October 2017

MagAttract® HMW DNA Handbook

For isolation of high-molecular-weight DNA from blood, tissue and bacteria for next-generation sequencing applications

Sample to Insight

QIAGEN

Contents

Handbook Revision History

Document	Changes	Date
HB-1523-005	Kit contents (page 5): Proteinase K volume changed from 1.25 mg to 1.25 ml. Kit contents (page 4): MagAttract Suspension G changed from 2 x 1.1 ml to 2 x 1 ml. Table 1 moved from "Product Specifications" section to "Introduction", page Table 19. Table 2 moved from "Handling guidelines for using the MagAttract HMW DNA Kit with magnetic racks from other suppliers" to "Quantification of starting material", page 16.	October 2017

Kit Contents

MagAttract HMW DNA Kit	**(48)**
Catalog no.	**67563**
Number of samples	**48**
MagAttract Suspension G	2 x 1 ml
Buffer ATL	11.2 ml
Buffer AL	12 ml
Buffer MB	15 ml
Buffer MW1 (concentrate)	77 ml
Buffer PE (concentrate)	20 ml
Buffer AE	22 ml
Proteinase K	1.25 ml
RNase A (100 mg/ml)	25 mg
Nuclease-Free Water	2 x 50 ml
Quick-Start Protocol	1

Storage

All kit components, buffers and RNase A stock solution can be stored at room temperature (15–25°C) for up to 1 year without showing any reduction in performance. The MagAttract HMW DNA Kit contains ready-to-use proteinase K solution, which is dissolved in a specially formulated storage buffer. Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of proteinase K, storage at 2–8°C is recommended.

Intended Use

The MagAttract HMW DNA 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/safety** 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, MB and MW 1 contain guanidine hydrochloride/guanidine thiocyanate, 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.

Quality Control

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

Introduction

The MagAttract HMW DNA Kit enables purification of high-molecular-weight (100–200 kb) DNA using a simple, magnetic bead-based protocol. The kit combines the speed and efficiency of silica-based DNA purification with protocols for convenient handling of magnetic particles. The MagAttract HMW DNA Kit is specifically designed to deliver high-molecular-weight DNA from a wide range of samples, including human or animal tissue, blood and bacteria. The kit uses an efficient combination of high-performance magnetic beads and innovative silica-based chemistry. High-performance magnetic particles ensure that the isolated DNA is of superior yield and purity, while the convenient protocol allows reproducible results in as little as 70 minutes. The bead-based binding technology is highly selective and minimizes shearing stress to the DNA, delivering high-purity DNA of superior size, which is ready for use in downstream applications including archiving, next-generation sequencing (NGS), and genotyping (Table 1). Due to its large size, the purified DNA is especially suited for the preparation of high-quality DNA libraries for NGS applications.

Table 1. Product specifications

Parameter	Specification
Sample volume/input	200 µl blood; up to 25 mg tissue; up to 2 x 10^9 bacterial cells
Elution volume	100–200 µl
Processing time	~70 minutes for 12 samples
DNA fragments recovered	>150 kb

Principle and procedure

The MagAttract HMW DNA Kit ensures reproducible isolation of genomic DNA >150 kb. Optimized buffers and enzymes gently lyse samples, while ensuring minimized fragmentation of genomic DNA. The procedure comprises four simple steps: lyse, bind, wash and elute. Following sample lysis, the DNA binds to the surface of magnetic beads. During the wash steps, contaminants and PCR inhibitors are effectively removed and pure, high-molecular-weight DNA is eluted in Buffer AE.

