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MagAttract® Mastitis Kit Handbook

For automated purification of bacterial DNA from milk using the BioSprint® 96 or equivalent workstations

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract Mastitis Kit (384)	
Catalog no.	947757
Number of preps	384
Buffer ML*	54 ml
Buffer MVL*† (concentrate)	2 x 88.2 ml
Reagent DX	1 ml
MagAttract Suspension G‡	13 ml
Buffer AW1*§ (concentrate)	2 x 98 ml
Buffer AW2§ (concentrate)	2 x 66 ml
Buffer ATE	2 x 20 ml
Pathogen Lysis Microtubes S (racked)	4
Caps for Collection Microtubes	4 x 55
Quick-Start Protocol	1

* CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 4 for safety information.

† Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.

‡ CAUTION: Contains sodium azide as a preservative.

§ Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Storage

The MagAttract Mastitis Kit can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

Intended Use

The MagAttract Mastitis Kit is intended for the automated extraction of bacterial DNA from ruminant milk using the BioSprint 96 or equivalent workstation.

For laboratory use. 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.

Buffer ML and Buffer AW1 contain guanidine hydrochloride and Buffer MVL contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

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

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 MagAttract Mastitis Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract Mastitis Kit is designed for purification of bacterial DNA from ruminant milk using BioSprint workstations. The MagAttract Mastitis 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.

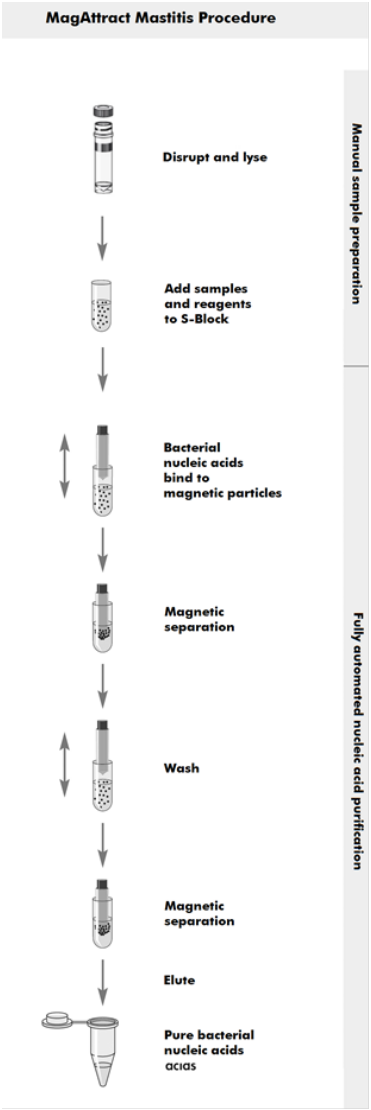
Principle and procedure

The MagAttract Mastitis Kit uses MagAttract magnetic-particle technology for nucleic acid purification. This technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (see flowchart). The MagAttract Mastitis Kit uses a combination of mechanical and chemical lysis to homogenize samples. To ensure efficient lysis of Gram-positive and Gram-negative bacteria, milk samples are disrupted using Pathogen Lysis Microtubes S and a lysis buffer. The Pathogen Lysis Microtubes S included in the kit contains small beads.

After homogenization and lysis, buffers added to the lysate allow optimal binding of the DNA to the silica surface of the MagAttract magnetic particles. DNA bound to the magnetic particles is then efficiently washed. Two different wash buffers are used, followed by an air drying step, which considerably improves the purity of the nucleic acids. High-quality nucleic acids are eluted in Buffer ATE.

DNA purified using the MagAttract Mastitis Kit is ready for use for real-time PCR and other downstream applications. The MagAttract Mastitis Kit is highly suited for use with *bactotype*® Mastitis PCR assays.

MagMAX™ Express-96 Magnetic Particle Processor and KingFisher® 96 (Thermo Fischer Scientific, Inc.) users can also use the MagAttract Mastitis Kit on these instruments by simply following the protocol on page 12. The appropriate software protocol is available from QIAGEN Technical Services.


MagAttract Mastitis Procedure
Manual sample preparation
Fully automated nucleic acid purification
Disrupt and lyse
Add samples and reagents to S-Block
Bacterial nucleic acids bind to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Pure bacterial nucleic acids

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
- Magnetic head for use with Large 96-Rod Covers (supplied with the BioSprint 96)
- S-Blocks (see page 20 for ordering information)
- Large 96 Rod-Cover (see page 20 for ordering information)
- 96-Well Microplates MP (see page 20 for ordering information)
- Equipment for homogenization of milk samples. We recommend the TissueLyser II with the TissueLyser Adapter Set 2 x 96 (see page 20 for ordering information)
- Centrifuge 4-16S or 4-16KS with Plate Rotor 2 x 96 (see page 21 for ordering information)
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Ethanol (96–100%)*
- Isopropanol (100%)
- Multichannel pipettor and disposable 1000 µl pipet tips with aerosol barriers
- Multidispenser
- Disposable gloves
- Vortexer
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the BioSprint 96 worktable

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.

