February 2021 | EN

IndiMag® Mastitis Kit Handbook

For automated purification bacterial DNA from from milk using magnetic particle processors (e.g., BioSprint® 96, KingFisher®)

REF IndiMag Mastitis Kit (cat no SP947757), *formerly* MagAttract® Mastitis Kit)

Manufactured by QIAGEN® GmbH for INDICAL BIOSCIENCE
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INDICAL BIOSCIENCE

Contents

Kit contents

IndiMag Mastitis Kit **Cat. no.** **Number of preps**	 **SP947757** **384**
Buffer ML[1]	1 x 54 ml
Buffer MVL (concentrate) [1,2]	2 x 88.2 ml
Reagent DX	1 x 1 ml
MagAttract Suspension G[3]	1 x 13 ml
Buffer AW1 (concentrate) [1,4]	2 x 98 ml
Buffer AW2 (concentrate) [4]	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 (PCard)	1

1 CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.
2 Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.
3 CAUTION: Contains sodium azide as a preservative
4 Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Storage

The IndiMag 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 IndiMag Mastitis Kit is intended for the automated extraction of bacterial DNA from ruminant milk using magnetic particle processors such as the BioSprint 96, KingFisher 96 or equivalent workstations.

For laboratory use.

Symbols


Legal manufacturer

Lot number


Use by date

Temperature limitations for storage


Handbook

Catalog number

Material number

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 from your local sales representative or by Email request under **compliance@indical.com**.

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.

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of IndiMag Mastitis Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The IndiMag Mastitis Kit is designed for purification of bacterial DNA from ruminant milk using magnetic particle processors. The IndiMag 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 IndiMag 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 (Figure 1, page 7)

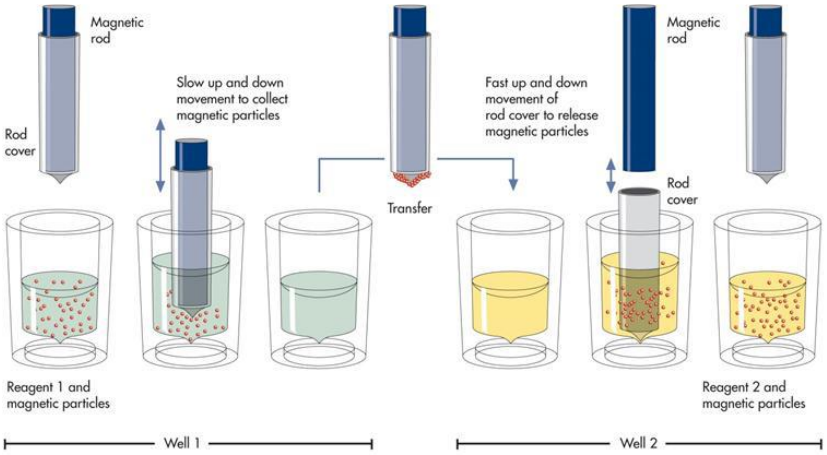

Figure 1. Schematic of the magnetic bead principle. The workstation processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The magnetic rod cover is positioned above another well (see well 2 in the figure) and the magnetic particles are released. Steps 1 and 2 are repeated several times during sample processing.

The IndiMag 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 IndiMag 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 (Figure 2, page 8).

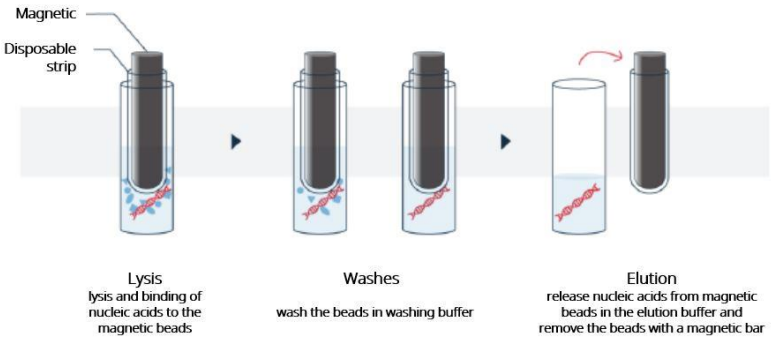

Figure 2. Schematic description of protocol steps.

DNA purified using the IndiMag Mastitis Kit is ready for use for real-time PCR and other downstream applications. The IndiMag 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 IndiMag Mastitis Kit on these instruments by simply following the protocol on page 13. The appropriate software protocol is available from INDICAL Support (**support@indical.com**).

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.

- Magnetic particle processors such as BioSprint 96, KingFisher 96
- Magnetic head for use with Large 96-Rod Covers
- S-Blocks
- Large 96 Rod-Cover
- 96-Well Microplates MP
- Equipment for homogenization of milk samples. We recommend the TissueLyser II with the TissueLyser Adapter Set 2 x 96
- Centrifuge 4-16S or 4-16KS with Plate Rotor 2 x 96
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Ethanol (96–100%)[1]
- 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

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

Important notes

Starting material

The IndiMag 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, please contact INDICAL Support at **support@indical.com**.

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.

