

June 2012

BioSprint® 96 DNA Handbook

For purification of DNA from

human whole blood

animal whole blood

buffy coat

cultured cells

tissues

rodent tails

buccal swabs

dried blood spots

using the BioSprint 96 workstation



QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

BioSprint 96 DNA Blood Kit	(48)	(384)
Catalog no.	940054	940057
Number of preps*	48	384
Buffer AL [†]	12 ml	3 x 33 ml
QIAGEN [®] Protease	1 vial [‡]	2 vials [§]
Protease Solvent [¶]	1.2 ml	2 x 4.4 ml
MagAttract [®] Suspension G [¶]	1.6 ml	13 ml
Buffer AW1 [†] (concentrate)	27 ml	2 x 98 ml
Buffer AW2 (concentrate)	17 ml	2 x 81 ml
RNase-free water	4 x 10 ml	2 x 250 ml
Buffer AE	15 ml	128 ml
Large 96-Rod Cover	1	4
96-Well Microplate MP	2	8
S-Block	6	24
Quick-Start Protocol	1	1

* When each prep is from 200 μ l blood.

[†] Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

[‡] Resuspension volume 1.2 ml.

[§] Resuspension volume 4.4 ml.

[¶] Contains sodium azide as a preservative.

Storage

All buffers and reagents can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

Lyophilized QIAGEN Protease can be stored dry at room temperature for up to 1 year without any decrease in performance. For storage longer than 1 year or if ambient temperatures constantly exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for up to 2 months when stored at 2–8°C. Storing reconstituted QIAGEN Protease at room temperature for prolonged periods should be avoided. Reconstituted QIAGEN Protease can be stored at –20°C for up to 6 months, but repeated freezing and thawing should be avoided. We recommend dividing the reconstituted QIAGEN Protease into aliquots before storing at –20°C.

Intended Use

The BioSprint 96 DNA Blood Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers AL and AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the BioSprint 96 workstation, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of BioSprint 96 DNA Blood Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The BioSprint 96 DNA Blood Kit is designed for purification of total DNA (i.e., genomic and mitochondrial DNA) from whole blood, buffy coat, cultured cells, tissues, rodent tails, buccal swabs, dried blood spots, and other sample types using the BioSprint 96 workstation. The BioSprint 96 DNA Blood Kit provides high-quality DNA that is free of protein, nucleases, and other contaminants or inhibitors. The DNA is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions.

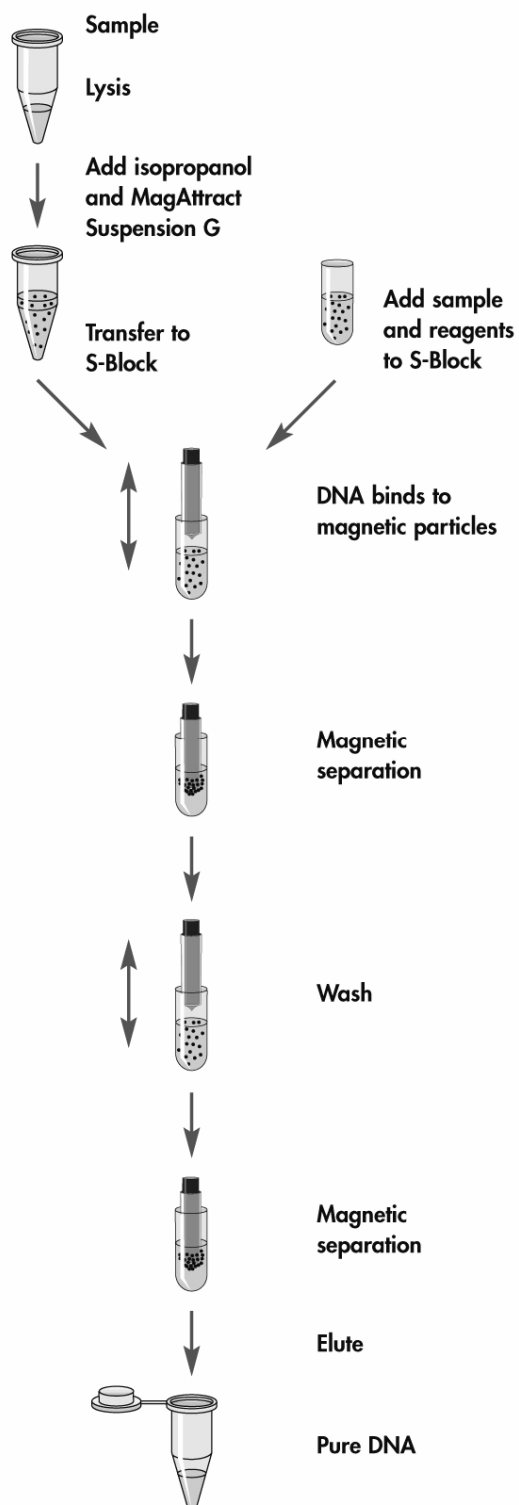
Principle and procedure

The BioSprint 96 DNA Blood Kit uses MagAttract magnetic-particle technology for DNA purification. MagAttract technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, next page). DNA binds to the silica surface of MagAttract magnetic particles in the presence of a chaotropic salt. DNA bound to the magnetic particles is then efficiently washed. Two different wash buffers are used, followed by a rapid rinse with distilled water or an air drying step, which considerably improves the purity of the DNA. High-quality DNA is eluted in Buffer AE. DNA yields depend on sample type, sample storage, and, if purifying from whole blood, white blood cell content.

Supplementary protocols for processing other sample types or for purification of different target molecules using the BioSprint 96 workstation are available at www.qiagen.com/literature/protocols or from QIAGEN Technical Services. BioSprint Software protocols for automated sample processing are available from QIAGEN Technical Services.