Important Notes

Starting material

The MagAttract Mastitis Kit procedure is suitable for use with fresh, frozen or stabilized (e.g., Bronopol, boric acid) milk samples.

As a starting material, 400 µl of fresh, frozen or stabilized milk should be used.

Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

If you need further information, contact QIAGEN Technical Services.

Homogenization and disruption of bacteria from milk

For homogenization and disruption of bacteria from milk, optimal results are obtained using the TissueLyser II together with the TissueLyser Adapter Set 2 x 96 and Pathogen Lysis Microtubes S (racked). The TissueLyser provides rapid and efficient disruption of 2 x 96 samples in 16 minutes.

Sample material and ML mixture are added to each of up to 192 Pathogen Lysis Microtubes S in two racks. The racks are fixed into the clamps on the TissueLyser using adapter plates and disrupted by two 8-minute high-speed (30 Hz) shaking steps.

Yield and quality of purified DNA

DNA yields depend on the sample type, the sample collection method used, and the method of disruption. The MagAttract Mastitis Kit procedure is optimized for 400 µl fresh, frozen or stabilized milk. Exceeding the recommended maximum amount of starting material can result in inefficient lysis, resulting in low DNA yield and purity.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified bacterial DNA at 2–8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at –15 to –30°C.

Preparing reagents

Buffer MVL

Buffer MVL is supplied as a concentrate. Before using for the first time, the appropriate amount of isopropanol (100%) must be added, as indicated on the bottle. Tick the check box on the bottle label to indicate that isopropanol has been added. Mix well after adding isopropanol.

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.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, the appropriate amount of ethanol (96–100%) must be added to Buffer AW1, as indicated on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year. Mix well after adding ethanol.

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, the appropriate amount of ethanol (96–100%) must be added to Buffer AW2, as indicated on the bottle.

Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 can be stored at room temperature (15–25°C) for up to 1 year. Mix well after adding ethanol.

Protocol: Purification of DNA from Milk

This protocol is for the purification of bacterial DNA from 400 µl milk using the BioSprint 96 workstation and the MagAttract Mastitis Kit with the "BS96 mastitis" protocol.

Important points before starting

- Ensure that you are familiar with operating the TissueLyser II and BioSprint 96. Refer to the TissueLyser II Handbook and BioSprint 96 User Manual for operating instructions.
- Before beginning the procedure, read "Important Notes" (page 9).
- Check that Buffer MVL, Buffer AW1 and Buffer AW2 have been prepared according to the instructions in "Preparing reagents" (page 10).
- The 96-rod covers are supplied either as packets of 2, or as packets of 1 inserted into an S-Block. If using a new packet of 2, store the second 96-rod cover on another S-block or plate. Care should be taken to not bend the 96-rod covers.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge/Centrifuge 4-16S.
- Use of a multichannel pipet is recommended.

Things to do before starting

- Thaw and equilibrate up to 96 samples at room temperature (15–25°C).
- Prepare the ML and MVL mixture according to Table 1 and Table 2 for use in step 2 and 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 use.

  Note: Prepare a volume of the Buffer MVL mixture that is 5% greater than that required for the total number of sample purifications to be performed; 500 µl mixture is required per sample (see step 3 of the procedure).

  The excess Buffer should be discarded.

Table 1. Preparation of MVL mixture

	Number of samples*			
Reagent	**1**	**8**	**48**	**96**
Buffer MVL	0.5 ml	4 ml	24 ml	48 ml
MagAttract Suspension G	25 µl	200 µl	1200 µl	2400 µl

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation.

Table 2. Buffer ML mixture preparation

	Number of samples†			
Reagent	**1**	**8**	**48**	**96**
Buffer ML	80 µl	640 µl	3840 µl	7680 µl
Reagent DX	1 µl	8 µl	48 µl	96 µl

† Calculate 1–2 extra reactions to ensure sufficient volume.

Procedure

1. Label 6 x S-Blocks.
2. Prepare Buffer MVL mixture (see Table 1) and mix thoroughly by flicking the tube.
3. Add 500 µl Buffer MVL mixture to each well in the S-Block.
4. Prepare 5 additional S-Blocks (slots 2–6) according to Table 3.

   In each plate or block, the number of wells 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 3. BioSprint 96 worktable setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well (µl)
6	Load Rod Cover	S-Block	Large 96-Rod Cover	–
5	Load Elution	96-well microplate MP	Buffer ATE	100
4	Load Wash 3	S-Block	Ethanol (96–100%)	1000
3	Load Wash 2	S-Block	Buffer AW2	1000
2	Load Wash 1	S-Block	Buffer AW1	1000
1	Load Lysate	S-Block	Lysate*	920

* Includes 420 µl lysate and 500 µl Buffer MVL mixture.