Yields of nucleic acids

DNA yields depend on the sample type, the sample collection method used, and the method of disruption. The IndiMag 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 (or equivalent) and the IndiMag Mastitis Kit with the “BS96 mastitis” protocol.

Important points before starting

- Ensure that you are familiar with the correct operation of the workstation. Refer to the respective user manual for operating instructions. Read the safety information in the instrument use manual before use.
- Before beginning the procedure, read “Important notes” (page 10).
- Check that Buffer MVL, Buffer AW1 and Buffer AW2 have been prepared according to the instructions in “Preparing reagents**Fehler! Verweisquelle konnte nicht gefunden werden.**” (page 11).
- 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 96-well deep well 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 (micro-) centrifuge 4-16S.
- Use of a multichannel pipet is recommended.

Things to do before starting

- Thaw and equilibrate samples at room temperature (15-25°C).

Procedure

1. Label and prepare 4 x 96-well deep well plates and 1 x 96-well microplate (columns 2-6) according to Table 1.

Table 1: Instrument setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well
6	Load Rod Cover	96-well deep well plate	Cover for 96 tip comb	—
5	Load Elution	96-Well microplate	Buffer ATE	100 µl
4	Load Wash 3	96-well deep well plate	Ethanol (96–100%)	1000 µl
3	Load Wash 2	96-well deep well plate	Buffer AW2	1000 µl
2	Load Wash 1	96-well deep well plate	Buffer AW1	1000 µl
1	Load Lysate	96-well deep well plate	Lysate*	920 µl

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

2. Prepare Buffer ML mixture according to Table 2 and mix thoroughly for 30 sec.

Table 2: Buffer ML mixture preparation

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

3. Prepare Buffer MVL mixture according to Table 3.

Table 3: Buffer MVL mixture preparation

Reagent	Number of samples *			
	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

* Prepared volume is 105% to compensate pipetting variation and possible evaporation.

4. **Mix the sample thoroughly by vortexing.**
5. **Open Pathogen Lysis Microtubes S and discard caps.**
6. **Pipet 80 µl Buffer ML mixture into each tube and add 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 which the samples are loaded.
7. **Cover the rack with new caps for collection microtubes (provided).**
8. **Homogenize the sample until it is thoroughly homogenized.**

   Disruption and homogenization using the TissueLyser II

   a. Place the Pathogen Lysis Microtubes S in the TissueLyser Adapter Set 2 x 96.
   b. Operate the TissueLyser II for 8 min at 30 Hz.
   c. Rearrange the tubes so that the outermost tubes are innermost, and the innermost tubes are outermost.
   d. Operate the TissueLyser II for another 8 min at 30 Hz.
9. **Centrifuge briefly to remove drops from the inside of the tube lid.**
10. **Carefully apply all of the lysate (approximately 420 µl) from step 9 to a new 96-well deep well plate.**

    Transfer of small quantities of glass beads will not affect the procedure.
11. **Mix Buffer MVL mixture thoroughly for 30 sec and add 500 µl Buffer MVL mixture to each sample in the 96-well deep well plate.**
12. **Immediately load prepared plates onto the processor and start the appropriate script.**

    a. Switch the BioSprint 96 on at the power switch.
    b. Slide the front door of the protective cover open.
    c. Select the protocol ‘‘BS96 mastitis’’ using the ▲ and ▼ keys.
    d. Press “Start” and follow the messages for loading the worktable as shown in Table 1, page 14.

e. The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see Table 1 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).

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

   For safety information, see the respective user manual for the device you are using.

g. Press “Start” to start sample processing.

13. After the samples are processed, remove the plates and blocks as instructed by the display of the processor. 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 19).

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

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

See page 5 for safety information.

16. Switch off the processor 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.

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 or help please contact INDICAL Support at **support@indical.com**.

		Comments and suggestions
Low yield of DNA and RNA		
1	MagAttract Suspension G not completely resuspended	Ensure that the MagAttract Suspension G is fully resuspended before adding to the Buffer MVL mixture. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
2	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 14). Repeat the DNA purification procedure with new samples.
3	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.
4	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.
5	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.

6	Frozen samples not mixed properly after thawing	Thaw frozen samples quickly in a 37ºC water bath with mild agitation to ensure thorough mixing
7	Nucleic acids in samples already degraded prior to purification	Samples were frozen and thawed more than once or stored at room temperature (15–25ºC) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
DNA or RNA does not perform well in downstream applications		
1	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.
2	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;) 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.
3	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.

Order information

Product name	Cat. no.
IndiMag Mastitis Kit (384) *formerly MagAttract Mastitis Kit (384)*	SP947757
bactotype Mastitis Screening PCR Kit (96)	BT280005
bactotype Mastitis HP3 PCR Kit (96)	BT280045
bactotype Mastitis HP3 PCR Kit	BT280025
bactotype Mastitis Env PCR Kit	BT280035

INDICAL offers a broad range of ready-to-use pathogen specific ELISA kits, qPCR/ RT-qPCR assays and reagents.

To optimize your workflow, and to handle your sample and throughput needs, INDICAL additionally offers instruments and kits for the efficient extraction of nucleic acids from a variety of sample types.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, IndiMag, IndiSpin, intype, pigtype and virotype products.

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Change index

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HB-2290-EN-002	February 2021	INDICAL design