BioSprint 96 DNA Procedure

Standard protocol Rapid blood protocol*



* Rapid protocol only available for blood.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- BioSprint 96 workstation (cat. no. 9000852)
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 μ l)
- Multidispenser (e.g., Finnpiquette® Stepper from Thermo Electron see www.thermo.com)*
- Ethanol (96–100%)†
- Isopropanol (100%)
- Phosphate-buffered saline (PBS) (may be required for diluting samples)
- Buffer AE, cat. no. 19077 (may be required for diluting samples)
- Tween® 20
- Disposable gloves
- Vortexer
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the BioSprint worktable
- Buffer ATL, cat. no. 19076, if processing tissues, rodent tails, swabs, and dried blood spots
- QIAGEN Proteinase K (2 ml), cat. no. 19131, or QIAGEN Proteinase K (10 ml), cat. no. 19133, if processing tissues, rodent tails, swabs, and dried blood spots
- Tape Pads (5), cat. no. 19570, for sealing S-Blocks (not needed for rapid blood protocol)
- DNase-free RNase A (required if purified DNA needs to be RNA-free) (not required if processing swabs or dried blood spots)
- Swabs, such as sterile Omni Swabs (available from Whatman, www.whatman.com), or Puritan® applicators with plastic shafts and cotton or Dacron® tips (available from Hardwood Products, www.hwppuritan.com) if processing buccal swabs*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Filter paper (e.g., 903[®] Specimen Collection Paper, Blood Stain Card, or FTA[®] Card [Whatman, www.whatman.com], or comparable blood cards) if processing dried blood spots. We recommend using 903 Specimen Collection Paper with the BioSprint 96 workstation*
- Shaker-incubator (e.g., Eppendorf[®] Thermomixer Comfort, cat. no. 5355 000.011, with adapter for deep-well blocks [S-Blocks], cat. no. 5363 000.012) (not needed for rapid blood protocol)*
- Additional S-Blocks (24), cat. no. 19585, if processing swabs
- Multichannel pipettor and disposable pipet tips with aerosol barriers (200 μ l) (e.g., Finnpiptette Digital and Finntip Filters, see www.thermo.com) if processing swabs*
- Centrifuge with rotor capable of holding S-Blocks (e.g., Centrifuge 4-16, cat. nos. 81300, 81310, 81325, and 81320, and Plate Rotor 2 x 96, cat. no. 81031, from QIAGEN)
- Manual paper punch, 6 mm (1/4 inch) if processing dried blood spots

Optional: If lysis steps are not carried out in an S-Block

- Shaker-incubator (e.g., Eppendorf Thermomixer Comfort, cat. no. 5355 000.011) (not needed for the rapid blood protocol)*
- Microcentrifuge tubes (1.5 ml) if processing blood (not needed for rapid blood protocol), cells, tissues, or rodent tails
- Microcentrifuge tubes (2 ml) if processing buffy coat
- Microcentrifuge

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Starting material

The amounts of starting material for use in BioSprint 96 DNA procedures are shown in Table 1.

Table 1. Sample volumes used in BioSprint 96 DNA procedures

Sample	Amount of sample
Whole blood*	100–250 μ l
Buffy coat	100–200 μ l
Cultured cells	Up to 5×10^6 diploid cells
Tissues	Up to 25 mg
Rodent tails	1.2 cm (approximately 25 mg)
Dried blood spots	1–2 punches (6 mm [1/4 inch] diameter)
Buccal swabs	1 swab

* We recommend using 100–200 μ l animal blood containing non-nucleated erythrocytes. If necessary, the volume of animal blood used can be reduced and the sample volume adjusted to 200 μ l with Buffer AE. For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 20 μ l blood and adjust the sample volume to 200 μ l with Buffer AE.

Storing blood samples

Whole blood samples treated with EDTA, ACD, or heparin can be used, and may be either fresh or frozen. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results.

For short-term storage of up to 10 days, collect blood in tubes containing EDTA as an anticoagulant, and store at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –70°C.

Preparing buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 900–1100 x g for 10 minutes at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should be harvested from 10 ml centrifuged whole blood, which gives 10x enrichment. To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10x enrichment. If buffy coat samples are of >10x enrichment, use less starting material in the DNA purification procedure.

Yield and quality of purified DNA

DNA yields depend on the sample type, the number of nucleated cells in the sample, and the protocol used for DNA purification. Typical DNA yields obtained from a variety of sample types are shown in Table 2, page 13. Elution in smaller volumes increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

The BioSprint 96 DNA procedure yields pure DNA, with A_{260}/A_{280} ratios greater than 1.7. The purified DNA is up to 50 kb in size (Figures 1 and 2, pages 14 and 15), and is suitable for all downstream applications, including Southern blotting, PCR, and real-time PCR (Figure 3, page 15).

Table 2. Typical DNA yields from a range of sample types

Sample type	Amount of sample	Typical DNA yield (μg)*
Bovine tissue		
Muscle	25 mg	13.5 ± 1.5
Heart	25 mg	3.4 ± 0.6
Spleen	25 mg	59.1 ± 4.8
Lung	25 mg	14.7 ± 5.5
Liver	25 mg	74.0 ± 22.3
Kidney	25 mg	33.5 ± 5.4
Mouse tissue		
Tail	1.2 cm (~25 mg)	30.9 ± 4.5
Cultured cells		
HL-60	2×10^6 cells	9.6 ± 5.6
Blood		
Human ($5\text{--}7 \times 10^6$ cells/ml)	200 μl	4.5–9.0
Horse	200 μl	4.0–4.9
Bovine	200 μl	4.4–7.0
Sheep	200 μl	1.2–8.0
Pig	200 μl	4.5–9.0
Dog	200 μl	6.9–16.1
Cat	100 μl	2.9–6.3
Mouse	100 μl	1.5–3.0
Rat	100 μl	1.0–3.0
Bird [†]	10 μl	14.7 ± 2.9

* Genomic DNA was purified from the indicated samples.

[†] Sample volume adjusted to 200 μl with Buffer AE.

Table 2 continued on next page.

Table 2 continued from previous page.

Sample type	Amount of sample	Typical DNA yield (μg)*
Dried blood spots		
903 Specimen Collection Paper	1 punch (6 mm [1/4 inch] diameter)	0.10
FTA Card	1 punch (6 mm [1/4 inch] diameter)	0.05
Swabs		
Buccal swabs	1 swab	0.8–2.0

* Genomic DNA was purified from the indicated samples.