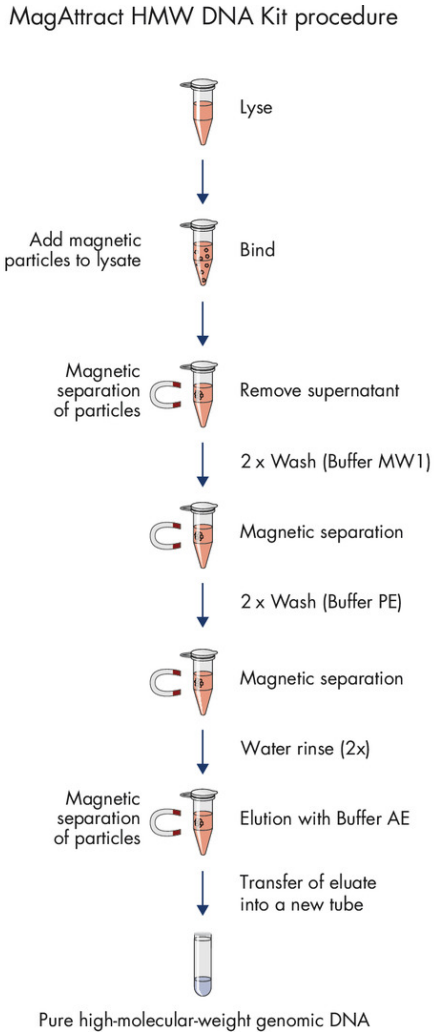

Figure 1. MagAttract HMW DNA Kit workflow.

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.

- Standard laboratory shaker (mixer) (e.g., Eppendorf® Thermomixer® or MixMate®)
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- MagAttract Magnetic Rack (cat. no. 19606) or other magnetic separation device
- 2 ml sample tubes (e.g., 2 ml SafeSeal microtubes, Sarstedt cat. no. 72.695.500)
- Pipet tips with aerosol barrier
- Ethanol (96–100%)

For tissue samples

- Equipment for tissue preparation, for example, scalpel, dry ice for frozen tissue material
- Scale
- Heating block (56°C) (e.g., Eppendorf Thermomixer)

For bacterial samples:

- Equipment for growing of bacterial cultures
- Microcentrifuge (with a rotor for 2 ml tubes)
- Lysozyme stock solution (100 mg/ml)
- Buffer P1 (QIAGEN, cat. no.19051)
- Heating block (56°C) (e.g., Eppendorf Thermomixer)

Important Notes

Handling guidelines for the MagAttract Magnetic Rack

The MagAttract Magnetic Rack consists of three separate components: a tube holder, a magnetic base and an adapter plate (Figure 2). The MagAttract Magnetic Rack is designed for the handling of up to 12 samples in parallel in 2 ml reaction tubes. The magnetic bead-based purification is performed on a standard laboratory shaker (e.g., Eppendorf Thermomixer) suited for the shaking of reaction tubes, as well as microtiter plates in SBS format. The tube holder of the MagAttract Magnetic Rack is fixed onto the shaker by the SBS adapter plate.

Figure 2. Convenient parallel processing of up to 12 samples with the MagAttract Magnetic Rack. The MagAttract Magnetic Rack has a removable tube holder that fits onto the Eppendorf Thermomixer or common microtiter plate mixers using the included adapter plate. The MagAttract Magnetic Rack renders tedious processing of individual tubes unnecessary.

The 2 ml reaction tubes are plugged into the tube holder of the MagAttract Magnetic Rack. Up to 12 sample tubes fixed to the tube holder are then transferred in a single step to a 24-well block of the shaker. The tube holder with 2 ml sample tubes fits onto, for example, the Eppendorf Thermomixer 24-well block for1.5 ml tubes. If using a 24-well block for 2 ml tubes, note that the tube holder of the MagAttract Magnetic Rack may slip out of the block at high shaker speeds during wash steps.

To use a shaker suited for the shaking of microtiter plates in SBS format, the adapter plate of the MagAttract Magnetic Rack must be fixed onto the shaker first. The tube holder of the MagAttract Magnetic Rack (with up to 12 reaction tubes) is then attached to adapter plate of the MagAttract Magnetic Rack.

The magnetic beads are easily separated by removing the tube holder from the shaker and placing it onto the magnetic base. Magnetic beads are separated by strong magnets in <1 minute. Supernatants can be easily removed by pipetting (Figure 3).

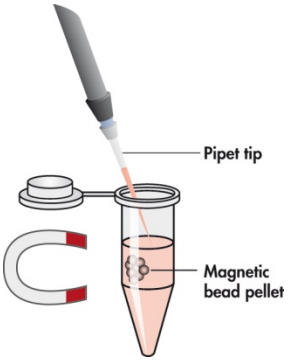

Figure 3. Pipetting during the water rinse step of the MagAttract HMW DNA Kit procedure.

Handling guidelines for using the MagAttract HMW DNA Kit with magnetic racks from other suppliers

The MagAttract HMW DNA Kit can be also used with magnetic racks from other suppliers. The necessary mixing steps during purification should be performed on a standard laboratory shaker (e.g., Eppendorf Thermomixer) with speed (rpm) and duration (min) specified in the protocols.

