage of the mix 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.

8. Remove the plate from the shaker, then add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

9. Remove the sealed plate from the shaker, carefully remove the cover, then 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 on the titer plate shaker.

   Note: Do not mix the DNA Lysis Buffer and isopropanol before adding them to the sample.

10. Place the sealed plate on the magnetic stand for 1 minute or until the beads are pelleted against the magnets.

11. Repeat step 4 twice using 150 µL of Wash Solution 2.

12. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

13. Dry the samples by shaking the *uncovered* plate 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 on the plate.

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate for 5 minutes at 70 °C in a thermomixer (or another heated shaking device). 2. Shake the sealed plate at 900 rpm for 5 minutes at 70 °C on the thermomixer.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the sealed plate from the thermomixer or shaker, then carefully remove the cover. Add 100 µL of DNA Elution Buffer 2 to each sample on the plate.

4. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

5. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 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.
- Preheat a heated block (or alternate heat source) to 70 °C.
- 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 the mix 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.

Disrupt the samples

1. If necessary, thaw the cell pellets to 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.
3. Vortex and mix each sample by pipetting up/down until the cell pellet is completely dissolved.

Perform the DNA extraction

1. Transfer 200 µL of each homogenate (1×10^6 cells) to a well on the processing plate. Mix each sample thoroughly prior to aliquotting.
2. Add 160 µL of 100% isopropanol to each sample, seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 7 on a titer plate shaker.
3. Remove the plate from the shaker, then carefully remove the cover. Add 20 µL of prepared DNA Binding Bead Mix to each sample, reseal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.
4. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.
5. Wash the beads using 150 µL of Wash Solution 1:

IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

b. Remove the plate from the magnetic stand, add 150 µL of Wash Solution 1 to each sample on the plate, reseal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

c. Place the plate on the magnetic stand for approximately 1 minute to collect the beads.

6. Repeat step 5 once using 150 µL of Wash Solution 2.

7. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads. Shake the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

8. While the plate is drying, prepare sufficient RNase A mix.

IMPORTANT! Prepare the RNase A mix up to 20 minutes before use. Prolonged storage of the mix 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.

9. Remove the plate from the shaker, then add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

10. Remove the sealed plate from the shaker, carefully remove the cover, then 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 on the titer plate shaker.

Note: Do not mix the DNA Lysis Buffer and isopropanol before adding them to the sample.

11. Place the sealed plate on the magnetic stand for 1 minute or until the beads are pelleted against the magnets.

12. Repeat step 5 twice using 150 µL of Wash Solution 2.

13. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

14. Dry the samples by shaking the *uncovered* plate 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 on the plate.

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate for 5 minutes at 70 °C in a thermomixer (or another heated shaking device). 2. Shake the sealed plate at 900 rpm for 5 minutes at 70 °C on the thermomixer.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the sealed plate from the thermomixer or shaker, then carefully remove the cover. Add 100 µL of DNA Elution Buffer 2 to each sample on the plate.

4. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

5. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 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.
- Preheat a heated block (or alternate heat source) to 70 °C.
- 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 the mix 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.

Disrupt the samples

1. Centrifuge 10 mL of whole blood (in EDTA or other anticoagulant) at 3200 rpm for 15 minutes at room temperature (or preferred protocol).
2. Add 50 µL of each buffy coat sample to a well on the processing plate.
3. Add 50 µL of Multi-Sample DNA Lysis Buffer to each sample and mix by pipetting up/down until the sample is homogenous.

Perform the DNA extraction

1. Add 80 µL of 100% isopropanol to each sample, seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 7 on a titer plate shaker.
2. Remove the plate from the shaker, then carefully remove the cover. Add 20 µL of prepared DNA Binding Bead Mix to each sample, reseal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.
3. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.
4. Wash the beads using 150 µL of Wash Solution 1:

IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

b. Remove the plate from the magnetic stand, add 150 µL of Wash Solution 1 to each sample on the plate, reseal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

c. Place the plate on the magnetic stand for approximately 1 minute to collect the beads.

5. Repeat step 4 once using 150 µL of Wash Solution 2.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads. Shake the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

7. While the plate is drying, prepare sufficient RNase A mix.

IMPORTANT! Prepare the RNase A mix up to 20 minutes before use. Prolonged storage of the mix 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.

8. Remove the plate from the shaker, then add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

9. Remove the sealed plate from the shaker, carefully remove the cover, then 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 on the titer plate shaker.

Note: Do not mix the DNA Lysis Buffer and isopropanol before adding them to the sample.