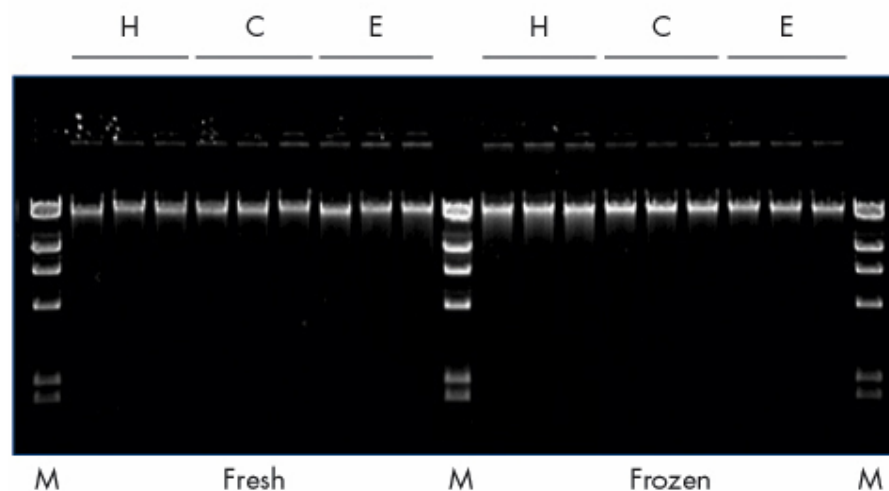


Figure 1. Purification of high-quality DNA from fresh and frozen blood. Human blood was collected and treated with one of 3 anticoagulants: heparin (**H**), citrate (**C**), or EDTA (**E**). DNA was purified from 200 μl blood immediately after blood collection (**Fresh**) and after one cycle of freezing and thawing (**Frozen**) using the BioSprint 96 DNA Blood Kit. DNA was eluted in 200 μl elution buffer. Eluates (15 μl) were run on a 0.8% agarose gel in 1x TBE. **M**: markers (Lambda *Hind*III).

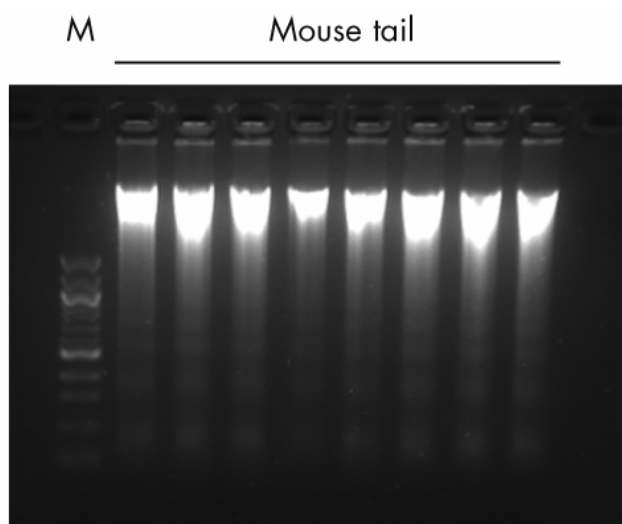


Figure 2. Purification of high-quality genomic DNA from mouse tail. Mouse tail samples were treated with 180 μ l Buffer ATL and 20 μ l QIAGEN Proteinase K at 56°C overnight. Genomic DNA was purified from the lysed tissue samples using the BioSprint 96 DNA Blood Kit with the BioSprint 96 DNA Tissue protocol. DNA was eluted in 200 μ l elution buffer. Eluates (2 μ l) from 8 out of 96 samples were visualized by agarose gel electrophoresis. **M**: markers (1 kb ladder).

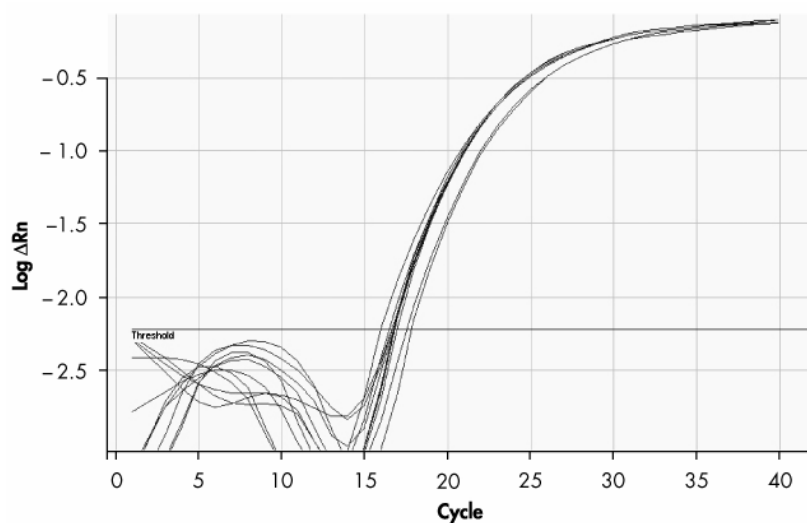


Figure 3. Efficient and sensitive real-time PCR. Genomic DNA was purified from mouse tail samples after overnight lysis at 56°C with 180 μ l Buffer ATL and 20 μ l QIAGEN Proteinase K. Purification was carried out using the BioSprint 96 DNA Blood Kit with the BioSprint 96 DNA Tissue protocol. The c-jun gene was amplified using 5 μ l purified DNA. Amplification reactions (50 μ l) were carried out on the Rotor-Gene™ system using the QuantiTect® Probe PCR Kit with gene-specific primers and probe.

Preparing reagents

QIAGEN Protease

Pipet Protease Solvent (which is nuclease-free water containing 0.04% (w/v) sodium azide) into the vial containing lyophilized QIAGEN Protease, as described on the vial label.

Reconstituted QIAGEN Protease is stable for up to 2 months when stored at 2–8°C. Storing reconstituted QIAGEN Protease at room temperature (15–25°C) for prolonged periods should be avoided. Reconstituted QIAGEN Protease can be stored at –20°C for up to 6 months, but repeated freezing and thawing should be avoided. We recommend dividing the reconstituted QIAGEN Protease into aliquots before storing at –20°C.

Buffer AL

Mix Buffer AL thoroughly by shaking before use. Buffer AL is stable for up to 1 year when stored at room temperature (15–25°C).

Note: Do not add QIAGEN Protease directly to Buffer AL.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as described on the bottle label; see also Table 3.

Table 3. Preparing Buffer AW1

Volume of AW1 concentrate (ml)	Volume of ethanol to add (ml)	Final volume (ml)
27	35	62
98	130	228

Tick the check box on the bottle to indicate that ethanol has been added. Store reconstituted Buffer AW1 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW1 before use by shaking the bottle 5 times.