Protocols are optimized for use with reaction tubes in 2 ml format. The duration of magnetic bead separation depends on the magnetic strength of rack used.

Storage of blood samples

Whole blood samples treated with EDTA, citrate or heparin can be used, and may be either fresh or frozen. 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. For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant, and store at –90 to –60°C.

Lysis with proteinase K

The MagAttract HMW DNA Kit includes proteinase K, which possesses a highly specific activity that remains stable over a wide range of temperatures and pH conditions. Enzymatic activity is substantially increased at higher temperatures.

Preparation of reagents

Buffer AL

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

Buffer MW1

Buffer MW1 is supplied as a concentrate. Before using for the first time, be sure to add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, be sure to add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Quantification of starting material

Weighing tissue or counting cells is the most accurate way to quantify starting material (recommended amounts are shown in Table 2, next page). Guidelines can be found below.

Animal tissue

A 2 mm cube (approximately this size: ■; volume, approximately 8 mm^3) of most animal tissues weighs approximately 10–15 mg.

Bacteria

Bacterial growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the correlation between OD values and cell numbers in bacterial cultures. Cell density is influenced by a variety of factors (e.g., species, media and shaker speed) and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the

distance between the sample and the detector and therefore readings vary between different types of spectrophotometers. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm). We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. [1991] Current Protocols in Molecular Biology, New York: John Wiley & Sons, Inc.). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range and the dilution factor should be used in calculating the number of cells per milliliter.

The following calculation can be considered as a rough guide, which may be helpful:

An *E. coli* culture of 1 x 10^9 cells per milliliter, diluted 1 in 4, gives OD_{600} values of 0.25 measured using a Beckman DU-7400 or 0.125 with a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5, respectively, for 1 x 10^9 cells per milliliter.

Table 2. Recommended starting amounts and expected yields from various sources

Sample	Input amount	Yield (µg DNA/mg tissue)	Total yield (µg)	Concentration (ng/µl)
Blood	200 µl	–	4–8	20–40
Kidney	25 mg	Up to 2	Up to 50	Up to 250
Liver	25 mg	Up to 0.5	Up to 13	Up to 65
Stomach	25 mg	Up to 1.8	Up to 45	Up to 225
Colon	25 mg	Up to 2.8	Up to 70	Up to 350
Lung	25 mg	Up to 0.5	Up to 12	Up to 60
Brain	25 mg	Up to 0.65	Up to 16	Up to 80
Spleen	25 mg	Up to 2.8	Up to 70	Up to 350
Gram-negative bacteria (*E. coli*)	Up to 2 x 10^9 bacterial cells	–	Up to 14	–
Gram-positive bacteria (*B. subtilis*)	Up to 2 x 10^9 bacterial cells	–	Up to 3.5	–

Protocol: Manual Purification of High-Molecular-Weight Genomic DNA from Whole Blood

This protocol enables purification of genomic DNA from whole blood. Whole blood samples treated with EDTA, citrate, 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 will depend on the storage conditions of the blood. Fresher blood samples may yield better results.

Important points before starting

- To ensure that the extracted DNA is not fragmented, avoid shearing stress such as fast or unnecessary pipetting steps.

Things to do before starting

- If using frozen blood samples, thaw and equilibrate to room temperature.
- If using fresh blood samples in primary tubes, mix the blood samples carefully (e.g., by inverting the tubes several times).
- Check that Buffers AL and MB do not contain a precipitate. If necessary, dissolve by incubating at 37°C with occasional shaking.
- Ensure that Buffers MW1 and PE have been prepared according to the instructions on page 16.
- Before starting, ensure that the magnetic particles are fully resuspended. Vortex the vessel containing the magnetic particles vigorously for at least 3 minutes before first use.

Procedure

1. Pipet 20 µl proteinase K into the bottom of a 2 ml sample tube.
2. Add 200 µl of total blood to the sample tube.
3. Add 4 µl RNase A solution and 150 µl Buffer AL to the sample. Mix carefully by vortexing.

   Note: Do not add proteinase K directly to Buffer AL.

   Note: To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed to yield a homogeneous solution.
4. Incubate at room temperature (15–25°C) for 30 min.

   Note: During lysis, the color of the solution will change to dark brown or black.