5. Mix the sample thoroughly by vortexing.
6. Open Pathogen Lysis Microtubes S and discard caps.
7. Add 80 µl Buffer ML mixture (see Table 2) to each tube.
8. Pipet 400 µl sample into the Pathogen Lysis Microtubes S by touching the insides of the tubes without wetting the rims.

   Cut the end of the pipet tip to make pipetting easier. Avoid pipetting large milk clots into the lysis tubes.

   Record the wells into to which you load the samples.
9. Cover the rack with new caps for collection microtubes (provided).
10. Homogenize the sample until the sample is thoroughly homogenized.

    Homogenize the sample using a conventional homogenizer until it is uniformly homogeneous.

    Disruption and homogenization using the TissueLyser II

    10a. Place the Pathogen Lysis Microtubes S in the TissueLyser Adapter Set 2 x 96.

    10b. Operate the TissueLyser II for 8 min at 30 Hz.

    10c. Rearrange the tubes so that the outermost tubes are innermost, and the innermost tubes are outermost.

    10d. Operate the TissueLyser II for another 8 min at 30 Hz.
11. Centrifuge briefly to remove drops from the inside of the tube lid.

12. Carefully apply all of the lysate (approximately 420 µl) from step 11 to the first S-block (Lysate).

    Transfer of small quantities of glass beads will not affect the procedure.

13. Switch the BioSprint 96 on at the power switch.

14. Slide the front door of the protective cover open.

15. Select the protocol ''BS96 mastitis'' using the ▲ and ▼ keys.

16. Press "Start" and follow the messages for loading the worktable as shown in Table 3.

17. The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see Table 3 above). After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.

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

18. 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.

19. Press "Start" to start sample processing.

20. 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 does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet and transferring the eluates to a clean microplate (see "Carryover of magnetic particles" on page 18).

21. Press "Stop" after all plates and blocks are removed.

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

    See page 4 for safety information.

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

24. 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.

    Do not use bleach as disinfectant. See page 4 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 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 yield of DNA	
a) MagAttract Suspension G not completely resuspended	Ensure that the MagAttract Suspension G is fully resuspended before adding to the Buffer VXL mixture. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
b) Buffer ML or MVL mixture prepared incorrectly	Ensure that Buffer ML or MVL mixture was prepared with the correct volumes of additional reagents as indicated on the buffer bottle or according to the tables in the protocol (page 13). Repeat the DNA purification procedure with new samples.
c) Buffer MVL prepared incorrectly	Check that Buffer MVL concentrate was diluted with the correct volumes of isopropanol as indicated on the bottle. Repeat the purification procedure with new samples.
d) Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW1 or Buffer AW2 concentrate was diluted with the correct volume of ethanol, as indicated on the bottle. Use 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol with new samples.
e) Reagents loaded onto worktable in wrong order	Ensure that all reagents are loaded onto the BioSprint 96 worktable in the correct order. Repeat the purification protocol with new samples.
f) Frozen samples not mixed properly after thawing	Thaw frozen samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.
g) Nucleic acids in samples already degraded prior to purification	Samples were frozen and thawed more than once or stored at room temperature (15–25°C) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.

		Comments and suggestions
DNA or RNA does not perform well in downstream applications		
a)	Little or no DNA in the eluate	See "Low yield of DNA" (above) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b)	Carryover of magnetic particles	Carryover of magnetic particles in eluates does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet (e.g., 96-Well Magnet Type A or 12-Tube Magnet; see ordering information, page 20) for 1 min, and transferring the eluates to a clean microplate. If a suitable magnet is not available, centrifuge the microplate containing eluates at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to a clean microplate.
c)	Too much eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN® products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at **www.qiagen.com/RefDB/search.asp** or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
MagAttract Mastitis Kit (384)	For 384 preps: Pathogen Lysis Microtubes S (racked), MagAttract Suspension G, Buffers and Reagents	947757
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate for 1000 spin, 250 midi, or 100 maxi preps	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
bactotype Mastitis Screening PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280045
bactotype Mastitis HP3 PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280045
bactotype Mastitis AMR PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280015
bactotype Mastitis HP2+ PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280025
bactotype Mastitis Env PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	280035

Accessories

TissueLyser II	Bead mill, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)*	1031656
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	19585
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	36912
Large 96-Rod Cover (16)	16 x Large 96-Rod Covers for use with the BioSprint 96 workstation	1031668
S-Blocks (24)	96-well blocks with 2.2 ml square wells, for collecting wash and lysis fractions from 96-well plates. Contents: 96-well blocks with 2.2 ml wells, 24 per case	19585
96-Well Microplates MP (20)	96-well microplates, 20 per case, for use with the 96-Well Magnet	1031656
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

QIAGEN 96-Well Centrifugation System

Centrifuge 4-16S	Universal laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81500* 81510† 81525‡ 81520§
Centrifuge 4-16KS	Refrigerated universal laboratory centrifuge with brushless motor	81600* 81610† 81625‡ 81620§
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031

* Japan;
† North America;
‡ UK;
§ Rest of world.

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

Limited License Agreement for MagAttract Mastitis Kit

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