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as described on the bottle label; see also Table 4.

Table 4. Preparing Buffer AW2

Volume of AW2 concentrate (ml)	Volume of ethanol to add (ml)	Final volume (ml)
17	40	57
81	190	271

Tick the check box on the bottle to indicate that ethanol has been added. Store reconstituted Buffer AW2 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW2 before use by shaking the bottle 5 times.

MagAttract Suspension G

To ensure that the magnetic silica particles are fully resuspended, MagAttract Suspension G must be shaken and vortexed before use. Before the first use, shake the vial or bottle, and vortex for 3 minutes. Before subsequent uses, shake the bottle, and vortex for 1 minute.

RNase-free water

In the BioSprint 96 DNA procedure, magnetic particles are briefly washed with RNase-free water to remove residual ethanol from the previous wash step with Buffer AW2. Tween 20 must be added to the RNase-free water to a final concentration of 0.02% (v/v) (e.g., add 6 μ l and 50 μ l Tween 20 to 30 ml and 250 ml RNase-free water, respectively).

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA, but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See the appendix, page 50, for more information.

Protocol: Purification of DNA from Blood

This protocol is for purification of total (genomic and mitochondrial) DNA from whole blood or blood products using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit. Human blood samples can be 100 μ l, 200 μ l, or 250 μ l. Animal blood samples containing non-nucleated erythrocytes can be 100 μ l or 200 μ l. Buffy coat samples can be 100 μ l or 200 μ l.

Important points before starting

- Tape sheets are required for this protocol. See “Equipment and Reagents to Be Supplied by User”, page 9
- Check that QIAGEN Protease, Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate by shaking the bottle. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Blood samples must be in the range of 100–250 μ l. Animal blood samples must be in the range 100–200 μ l. If necessary, the volume of animal blood used can be reduced and the sample volume adjusted to 100 μ l or 200 μ l with Buffer AE. Since bird and fish blood contain nucleated erythrocytes, use less than 20 μ l blood and adjust the sample volume to 200 μ l with Buffer AE. Buffy coat samples must be 100–200 μ l.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.
- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 100 μ l blood samples; choose ◆ if processing 200 μ l blood samples; choose ● if processing 250 μ l blood samples.

Things to do before starting

- Thaw and equilibrate up to 96 whole blood samples at room temperature (15–25°C), or prepare buffy coat samples according to page 12.
- Set a shaker-incubator with an adapter for S-Blocks to 70°C for use in step 4 of the procedure.

- All samples in a single procedure must have the same volume (100 μ l, 200 μ l, or 250 μ l). If the volume of a sample needs to be increased, add the appropriate volume of PBS (human blood samples) or Buffer AE (animal, bird, and fish blood samples).
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 μ l of a 100 mg/ml RNase A solution to a 200 μ l sample).

Procedure

- 1. Prepare five S-Blocks and two 96-well microplates according to Table 5 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 11.**

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 5. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)		
				■	◆	●
8	Load Rod Cover	96-well microplate MP	Large 96-Rod Cover	—	—	—
7	Load Elution	96-well microplate MP	Buffer AE	100	200	200
6	Load Wash 5	S-Block	RNase-free water*	500	500	500
5	Load Wash 4	S-Block	Buffer AW2	500	500	500
4	Load Wash 3	S-Block	Buffer AW2	500	500	500
3	Load Wash 2	S-Block	Buffer AW1	500	500	500
2	Load Wash 1	S-Block	Buffer AW1	500	650	800
1	Load Lysate	S-Block	Lysate [†]	325	650	810

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 2, 3, 6, and 7; includes volume of QIAGEN Protease, sample, Buffer AL, isopropanol, and MagAttract Suspension G.

- 2. Pipet ■ 10 μl, ◆ 20 μl, or ● 25 μl QIAGEN Protease into the bottom of a well of an S-Block. Add ■ 100 μl, ◆ 200 μl, or ● 250 μl sample to the QIAGEN Protease.**

Note: It is possible to add QIAGEN Protease to samples that have already been dispensed into the S-Block. In this case, it is important to ensure proper mixing after adding QIAGEN Protease.

Note: Record in which wells you load the samples.

3. **Add ■ 100 μ l, ◆ 200 μ l, or ● 250 μ l Buffer AL, and seal the S-Block with a tape sheet (not supplied). Mix by pulse-vortexing for 10 s.**

To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 70°C for 10 min.**

Maximum DNA yields are achieved after lysis at 70°C for 10 min. Longer incubation times should be avoided.

5. **Briefly centrifuge the S-Block to remove drops from underneath the tape. Remove the tape sheet from the S-Block.**

6. **Add ■ 100 μ l, ◆ 200 μ l, or ● 250 μ l isopropanol.**

7. **Add ■ 15 μ l, ◆ 30 μ l, or ● 35 μ l MagAttract Suspension G.**

Note: Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.

8. **Switch on the BioSprint 96 at the power switch.**

9. **Slide open the front door of the protective cover.**

10. **Select the protocol ■ “BS96 DNA Blood 100”, ◆ “BS96 DNA Blood 200”, or ● “BS96 DNA Blood 250” using the ▲ and ▼ keys. Press “Start” to start the protocol run.**

11. **The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover. After loading slot 8, press “Start”. The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press “Start” again. Continue this process of pressing “Start” and loading a particular slot until all slots are loaded.**

Table 5, page 20 shows in which slots the plates and blocks should be loaded.

Note: Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot’s label (i.e., well A1 faces inward).

12. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

See the *BioSprint 96 User Manual* for safety information.

13. **Press “Start” to start sample processing.**

- 14. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block. The first item to be removed contains the purified samples.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

- 15. Press “Stop” after all plates and blocks are removed.**
- 16. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

Note: See page 5 for safety information.

- 17. Switch off the BioSprint 96 at the power switch.**
- 18. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Rapid Purification of DNA from Human Whole Blood

This protocol is for rapid purification of total (genomic and mitochondrial) DNA from human whole blood using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit. Blood samples can be 100 μ l or 200 μ l. This shorter protocol has less manual handling steps than the standard protocol (see “Protocol: Purification of DNA from Blood Using the BioSprint 96”, page 18), but yield and purity of the purified DNA may be lower.

Important points before starting

- Check that QIAGEN Protease, Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- This protocol is suitable for human whole blood. Blood samples must be in the range of 100–200 μ l.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.
- In some steps of the procedure, one of two choices can be made. Choose ■ if processing 100 μ l blood samples; choose ◆ if processing 200 μ l blood samples.