   Note: To shorten the lysis time, it is possible to incubate for 10 min at 65°C.
5. Briefly centrifuge the 2 ml sample tube to remove drops of liquid from the inside of the lid.
6. Add 15 µl MagAttract Suspension G to the sample.

   Note: Ensure that the magnetic particles are fully resuspended.
7. Add 280 µl Buffer MB to the sample and place the microcentrifuge tube with the sample in the tube holder of the MagAttract Magnetic Rack.
8. Place the tube holder of the MagAttract Magnetic Rack onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.
9. Place the tube holder of the MagAttract Magnetic Rack on its magnetic base, wait (~1 min) until bead separation has been completed, and remove the supernatant.

   Note: Due to the dark color of the solution, the bead pellet is not easily visible. While aspirating the supernatant, avoid disturbing the magnetic bead pellet. Remove the supernatant completely.

10. Add 700 µl Buffer MW1 to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.

    Note: To increase the efficiency of wash step, remove the tube holder from magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet

11. Repeat steps 9–10, followed by step 9 again.
12. Add 700 µl Buffer PE to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.

    Note: To increase the efficiency of the wash step, remove the tube holder from the magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet.

13. Repeat steps 9 and 12, followed by step 9 again.

    Note: Remove all the supernatant. Use a small pipet tip to remove any traces of Buffer PE.

14. Rinse the particles with 700 µl distilled water while the tube holder is on the magnetic base and the beads are fixed to the wall of the sample tube. Incubate for 1 min at room temperature (15–25°C) and remove the supernatant completely.

    IMPORTANT: Do not pipet water directly onto the bead pellet, pipet it into the sample tube against the side facing away from the bead pellet. All pipetting steps must be performed carefully to avoid disturbing the fixed bead pellet (see Figure 2, page 13).

15. Repeat step 14.
16. Remove the tube holder of the MagAttract Magnetic Rack from its magnetic base and add an appropriate volume of Buffer AE (100–200 µl). Place the tube holder onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.

    Optional: Elute the DNA with distilled pure water if the DNA will be used in enzymatic downstream applications.

17. Place the tube holder of the MagAttract Magnetic Rack on its magnetic base, wait (~1 min) until bead separation has been completed and transfer the supernatant with the high-molecular-weight DNA to a new sample tube.

    Note: The yield of genomic DNA depends on the sample type and the number of cells in the sample. Typically, a 200 µl sample of whole blood from a healthy individual will yield 5–6 µg of DNA. For most whole blood samples, a single elution with 200 µl Buffer AE is sufficient.

18. For some downstream applications, concentrated DNA may be required.

    Note: In general, magnetic particles need to be removed before performing downstream applications. Tubes containing eluates should first be placed in the MagAttract Magnetic Rack and the eluates transferred to a clean tube.

Protocol: Disruption/Lysis of Tissue

This protocol is for enzymatic lysis of tissue samples. Fresh and frozen tissue can be used for DNA purification. Yield and DNA quality obtained will depend on the tissue type, source and storage conditions.

Important points before starting

- To ensure that the extracted DNA is not fragmented, avoid shearing stress such as fast or unnecessary pipetting steps.

Things to do before starting

- Heat the mixer (e.g., Eppendorf Thermomixer or an equivalent mixer) to 56°C for the lysis step.
- If a precipitate has formed in Buffer ATL, dissolve by incubating at 37°C with occasional shaking.

Procedure

1. Excise the tissue sample or remove it from storage. Cut up to 25 mg tissue into small pieces and place in a 2 ml microcentrifuge tube.

   Note: Weighing tissue is the most accurate way to determine the amount.

   Note: The yield of DNA will depend on both the amount and the type of tissue processed.
2. Add 220 µl Buffer ATL to the sample.
3. Add 20 µl proteinase K and mix by vortexing.
4. Incubate overnight (12–16 h) at 56°C, shaking at 900 rpm until the tissue is completely lysed.

   Spin down the tube for a few seconds at maximum speed to remove droplets from the lid of the tube, and to ensure that the incompletely lysed tissue particles are settled at the bottom of the tube.

Protocol: Manual Purification of High-Molecular-Weight Genomic DNA from Fresh or Frozen Tissue

Fresh and frozen tissue can be used for DNA purification. Yield and the DNA quality obtained will depend on the tissue type, source and storage conditions.

Important points before starting

- To ensure that the extracted DNA is not fragmented, avoid shearing stress such as fast or unnecessary pipetting steps.