10. Place the sealed plate on the magnetic stand for 1 minute or until the beads are pelleted against the magnets.

11. Repeat step 4 twice using 150 µL of Wash Solution 2.

12. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

13. Dry the samples by shaking the *uncovered* plate 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 on the plate.
2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate for 5 minutes at 70 °C in a thermomixer (or another heated shaking device). 2. Shake the sealed plate at 900 rpm for 5 minutes at 70 °C on the thermomixer.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the sealed plate from the thermomixer or shaker, then carefully remove the cover. Add 50 µL of DNA Elution Buffer 2 to each sample on the plate.
4. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.
5. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.
6. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 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.
- Preheat a thermomixer (or alternate heat source) to 70 °C.
- 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 the mix 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.

Disrupt the samples

1. For each buccal swab sample:
   a. Add 400 µL of Multi-Sample DNA Lysis Buffer to a well of the processing plate.
   b. Cut and remove the stick portion of the swab (to make shaking easier), then place the swab into the lysis buffer.
2. Shake the unsealed plate for 3 minutes at speed 7 on the titer plate shaker.
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

1. Add 160 µL of 100% isopropanol to each sample, seal the plate using a MicroAmp® Clear Adhesive Film, then shake the sealed plate for 3 minutes at speed 7 on a titer plate shaker.
2. Remove the plate from the shaker, then carefully remove the cover. Add 20 µL of prepared DNA Binding Bead Mix to each sample, reseal the plate, then shake for 3 minutes at speed 7 on the titer plate shaker.
3. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

4. Wash the beads using 150 µL of Wash Solution 1:

   IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

   b. Remove the plate from the magnetic stand, add 150 µL of Wash Solution 1 to each sample on the plate, reseal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

   c. Place the plate on the magnetic stand for approximately 1 minute to collect the beads.

5. Repeat step 4 once using 150 µL of Wash Solution 2.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads. Shake the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

7. While the plate is drying, prepare sufficient RNase A mix.

   IMPORTANT! Prepare the RNase A mix up to 20 minutes before use. Prolonged storage of the mix 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.

8. Remove the plate from the shaker, then add 100 µL of RNase A mix to each sample. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

9. Remove the sealed plate from the shaker, carefully remove the cover, then 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 on the titer plate shaker.

   Note: Do not mix the DNA Lysis Buffer and isopropanol before adding them to the sample.

10. Place the sealed plate on the magnetic stand for 1 minute or until the beads are pelleted against the magnets.

11. Repeat step 4 twice using 150 µL of Wash Solution 2.

12. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

13. Dry the samples by shaking the *uncovered* plate 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 on the plate.

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate for 5 minutes at 70 °C in a thermomixer (or another heated shaking device). 2. Shake the sealed plate at 900 rpm for 5 minutes at 70 °C on the thermomixer.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the sealed plate from the thermomixer or shaker, then carefully remove the cover. Add 50 µL of DNA Elution Buffer 2 to each sample on the plate.

4. Seal the plate, then shake for 2 minutes at speed 7 on the titer plate shaker.

5. Place the sealed plate on the magnetic stand for 3 minutes or until the solution clears and the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 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, and 70 °C after 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)	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.

Perform the DNA extraction

1. Perform the proteinase K digestion:

   a. Prepare the PK buffer/enzyme mix:

Component	Volume (µL)	
	1 well	96-well plate‡
Proteinase K Solution	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 the processing plate followed by 50 µL of whole 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 or Thermomixer (without shaking) for 20 minutes at 60 to 65 °C.

2. Remove the plate from the heat source, then carefully remove the cover. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample, seal the plate, then shake for 3 minutes at speed 7 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 sample on the plate. Seal the plate, then shake for 3 minutes at speed 6 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. Seal the plate, then shake for 3 minutes at speed 3 or 4 on the titer plate shaker.

   Note: If speed 4 shakes the samples too vigorously, use speed 3.

5. Place the sealed plate on the magnetic stand for 5 minutes until the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

   Note: The sample is dark and opaque during the first binding. When removing the supernatant, note where the magnet contacts the wells of the plate so that you can angle the pipet tips away from the magnetic beads.

7. Wash the samples using 150 µL of Wash Solution 1:

   IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Remove the plate from the magnetic stand, then add 150 µL of Wash Solution 1 to each sample on the plate. Seal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

   b. Place the sealed plate on the magnetic stand for 1 minute until the beads are pelleted against the magnets.

   c. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

8. Repeat step 7 *twice* using 150 µL of Wash Solution 2.

9. Dry the samples by shaking the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Remove the plate from the shaker, carefully remove the cover, then add 50 µL of DNA Elution Buffer 1 to each sample.