Things to do before starting

- Thaw and equilibrate up to 96 whole blood samples at room temperature (15–25°C).
- All samples in a single procedure must have the same volume (100 μ l or 200 μ l). If the volume of a sample needs to be increased, add the appropriate volume of PBS.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 μ l of a 100 mg/ml RNase A solution to a 200 μ l sample).

- Prepare a master mix according to the table below for use in step 3 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, ■ 225 μ l or ◆ 450 μ l master mix is required per sample (see step 3 of the procedure). The starting volume of master mix should be increased accordingly.

Reagent	Volume of reagent per sample (μ l)	
	■	◆
Buffer AL	100	200
Isopropanol	100	200
MagAttract Suspension G	15	30

Procedure

- 1. Prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 6 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 7.**

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 6. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)	
				■	◆
8	Load Rod Cover	96-well microplate MP	Large 96-Rod Cover	—	—
7	Load Elution	96-well microplate MP	Buffer AE	100	200
6	Load Wash 5	S-Block	RNase-free water*	500	500
5	Load Wash 4	S-Block	Buffer AW2	500	500
4	Load Wash 3	S-Block	Buffer AW2	500	500
3	Load Wash 2	S-Block	Buffer AW1	500	500
2	Load Wash 1	S-Block	Buffer AW1	500	650
1	Load Lysate	S-Block	Lysate [†]	325	650

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 2 and 3; includes volume of QIAGEN Protease, sample, Buffer AL, isopropanol, and MagAttract Suspension G.

- 2. Pipet ■ 10 μl or ◆ 20 μl QIAGEN Protease into the bottom of a well of an S-Block. Add ■ 100 μl or ◆ 200 μl sample to the QIAGEN Protease.**

Note: Record in which wells you load the samples.

- 3. Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see “Things to do before starting”). Add ■ 215 μl or ◆ 430 μl master mix to each sample in the S-Block.**

Note: If using a multidispenser, add ■ 225 μl or ◆ 450 μl master mix to each sample.

4. Switch on the BioSprint 96 at the power switch.
5. Slide open the front door of the protective cover.
6. Select the protocol ■ "BS96 DNA Blood 100" or ♦ "BS96 DNA Blood 200" using the ▲ and ▼ keys. Press "Start" to start the protocol run.
7. The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 6, page 25). After loading slot 8, press "Start". The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.

Note: Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

8. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.

See the *BioSprint 96 User Manual* for safety information.

9. Press "Start" to start sample processing.
10. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

11. Press "Stop" after all plates and blocks are removed.
12. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.

Note: See page 5 for safety information.

13. Switch off the BioSprint 96 at the power switch.
14. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of DNA from Cultured Cells

This protocol is for purification of total (genomic and mitochondrial) DNA from up to 5×10^6 diploid cells per sample using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit.

Important points before starting

- Tape sheets are required for this protocol. See “Equipment and Reagents to Be Supplied by User”, page 9
- Check that QIAGEN Protease, Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaker–incubator with an adapter for S-Blocks or 1.5 ml microcentrifuge tubes (if lysis is not to be performed in an S-Block) to 70°C for use in step 5 of the procedure.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in step 2 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 μ l of a 100 mg/ml RNase A solution to a 200 μ l sample).
- Prepare a master mix according to the table on the next page for use in step 7 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Reagent	Volume of reagent per sample (μl)
Isopropanol	200
MagAttract Suspension G	30

Procedure

- 1. Prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 7 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 11.**

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 7. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	200
6	Load Wash 5	S-Block	RNase-free water*	500
5	Load Wash 4	S-Block	Buffer AW2	500
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	650
1	Load Lysate	S-Block	Lysate [†]	650

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 2–4, and 7; includes volume of sample, QIAGEN Protease, Buffer AL, isopropanol, and MagAttract Suspension G.

2. Centrifuge the appropriate number of cells (up to 5 x 10⁶ per sample) for 5 min at 300 x g. Discard the supernatant and resuspend the cell pellet in 200 μl PBS (not supplied). Lysis can either be performed in an S-Block or a 1.5 ml microcentrifuge tube (not supplied).

Cells can be grown in an S-Block in 1 ml of growth medium and incubated under appropriate growth conditions.

When using a frozen cell pellet, allow cells to thaw until the pellet can be dislodged before adding PBS.

Ensure that an appropriate number of cells are used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells.

Optional: RNase treatment of the sample. Add 4 μl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).

3. **Add 20 μ l QIAGEN Protease to the sample.**
4. **Add 200 μ l Buffer AL, and seal the S-Block with a tape sheet (not supplied) or close the 1.5 ml microcentrifuge tube. Mix by pulse-vortexing for 15 s.**

To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogenous solution.

Note: Do not add QIAGEN Protease directly to Buffer AL.

5. **Incubate at 70°C for 10 min.**

Maximum DNA yields are achieved after lysis at 70°C for 10 min. Longer incubation times should be avoided.

6. **Briefly centrifuge the S-Block or 1.5 ml microcentrifuge tube to remove drops from underneath the tape or lid. Remove the tape sheet from the S-Block. If lysis was performed in microcentrifuge tubes, transfer the lysates to an S-Block.**
7. **Vortex the master mix containing isopropanol and MagAttract Suspension G for 1 min (see “Things to do before starting”). Add 230 μ l master mix to each sample in the S-Block.**

Note: If using a multidispenser, add 225 μ l master mix to each sample.

8. **Switch on the BioSprint 96 at the power switch.**
9. **Slide open the front door of the protective cover.**
10. **Select the protocol “BS96 DNA Blood 200” using the $\blacktriangle$ and $\blacktriangledown$ keys on the BioSprint 96 workstation. Press “Start” to start the protocol run.**
11. **The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 7, page 29). After loading slot 8, press “Start”. The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press “Start” again. Continue this process of pressing “Start” and loading a particular slot until all slots are loaded.**

Note: Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot’s label (i.e., well A1 faces inward).

12. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

See the *BioSprint 96 User Manual* for safety information.

13. **Press “Start” to start sample processing.**

- 14. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block. The first item to be removed contains the purified samples.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

- 15. Press “Stop” after all plates and blocks are removed.**
- 16. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

Note: See page 5 for safety information.