Things to do before starting

- If a precipitate has formed in Buffer AL, dissolve by incubating at 37°C with occasional shaking.
- Ensure that Buffers MW1 and PE have been prepared according to the instructions on page 16.
- Before starting, ensure that the magnetic particles are fully resuspended. Vortex the vessel containing the magnetic particles vigorously for at least 3 minutes before first use.

Procedure

1. Transfer 200 µl of the lysate to a new 2 ml sample tube placed in the tube holder of the MagAttract Magnetic Rack.

   Note: We do not recommend the use of 1.5 sample tubes because of the increased risk of salt carryover into the eluate due to inefficient mixing caused by the conical shape of the tube.

   Note: If pieces of insoluble material are still present, centrifuge at 20,000 x *g* for 3 min and transfer the supernatant into a clean sample tube.

2. Add 4 µl RNase A to the sample. Mix by pulse-vortexing and incubate for 2 min at room temperature (15–25°C).
3. Add 150 µl Buffer AL to the sample. Mix by pipetting up and down.
4. Add 280 µl Buffer MB to the sample.
5. Add 40 µl of MagAttract Suspension G to the sample.

   Note: Ensure that the magnetic particles are fully resuspended.
6. Place the tube holder of the MagAttract Magnetic Rack onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.
7. Place the tube holder of the MagAttract Magnetic Rack onto the magnetic base, wait (~1 min) until bead separation has been completed, and remove the supernatant.

   Note: Avoid disturbing the magnetic bead pellet while aspirating the supernatant (see Figure 2, page 13). Remove the supernatant completely.
8. Add 700 µl Buffer MW1 to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 2 min at 1400 rpm.

   Note: To increase the efficiency of the wash step, remove the tube holder from magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet.
9. Repeat steps 7–8, followed by step 7 again.
10. Add 700 µl Buffer PE to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 2 min at 1400 rpm.

    Note: To increase the efficiency of the wash step, remove the tube holder from magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet.
11. Repeat steps 7 and 10, followed by step 7 again.

    Note: Remove all the supernatant. Use a small pipet tip to remove any traces of Buffer PE.

12. Rinse the particles with 700 µl distilled water while the tube holder is on the magnetic base and the beads are fixed to the wall of the sample tube. Incubate for 1 min at room temperature (15–25°C) and remove the supernatant.

    IMPORTANT: Do not pipet water directly onto the bead pellet, pipet it into the sample tube against the side facing away from the bead pellet. All pipetting steps must be performed carefully to avoid disturbing the fixed bead pellet (see Figure 2, page 13).

13. Repeat step 12.

14. Remove the tube holder of the MagAttract Magnetic Rack from its magnetic base and add an appropriate volume of Buffer AE (100–200 µl). Place the tube holder onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.

    Optional: Elute the DNA with distilled pure water if the DNA will be used in enzymatic downstream applications.

15. Place the tube holder of the MagAttract Magnetic Rack on its magnetic base, wait (~1 min) until bead separation has been completed, and transfer the supernatant with the high-molecular-weight DNA to a new sample tube.

    Note: The yield of genomic DNA depends on the sample type and the number of cells in the sample. For some downstream applications, concentrated DNA may be required. Elution with volumes of <200 µl (e.g., 150 µl or 100 µl) increases the DNA concentration in the eluate.

16. A second elution with Buffer AE will increase the total DNA yield. Due to the increased volume, the DNA concentration is reduced.

    Note: In general, magnetic particles need to be removed before performing downstream applications. Tubes containing eluates should first be placed in the MagAttract Magnetic Rack and the eluates transferred to a clean tube.

Protocol: Manual Purification of High-Molecular-Weight Genomic DNA from Gram-Negative Bacteria

This protocol enables extraction of high-molecular-weight DNA from Gram-negative bacterial cultures.

Important points before starting

- To ensure that the extracted DNA is not fragmented, avoid shearing stress such as fast or unnecessary pipetting steps.
- See "Quantification of starting material", page 16, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.

Things to do before starting

- Heat the mixer (e.g., Eppendorf Thermomixer or an equivalent mixer) to 56°C for the lysis step.
- If a precipitate has formed in Buffer ATL, dissolve by incubating at 37°C with occasional shaking.
- Ensure that Buffers MW1 and PE have been prepared according to the instructions on page 16.
- Before starting, ensure that the magnetic particles are fully resuspended. Vortex the vessel containing the magnetic particles vigorously for at least 3 minutes before first use.