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate the plate in a thermomixer (or another heated shaking device) for 5 minutes at 70 °C. 2. Shake the sealed plate on a thermomixer at 900 rpm for 5 minutes at 70 °C.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the plate from the thermomixer or shaker, carefully remove the cover, then add 50 µL of DNA Elution Buffer 2 to each sample on the plate. After the transfer, mix gently by pipetting up/down 10 times. Seal the plate, then shake for 2 minutes at speed 7 at room temperature on the titer plate shaker.

4. Place the sealed plate on the magnetic stand for 5 minutes or until the solution clears and the beads are pelleted against the magnets.

5. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 to 6 °C for up to 24 hours or at – 20 to – 80 °C for prolonged storage.

Isolation of genomic DNA from Whatman® FTA® or SS 903 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, and 70 °C after 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)	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.

Perform the DNA extraction

1. Perform the proteinase K digestion:
   a. Prepare the PK buffer/enzyme mix:

Component	Volume (µL)	
	1 well	96-well plate‡
Proteinase K Solution	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 the processing plate followed by 2 × 2-mm punches of an 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 or Thermomixer (without shaking) for 20 minutes at 60 to 65 °C.
2. Remove the plate from the heat source, carefully remove the cover, then transfer the liquid from each plate well to a new well of the same plate or the corresponding well of a new 1.2 mL 96-well plate, leaving behind the card material. Add 100 µL of Multi-Sample DNA Lysis Buffer to each sample, seal the plate, then shake for 3 minutes at speed 7 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 sample on the plate. Seal the plate, then shake for 3 minutes at speed 6 on the titer plate shaker.

4. Remove the plate from the shaker, carefully remove the cover, then add 160 µL of 100% isopropanol to each sample. Seal the plate, then shake for 3 minutes at speed 4 on the titer plate shaker.

5. Place the sealed plate on the magnetic stand for 5 minutes until the beads are pelleted against the magnets.

6. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well, being careful not to disturb the beads.

   Note: The sample is dark and opaque during the first binding. When removing the supernatant, note where the magnet contacts the wells of the plate so that you can angle the pipet tips away from the magnetic beads.

7. Wash the samples using 150 µL of Wash Solution 1:

   IMPORTANT! Prepare Wash Solution 1 and Wash Solution 2 before use according to the instructions on the reagent bottles.

   a. Remove the plate from the magnetic stand, then add 150 µL of Wash Solution 1 to each sample on the plate. Seal the plate, then shake for 1 minute at speed 7 on the titer plate shaker.

   b. Place the sealed plate on the magnetic stand for 1 minute until the beads are pelleted against the magnets.

   c. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

8. Repeat step 7 *twice* using 150 µL of Wash Solution 2.

9. Dry the samples by shaking the *uncovered* plate for 2 minutes at speed 9 on the titer plate shaker.

Perform the elution

1. Remove the plate from the shaker, carefully remove the cover, then add 50 µL of DNA Elution Buffer 1 to each sample.

2. Incubate the plate using one of the following methods:

Method	Action
Heated shaking	1. Seal the plate, then incubate the plate in a thermomixer (or another heated shaking device) for 5 minutes at 70 °C. 2. Shake the sealed plate on a thermomixer at 900 rpm for 5 minutes at 70 °C.
Non-heated shaking	1. Seal the plate, then incubate the sealed plate on a 96-well heated block for 5 minutes at 70 °C. **IMPORTANT!** If you use a hybridization oven to perform the incubation, elevate the plate to allow air to circulate around it so that the bottom is heated adequately. 2. Shake the sealed plate for 5 minutes at speed 7 on the titer plate shaker at room temperature.

3. Remove the plate from the thermomixer or shaker, carefully remove the cover, then add 50 µL of DNA Elution Buffer 2 to each sample on the plate. After the transfer, mix gently by pipetting up/down 10 times. Seal the plate, then shake for 2 minutes at speed 7 at room temperature on the titer plate shaker.

4. Place the sealed plate on the magnetic stand for 5 minutes or until the solution clears and the beads are pelleted against the magnets.

5. Keeping the plate on the magnet, carefully remove the cover, then transfer the eluates (which contain the purified DNA) to the corresponding wells of the elution plate. Seal the plate immediately after the transfers are complete.

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 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.

Part Number 4428201 Rev. B 08/2009

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

International Sales
For our office locations please call the division headquarters or refer to our Web site at
www.appliedbiosystems.com/about/offices.cfm