- 17. Switch off the BioSprint 96 at the power switch.**
- 18. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of DNA from Tissues

This protocol is for the purification of total (genomic and mitochondrial) DNA from up to 25 mg of tissue per sample using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit.

Important points before starting

- Buffer ATL, QIAGEN Proteinase K, and tape sheets are required for this protocol. See “Equipment and Reagents to Be Supplied by User”, page 9.
- Check that Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaker–incubator with an adapter for S-Blocks or 1.5 ml microcentrifuge tubes (if lysis is not to be performed in an S-Block) to 56°C for use in step 3 of the protocol.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in step 3 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table on the next page for use in step 6 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, 450 µl master mix is required per sample (see step 6 of the procedure). The starting volume of master mix should be increased accordingly.

Reagent	Volume of reagent per sample (μl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	30

Procedure

1. Cut ≤25 mg of each tissue sample into small pieces. Place the tissue sample into the well of an S-Block or into a 1.5 ml microcentrifuge tube (not supplied), and add 180 μl Buffer ATL (not supplied).
2. Add 20 μl QIAGEN Proteinase K (not supplied), and seal the S-Block with a tape sheet (not supplied) or close the 1.5 ml microcentrifuge tube.
3. Place the S-Block or 1.5 ml microcentrifuge tube in a shaker-incubator, and incubate at 56°C with shaking until the tissue is completely lysed.

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

Optional: Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. If RNA-free genomic DNA is required, add 4 μl of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).

4. Towards the end of proteinase K digestion, prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 8 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 10.

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 8. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	200
6	Load Wash 5	S-Block	RNase-free water*	500
5	Load Wash 4	S-Block	Buffer AW2	500
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	650
1	Load Lysate	S-Block	Lysate [†]	630

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 1, 2, and 6; includes volume of sample, QIAGEN Proteinase K, Buffer ATL, Buffer AL, isopropanol, and MagAttract Suspension G.

- Briefly centrifuge the S-Block or 1.5 ml microcentrifuge tube containing the sample to remove drops from underneath the tape or inside the lid. Remove the tape sheet from the S-Block. If lysis was performed in 1.5 ml microcentrifuge tubes, transfer the lysates to an S-Block.**
- Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see “Things to do before starting”). Add 430 μl of master mix to each sample in the S-Block.**
Note: If using a multidispenser, add 450 μl master mix to each sample.
- Switch on the BioSprint 96 at the power switch.**
- Slide open the front door of the protective cover.**
- Select the protocol “BS96 DNA Tissue” using the ▲ and ▼ keys on the BioSprint 96 workstation. Press “Start” to start the protocol run.**

- 10. The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 8, page 34). After loading slot 8, press "Start". The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

Note: Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

- 11. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

See the *BioSprint 96 User Manual* for safety information.

- 12. Press "Start" to start sample processing.**

- 13. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

- 14. Press "Stop" after all plates and blocks are removed.**

- 15. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

Note: See page 5 for safety information.

- 16. Switch off the BioSprint 96 at the power switch.**

- 17. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of DNA from Rodent Tails

This protocol is for the purification of total (genomic and mitochondrial) DNA from up to 1.2 cm (approximately 25 mg) of rodent tail per sample using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit.

Important points before starting

- Buffer ATL, QIAGEN Proteinase K, and tape sheets are required for this protocol. See “Equipment and Reagents to Be Supplied by User”, page 9.
- Check that Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaker–incubator with an adapter for S-Blocks or 1.5 ml microcentrifuge tubes (if lysis is not to be performed in an S-Block) to 56°C for use in step 3 of the procedure.
- MagAttract magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in step 3 of the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).
- Prepare a master mix according to the table on the next page for use in step 6 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed. If using a multidispenser, 450 µl master mix is required per sample (see step 6 of the procedure). The starting volume of master mix should be increased accordingly.

Reagent	Volume of reagent per sample (μl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	30

Procedure

1. Cut ≤ 1.2 cm (approximately 25 mg) of each rodent tail sample into small pieces. Place the tissue sample into the well of an S-Block or into a 1.5 ml microcentrifuge tube (not supplied), and add 180 μ l Buffer ATL (not supplied).
2. Add 20 μ l QIAGEN Proteinase K (not supplied), and seal the S-Block with a tape sheet (not supplied) or close the microcentrifuge tube.
3. Place the S-Block or 1.5 ml microcentrifuge tube in a shaker-incubator, and incubate at 56°C with shaking until the tissue is completely lysed.

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

Optional: If RNA-free genomic DNA is required, add 4 μ l of RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature (15–25°C).

4. Towards the end of proteinase K digestion, prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 9 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 10.

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 9. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	200
6	Load Wash 5	S-Block	RNase-free water*	500
5	Load Wash 4	S-Block	Buffer AW2	500
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	650
1	Load Lysate	S-Block	Lysate [†]	630

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 1, 2, and 6; includes volume of sample, QIAGEN Proteinase K, Buffer ATL, Buffer AL, isopropanol, and MagAttract Suspension G.

5. **Briefly centrifuge the S-Block or microcentrifuge tube containing the sample to remove drops from underneath the tape or inside the lid. Remove the tape sheet from the S-Block. If lysis was performed in microcentrifuge tubes, transfer the lysates to an S-Block**
6. **Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see “Things to do before starting”). Add 430 μl of master mix to each sample in the S-Block. Note:** If using a multidispenser, add 450 μl master mix to each sample.
7. **Switch on the BioSprint 96 at the power switch.**
8. **Slide open the front door of the protective cover.**
9. **Select the protocol “BS96 DNA Tissue” using the ▲ and ▼ keys on the BioSprint 96 workstation. Press “Start” to start the protocol run.**

- 10. The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 9, page 38). After loading slot 8, press "Start". The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

Note: Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

- 11. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

See the *BioSprint 96 User Manual* for safety information.

- 12. Press "Start" to start sample processing.**

- 13. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

- 14. Press "Stop" after all plates and blocks are removed.**

- 15. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

Note: See page 5 for safety information.

- 16. Switch off the BioSprint 96 at the power switch.**

- 17. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of DNA from Buccal Swabs

This protocol is for purification of total (genomic and mitochondrial) DNA from buccal swabs using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit. The procedure is optimized for air-dried buccal swabs with cotton or Dacron tips, and brushes or swabs with an ejectable head (e.g., Whatman® Omni Swab). Other swab types may also be used.