Procedure

1. Harvest up to 2 x 10^9 bacterial cells in a 2 ml microcentrifuge tube by centrifugation for 10 min at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.

   Note: The cell pellet can be stored at –30 to –15°C or –90 to –60°C for future use, or can be used immediately.

2. Resuspend the bacterial pellet in 180 µl Buffer ATL, add 20 µl proteinase K, and incubate for 30 min on a mixer at 56°C shaking at 900 rpm.
3. Add 4 µl RNase A to the sample, mix by pulse vortexing or tapping the tube several times, and incubate for 2 min at room temperature (15–25°C).
4. Add 15 µl MagAttract Suspension G and 280 µl Buffer MB to the sample. Mix by pulse vortexing. Transfer the sample tubes to the tube holder of the MagAttract Magnetic Rack.
5. Place the tube holder of the MagAttract Magnetic Rack onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.
6. Place the tube holder of the MagAttract Magnetic Rack on the magnetic base, wait (~1 min) until bead separation has been completed, and remove the supernatant.

   Note: Avoid disturbing the magnetic bead pellet while aspirating the supernatant. Remove the supernatant completely.

7. Add 700 µl Buffer MW1 to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.

   Note: To increase the efficiency of the wash step, remove the tube holder from the magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet.

8. Repeat steps 6–7, followed by step 6 again.
9. Add 700 µl Buffer PE to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.

Note: To increase the efficiency of the wash step, remove the tube holder from the magnetic base before adding the wash buffer. Add the wash buffer directly onto the magnetic bead pellet.

10. Repeat steps 6 and 9, followed by step 6 again.

    Note: Remove all the supernatant. Use a small pipette tip to remove any traces of Buffer PE.

11. Rinse the particles with 700 µl distilled water while the tube holder is on the magnetic base and the beads are fixed to the walls of the microcentrifuge tube. Incubate for 1 min at room temperature (15–25°C) and remove the supernatant.

    IMPORTANT: Do not pipet water directly onto the bead pellet, pipet it into the sample tube against the side facing away from the bead pellet. All pipetting steps must be performed carefully to avoid disturbing the fixed bead pellet (see Figure 2, page 13).

12. Repeat step 11.

13. Remove the tube holder of the MagAttract Magnetic Rack from its magnetic base and add an appropriate volume of Buffer AE (100–200 µl). Place the tube holder onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.

    Optional: Elute the DNA with distilled pure water if the DNA will be used in enzymatic downstream applications.

14. Place the tube holder of the MagAttract Magnetic Rack on its magnetic base, wait (~1 min) until bead separation has been completed, and transfer the supernatant with the high-molecular-weight DNA to a new sample tube.

    Note: The yield of genomic DNA depends on the sample type and the number of cells in the sample. For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200 µl (e.g., 150 µl, 100 µl) increases the DNA concentration in the eluate.

15. A second elution with Buffer AE increases the total DNA yield. Due to the increased volume, the DNA concentration is reduced.

    Note: In general, magnetic particles need to be removed before performing downstream applications. Tubes containing eluates should first be placed in the MagAttract Magnetic Rack and the eluates transferred to a clean tube.

Protocol: Manual Purification of High-Molecular-Weight Genomic DNA from Gram-Positive Bacteria

This protocol enables extraction of high-molecular-weight DNA from Gram-positive bacterial cultures.

Important points before starting

- To ensure that the extracted DNA is not fragmented, avoid shearing stress such as fast or unnecessary pipetting steps.
- See "Quantification of starting material", page 16, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.
- See "Equipment and Reagents to Be Supplied by User" on page 12 for details on reagents required.

Things to do before starting

- Heat the mixer (e.g., the Eppendorf Thermomixer or an equivalent mixer) to 37°C for the lysis step.
- Prepare the lysozyme solution. Lysozyme should be dissolved in distilled water to a concentration of 100 mg/ml and should be stored at –30 to –15°C.
- Prepare Buffer P1 (50 mM Tris, 10mM EDTA, pH 8.0) or order from QIAGEN (cat. no.19051).
- Ensure that Buffers MW1 and PE have been prepared according to the instructions on page 16.
- Before starting, ensure that the magnetic particles are fully resuspended. Vortex the vessel containing the magnetic particles vigorously for at least 3 minutes before first use.

Procedure

1. Harvest up to 2 x 10^9 bacterial cells in a 2 ml microcentrifuge tube by centrifugation for 10 min at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.

   Note: The cell pellet can be stored at –15 to –30°C or –90 to –60°C for future use, or can be used immediately.

2. Resuspend the bacterial pellet in 160 µl Buffer P1 and transfer the sample to a 2 ml sample tube (not supplied).
3. Add 20 µl lysozyme (100 mg/ml) and mix by tapping the tube.
4. Place the sample in a thermomixer or shaker–incubator, and incubate at 37°C with shaking at 900 rpm for 30 min to 2 h.

   Lysis time depends on the type of cells and the cell number.

5. Add 20 µl proteinase K, and mix by tapping the tube.
6. Incubate at 56°C with shaking at 900 rpm for 30 min.
7. If RNA-free DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature (15–25°C). Otherwise, proceed immediately with step 8.
8. Add 150 µl Buffer AL to the sample. Mix by pulse vortexing.
9. Add 15µl of MagAttract Suspension G and 280 µl Buffer MB to the sample. Mix by pulse vortexing. Transfer the sample tubes to the tube holder of the MagAttract Magnetic Rack.
10. Place the tube holder of the MagAttract Magnetic Rack onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.
11. Place the tube holder of the MagAttract Magnetic Rack on its magnetic base, wait (~1 min) until bead separation has been completed, and remove the supernatant.

    Note: Avoid disturbing the magnetic bead pellet while aspirating the supernatant. Remove the supernatant completely.

12. Add 700 µl Buffer MW1 to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.

13. Repeat steps 11–12, followed by step 11 again.
14. Add 700 µl Buffer PE to the sample and place the tube holder of the MagAttract Magnetic Rack onto the mixer. Incubate at room temperature (15–25°C) for 1 min at 1400 rpm.
15. Repeat steps 11 and 14, followed by 11 again.
16. Rinse the particles with 700 µl distilled water while the beads are fixed to the walls of the sample tube. Incubate for 1 min at room temperature (15–25°C) and remove the supernatant.

    IMPORTANT: Do not pipet water directly onto the bead pellet, pipet it into the sample tube against the side facing away from the bead pellet. All pipetting steps must be performed carefully to avoid disturbing the fixed bead pellet (see Figure 2, page 13).
17. Repeat step 16.
18. Remove the tube holder of the MagAttract Magnetic Rack from its magnetic base and add an appropriate volume of Buffer AE (100–200 µl). Place the tube holder onto the mixer and incubate at room temperature (15–25°C) for 3 min at 1400 rpm.

    Optional: Elute the DNA with distilled pure water if the DNA will be used in enzymatic downstream applications.
19. Place the tube holder of the MagAttract Magnetic Rack on the magnetic base, wait (~1 min) until bead separation has been completed, and transfer the supernatant with the high-molecular-weight DNA to a new sample tube.

    Note: The yield of genomic DNA depends on the sample type and the number of cells in the sample. For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200 µl (e.g., 150 µl, 100 µl) increases the DNA concentration in the eluate.

    A second elution with Buffer AE increases the total DNA yield. Due to the increased volume, the DNA concentration is reduced.

    Note: In general, magnetic particles need to be removed before performing downstream applications. Tubes containing eluates should first be placed in the MagAttract Magnetic Rack and the eluates transferred to a clean tube.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Low or no DNA recovery		
a)	Buffer storage	Storage of reagents under 15°C may lead to formation of precipitates.
b)	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers.
c)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
d)	Incomplete sample lysis	Before use, check that Buffers AL, ATL and MB do not contain precipitates. If necessary, incubate Buffers AL, ATL and MB for 30 min at 37°C with occasional shaking to dissolve precipitates.
e)	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.
f)	Incomplete digestion of tissue samples	Ensure that the tissue is completely digested by extending the time of incubation with proteinase K.
g)	Clogging of pipette tip due insoluble material	Insoluble material was not removed from the digestion. To remove insoluble material, centrifuge the sample at 20,000 x *g* for 3 minutes and transfer the supernatant to a new sample tube.
h)	Buffer MW 1 did not contain ethanol	Ethanol must be added to Buffer MW1 (concentrate) before use. Repeat procedure with correctly prepared Buffer MW1.
i)	Buffer PE did not contain ethanol	Ethanol must be added to Buffer PE (concentrate) before use. Repeat procedure with correctly prepared Buffer PE.
j)	Storage of starting material	DNA yield is dependent on the type, size, age and storage of starting material. Lower yields will be obtained from material that has been inappropriately stored.