Important points before starting

- Buffer ATL and QIAGEN Proteinase K are required for this protocol. If lysis is to be performed overnight, tape sheets for sealing the S-Block are also required. See “Equipment and Reagents to Be Supplied by User”, page 9.
- Check that Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaker–incubator with an adapter for S-Blocks to 56°C for use in step 4 of the procedure.
- Prepare a master mix according to the table on the next page for use in step 7 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Reagent	Volume of reagent per sample (µl)
Buffer AL	200
Isopropanol	200
MagAttract Suspension G	20

Procedure

1. Place the swab into the well of an S-Block.

If using an Omni Swab, eject the swab head by pressing the end of the inner shaft towards the swab head.

If using a cotton or Dacron swab, separate the swab head from its shaft by hand or by using scissors.

2. If using an Omni Swab, add 500 μ l Buffer ATL (not supplied) to the well containing the swab. If using a cotton or Dacron swab, add 400 μ l Buffer ATL to the well containing the swab.

3. Add 20 μ l QIAGEN Proteinase K (not supplied), and mix by pulse vortexing for 10 s.

Note: Since the S-Block is not covered, avoid vortexing at high speeds.

4. Place the S-Block in a shaker-incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.

If it is more convenient, samples can be lysed overnight; this will not affect the DNA quality.

Note: When lysis is performed overnight, we recommend sealing the S-Block using a tape sheet (not supplied). After incubation, briefly centrifuge the S-Block to remove drops from underneath the tape.

5. Towards the end of proteinase K digestion, prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 10 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 11.

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 10. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	200
6	Load Wash 5	S-Block	RNase-free water*	500
5	Load Wash 4	S-Block	Buffer AW2	500
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	650
1	Load Lysate	S-Block	Lysate [†]	620

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 6 and 7; includes volume of QIAGEN Proteinase K, Buffer ATL, Buffer AL, isopropanol, and MagAttract Suspension G.

6. Using a multichannel pipettor, carefully transfer 200 μl of the lysate from each well to a fresh S-Block. Do not transfer the swabs to the fresh S-Block.
7. Vortex the master mix containing Buffer AL, isopropanol, and MagAttract Suspension G for 1 min (see “Things to do before starting”). Add 420 μl master mix to each sample in the S-Block.
Note: If using a multidispenser, add 400 μl master mix to each sample.
8. Switch on the BioSprint 96 at the power switch.
9. Slide open the front door of the protective cover.

10. Select the protocol “BS96 DNA Swab” using the ▲ and ▼ keys on the BioSprint 96 workstation. Press “Start” to start the protocol run.

11. The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 10, page 42). After loading slot 8, press “Start”. The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press “Start” again. Continue this process of pressing “Start” and loading a particular slot until all slots are loaded.

Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot’s label (i.e., well A1 faces inward).

12. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.

Warning: To avoid contact with moving parts during operation of the BioSprint 96, do not insert your hands and fingers inside the workstation. See the *BioSprint 96 User Manual* for safety information.

13. Press “Start” to start sample processing.

14. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block. The first item to be removed contains the purified samples.

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

15. Press “Stop” after all plates and blocks are removed.

16. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.

Note: See page 5 for safety information.

17. Switch off the BioSprint 96 at the power switch.

18. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of DNA from Dried Blood Spots

This protocol is for purification of total (genomic and mitochondrial) DNA from blood card punches using the BioSprint 96 workstation and the BioSprint 96 DNA Blood Kit. This protocol is suitable for untreated blood or blood treated with anticoagulants such as EDTA, citrate, or heparin. The blood must be spotted and dried on filter paper according to the manufacturer's instructions. We recommend using 903 Specimen Collection Paper with the BioSprint 96.

Important points before starting

- Buffer ATL, QIAGEN Proteinase K, and tape sheets are required for this protocol. See "Equipment and Reagents to Be Supplied by User", page 9.
- Check that Buffer AW1, Buffer AW2, and RNase-free water have been prepared according to the instructions on pages 16–17.
- Check that Buffer AL and Buffer ATL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ATL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Things to do before starting

- Set a shaker–incubator with an adapter for S-Blocks to 56°C for use in steps 4 and 8 of the procedure.

Procedure

1. **Cut 6 mm (1/4 inch) diameter punches from a dried blood spot with a single-hole paper punch. Place 1 or 2 blood card punches into the well of an S-Block.**

Note: We do not recommend using punches with a diameter of less than 6 mm.

2. **Add 200 μ l Buffer ATL (not supplied).**
3. **Add 20 μ l QIAGEN Proteinase K (not supplied), and seal the S-Block with a tape sheet (not supplied). Mix by pulse-vortexing for 10 s.**

Note: Make sure that the punches are fully covered with buffer. If necessary, briefly centrifuge the S-Block after mixing.

4. Place the S-Block in a shaker-incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.
5. Towards the end of proteinase K digestion, prepare five S-Blocks (slots 2–6) and two 96-well microplates (slots 7 and 8) according to Table 11, below. The S-Blocks and microplates are loaded onto the worktable in step 15.

In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 11. BioSprint 96 worktable setup and reagent volumes

Slot	Message when loading	Plate/block	To add	Volume per well (μl)
8	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
7	Load Elution	96-well microplate MP	Buffer AE	125
6	Load Wash 5	S-Block	RNase-free water*	500
5	Load Wash 4	S-Block	Buffer AW2	500
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	650
1	Load Lysate	S-Block	Lysate [†]	640

* Contains 0.02% (v/v) Tween 20.

[†] Added at steps 1, 2, 3, 7, 10, and 11; includes volume of QIAGEN Proteinase K, Buffer ATL, Buffer AL, isopropanol, and MagAttract Suspension G.