		Comments and suggestions
k)	Too much starting material	In future preparations, reduce the amount of starting material used.
l)	DNA loss during water rinse	Perform the water rinse carefully while the magnetic bead pellet is separated by the magnet. Avoid adding water directly onto the bead pellet as this will disturb the magnetic bead pellet. Pipet water into sample tube against the side facing away from the magnetic bead pellet.
DNA does not perform well in downstream applications		
a)	Magnetic particle carryover	Carryover of magnetic particles in the eluates will not affect most downstream applications including RT-PCR. If the risk of magnetic-particle carryover must be minimized, first place the tubes containing eluates in the MagAttract Magnetic Rack, then transfer the eluates to clean tubes or centrifuge the tubes containing eluates in a microcentrifuge at full speed for 1 minute to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes.
b)	Salt carryover	Ensure that Buffer MW1 and Buffer PE were added in the correct order. Ensure that the supernatant after the binding step and the Buffer MW1wash step was removed completely. Use a smaller pipet to remove any remaining visible liquid, if necessary.
c)	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.
d)	Degraded DNA obtained from tissue samples	Too much sample may have been used. Overloading may lead to insufficient lysis and therefore insufficient inactivation of potential DNases.
***A_{260}/A_{280}* ratio for purified DNA is low**		
a)	Ethanol carryover	Aspirate as much of the ethanol in the final wash step as possible. Carefully rinse the magnetic bead pellet with water as described in the protocol.
b)	Salt carryover	Ensure that the supernatant after the binding step and the Buffer MW1wash step was removed completely. Use a smaller pipet to remove any remaining visible liquid, if necessary.
c)	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.
d)	Water used for elution instead of Buffer AE	Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio.

Appendix A: Handling, Quantification and Determination of Quality of DNA

Storage of DNA

Purified genomic DNA can be stored at –90 to –60°C, –30 to –15°C or at 2–8°C.

Quantification 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 milliliter (A_{260} = 1 → 50 µg/ml). The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity. Measure the absorbance at 320, 280 and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for effects of background absorbance.

Concentration of DNA sample = 50 µg/ml x (A_{260}–A_{320}) x dilution factor.

Total amount of DNA purified = concentration x volume of sample in milliliters

Carryover of magnetic particles in the eluate may affect the A_{260} reading. If the purified DNA is to be analyzed by, for example next-generation sequencing, the tube containing the eluate should first be applied to the MagAttract Magnetic Rack and the eluate transferred to a clean tube:

- Apply the tube containing the DNA to the MagAttract Magnetic Rack until the magnetic particles are separated.

- If the MagAttract Magnetic Rack or another suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw the purified DNA and transfer to a new tube or rack.

Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm; i.e., $(A_{260}–A_{320})/(A_{280}–A_{320})$. Pure DNA has a ratio of 1.7–1.9.

Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. We recommend 800 ng DNA.

Dissolve samples in Buffer AE and incubate for 15–30 minutes at 65°C with gentle shaking (max. 300 rpm). To ensure maximal size of the extracted DNA, avoid shearing stress such as fast or unnecessary pipetting steps.

Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer
- Switch intervals: 1–12 s
- Run time: 16 h
- Voltage: 6 V/cm

Ordering Information

Product	Contents	Cat. no.
MagAttract HMW DNA Kit (48)	For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	67563
Accessory		
MagAttract Magnetic Rack	Magnetic rack for convenient parallel processing of up to 12 samples	19606
QIAGEN GeneRead™ Kits — for next-generation sequencing applications		
GeneRead Size Selection Kit (50)	For 50 reactions: Spin columns and buffers	180514
GeneRead DNA Library I Core Kit (12)	For 12 reactions: Buffers and reagents for end-repair, A-Addition, and ligation, for use with Illumina® instruments	180432
GeneRead DNA I Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Illumina instruments	180455
GeneRead Adapter I Set 1-plex (12)	For 12 reactions: Adapters for DNA ligations, for use with Illumina instruments	180912

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

Limited License Agreement for MagAttract HMW DNA Kit

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