6. Briefly centrifuge the S-Block containing the samples to remove drops from underneath the tape. Remove the tape sheet from the S-Block.
7. Add 200 μ l Buffer AL, seal the S-Block with a tape sheet, and mix by pulse-vortexing for 10 s.
Note: Make sure that the punches are fully covered with buffer. If necessary, briefly centrifuge the S-Block after mixing.
8. Place the S-Block in a shaker-incubator, and incubate at 56°C with shaking at 900 rpm for 10 min.
9. Briefly centrifuge the S-Block containing the samples to remove drops from underneath the tape. Remove the tape sheet from the S-Block.
10. Add 200 μ l isopropanol.
11. Add 20 μ l MagAttract Suspension G.
Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.
12. Switch on the BioSprint 96 at the power switch.
13. Slide open the front door of the protective cover.
14. Select the protocol "BS96 DNA Dried Blood" using the $\blacktriangle$ and $\blacktriangledown$ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.
15. The LCD displays a message asking you to load slot 8 of the worktable with the 96-rod cover (see Table 11, page 45). After loading slot 8, press "Start". The worktable rotates and a new message appears, asking you to load slot 7 with the elution plate. Load slot 7 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.
Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).
16. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.
See the *BioSprint 96 User Manual* for safety information.
17. Press "Start" to start sample processing.

- 18. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block. The first item to be removed contains the purified samples.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see the appendix, page 50).

- 19. Press “Stop” after all plates and blocks are removed.**
20. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.

Note: See page 5 for safety information.

- 21. Switch off the BioSprint 96 at the power switch.**
22. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

Note: Do not use bleach as disinfectant. See page 5 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Comments and suggestions

Low DNA yield

- | | |
|---|--|
| a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL | Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing. |
| b) Inefficient cell lysis due to decreased protease activity | Repeat the DNA purification procedure with a new sample and with freshly reconstituted QIAGEN Protease. Be sure to store QIAGEN Protease at 2–8°C immediately after use. Ensure that QIAGEN Protease is not added directly to Buffer AL. |
| c) No isopropanol added to the lysate before adding MagAttract Suspension G | Repeat the DNA purification procedure with a new sample. |
| d) MagAttract Suspension G was not completely resuspended | Before starting the procedure, ensure that the MagAttract Suspension G is fully resuspended. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses. |
| e) Buffer AW1 or AW2 prepared incorrectly | Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of ethanol (96–100%) (see pages 16–17). Repeat the DNA purification procedure with a new sample. |
| f) Frozen blood samples were not mixed properly after thawing | Thaw frozen blood samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing. |

Comments and suggestions

DNA does not perform well in downstream applications

- | | |
|--|---|
| a) Insufficient DNA used in downstream application | Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 50). |
| b) Excess DNA used in downstream application | Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 50). |

A_{260}/A_{280} ratio for purified DNA is low

- | | |
|--|--|
| a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL | Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing. |
| b) Inefficient cell lysis due to decreased protease activity | Repeat the DNA purification procedure with a new sample and with freshly reconstituted QIAGEN Protease. Be sure to store QIAGEN Protease at 2–8°C immediately after use. Ensure that QIAGEN Protease is not added directly to Buffer AL. |
| c) No isopropanol added to the lysate before adding MagAttract Suspension G | Repeat the DNA purification procedure with a new sample. |
| d) Buffer AW1 or AW2 prepared incorrectly | Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of ethanol (96–100%) (see pages 16–17). Repeat the DNA purification procedure with a new sample. |
| e) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm | To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see the appendix, page 50). |

Appendix: Handling, Quantification, and Determination of Purity of DNA

Storage of DNA

Purified DNA may be stored at 2–8°C for 24 hours or at –20°C for longer periods.

Minimizing magnetic particle carryover in the DNA

If the purified DNA is to be analyzed by real-time PCR, any trace amounts of magnetic particles should be minimized using a magnet.

Transfer the eluates to 1.5 ml microcentrifuge tubes. Apply the tubes to a suitable magnet (e.g., QIAGEN 12-Tube Magnet) for 10 minutes, and carefully remove the supernatants. Alternatively, transfer the eluates to a flat-bottom microplate (e.g., QIAGEN 96-Well Microplate FB). Apply the microplate to a suitable magnet (e.g., QIAGEN 96-Well Magnet Type A) for 10 minutes, and carefully remove the supernatants.

If a suitable magnet is not available, transfer the eluates to microcentrifuge tubes, centrifuge for 1 minute at full speed to pellet any remaining magnetic particles, and carefully remove the supernatants.

Quantification and determination of purity of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 μg of DNA per ml ($A_{260} = 1 \rightarrow 50 \mu\text{g/ml}$). Use a low-salt buffer of neutral pH (e.g., 10 mM Tris·HCl,* pH 7) to dilute DNA samples and to calibrate the spectrophotometer.

The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity. For accurate results, use a slightly alkaline buffer (e.g., 10 mM Tris·HCl, pH 7.5) to dilute DNA samples and to calibrate the spectrophotometer. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Carryover of magnetic particles in the eluates may affect the A_{260} and A_{280} readings, but should not affect the performance of the DNA in downstream applications. Measure the absorbance at 320 nm, 280 nm, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Concentration of DNA sample = $50 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$

Total amount of DNA isolated = concentration $\times$ volume of sample in ml

Purity of DNA sample = $(A_{260} - A_{320}) / (A_{280} - A_{320})$

Ordering Information

Product	Contents	Cat. no.
BioSprint 96 DNA Blood Kit (48)	For 48 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	940054
BioSprint 96 DNA Blood Kit (384)	For 384 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	940057
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Buffer AE (240 ml)	240 ml Elution Buffer	19077
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Accessories		
Large 96-Rod Cover (16)	16 x 96-Rod Covers for use with the BioSprint 96	1031668
96-Well Microplates MP (20)	96-well microplates, 20 per case, for use with the BioSprint 96	1031656
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case, for use with the BioSprint 96	19585
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells for use with the 96-Well Magnet Type A; pack of 24	36985

Product	Contents	Cat. no.
Centrifuges		
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor	81300*; 81310†; 81325‡; 81320§
Centrifuge 4-16K	Refrigerated universal laboratory centrifuge with brushless motor	81400*; 81410†; 81425‡; 81420§
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Related products		
BioSprint 15 DNA Blood Kits — for rapid purification of DNA from cells, tissue, blood, buffy coat, buccal swabs, and dried blood spots using the BioSprint 15 workstation		
BioSprint 15 DNA Blood Kit (45)**	For 45 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	940014
BioSprint DNA Plant Kits — for rapid purification of total DNA from plant tissue using BioSprint workstations		
BioSprint 15 DNA Plant Kit (360)	For 360 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	941517
BioSprint 96 DNA Plant Kit (576)**	For 576 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941557

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* Japan.

† North America.

‡ UK.

§ Rest of World.

** Other kit sizes are available; see www.qiagen.com.

Notes

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