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DNeasy® Mastitis Mini Kit Handbook

For the purification of bacterial DNA from milk

Sample to Insight

QIAGEN

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Kit Contents

DNeasy Mastitis Mini Kit (192)	
Catalog no.	69805
Number of preps	192
DNeasy Mini Spin Columns	192
Buffer ML*	54 ml
Buffer MVL*† (concentrate)	6 x 14.7 ml
Reagent DX	1 ml
Buffer AW1*‡ (concentrate)	2 x 27 ml
Buffer AW2‡ (concentrate)	2 x 17 ml
Buffer ATE	2 x 12 ml
Collection Tubes (1.5 ml)	200
Collection Tubes (2 ml)	200
Pathogen Lysis Microtubes S (racked)	2
Caps for Collection Microtubes	1 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.

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

Storage

The DNeasy Mastitis Mini 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 DNeasy Mastitis Mini Kit is intended for extraction of bacterial DNA from ruminant milk.

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

Introduction

The DNeasy Mastitis Mini Kit is intended for the extraction of bacterial DNA from ruminant milk.

The DNeasy Mastitis Mini Kit uses a combination of mechanical and chemical lysis to homogenize milk samples. The mechanical disruption ensures complete lysis of Gram-positive and Gram-negative bacteria and is achieved by using the Pathogen Lysis Microtubes S included in the kit.

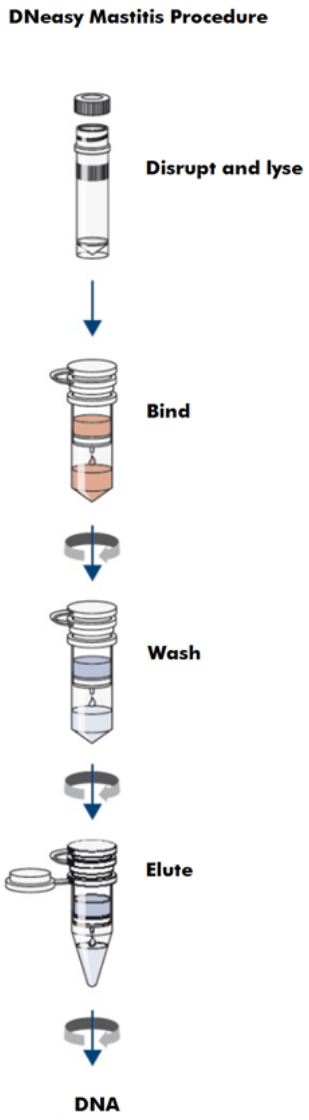
After homogenization and lysis, buffers added to the lysate allow optimal binding of the DNA to the DNeasy membrane before the sample is loaded onto the DNeasy Mini spin column. DNA is adsorbed onto the DNeasy silica membrane during a brief centrifugation step.

DNA bound to the DNeasy membrane is washed in two centrifugation steps. The use of two different wash buffers, Buffer AW1 and Buffer AW2, significantly improves the purity of the eluted DNA. Wash conditions ensure complete removal of any residual contaminants without affecting DNA binding.

Purified DNA is eluted from the DNeasy Mini spin column in a concentrated form in Buffer ATE. Elution buffer should be equilibrated to room temperature (15–25°C) before applying to the column. Eluted DNA is ready for direct addition to downstream applications.

Alternatively, eluted DNA can be safely stored at –20°C for later use. The purified DNA is free of protein, nucleases and other contaminants or inhibitors. Nucleic acid yields depend on sample type and sample storage.

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


DNeasy Mastitis Procedure
Disrupt and lyse
Bind
Wash
Elute
DNA

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.

- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Equipment for homogenization of milk samples. We recommend the TissueLyser II with the TissueLyser Adapter Set 2 x 96 (see page 18 for ordering information).
- Multichannel pipettor and disposable pipet tips with aerosol barriers
- Multidispenser
- Ethanol (96–100%)*
- Isopropanol
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Centrifuge 4-16S or 4-16KS with Plate Rotor 2 x 96 (see page 18 for ordering information).
- Disposable gloves
- Vortexer

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

Important Notes

Starting material

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

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

Do not overload the DNeasy membrane, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. In some downstream applications, such as PCR, very high background concentrations of nucleic acids may impair the reaction.

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 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 DNeasy Mastitis Mini 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.

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.

Important points before starting

- 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).
- 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 mixture according to Table 1 for use in step 3 of the procedure.

Table 1. 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. Mix the sample thoroughly by vortexing.
2. Open the Pathogen Lysis Microtubes S and discard caps.
3. Add 80 µl Buffer ML mixture (see Table 1) to each tube.

4. 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.
5. Cover the rack with new caps for collection microtubes (provided).
6. 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

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

   Operate the TissueLyser II for 8 min at 30 Hz.

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

   Operate the TissueLyser II for another 8 min at 30 Hz.
7. Centrifuge briefly to remove drops from the inside of the tube lid.
8. Add 530 µl Buffer MVL and mix by pipetting. Let beads settle down.

   Optional: Use of a multichannel pipet is recommended.
9. Label the lid of a new DNeasy spin column placed in a 2 ml collection tube. Carefully apply 500 µl lysate from step 8 to the DNeasy spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the DNeasy spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.

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

   Close each spin column to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the DNeasy spin column is empty.
10. Carefully open the DNeasy spin column, and repeat step 9.

11. Carefully open the DNeasy spin column and add 500 µl Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the DNeasy spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
12. Carefully open the DNeasy spin column and add 500 µl Buffer AW2. Close the cap and centrifuge at full speed for 1 min. Discard the collection tube containing the filtrate.

    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, contacting the DNeasy spin column. Removing the DNeasy spin column and collection tube from the rotor may also cause flow-through to come into contact with the DNeasy spin column.
13. Place the DNeasy spin column in a new 2 ml collection tube (provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.

    This step helps to eliminate the chance of possible Buffer AW2 carryover.
14. Transfer the DNeasy spin column into a new, labeled 1.5 ml microcentrifuge tube (provided). Carefully open the DNeasy spin column and pipet 100 µl Buffer ATE directly onto the DNeasy membrane. Close the cap and incubate for 1 min at room temperature, and then centrifuge at full speed for 1 min to elute DNA.

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
General handling	
Lysate not completely passed through the membrane	Centrifuge for 1 minute at full speed or until all the lysate has passed through the membrane
Low yield of DNA	
a) Buffer ML mixture prepared incorrectly	Ensure that Buffer ML mixture was prepared with the correct volumes of additional reagent according to the table in the protocol (page 12). Repeat the DNA purification procedure with new samples.
b) 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.
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 a new sample.
d) DNeasy Mini spin column not incubated at room temperature (15–25°C) for 1 minute	After addition of Buffer ATE, the DNeasy Mini spin column should be incubated at room temperature for at least 1 minute.
e) Frozen samples not mixed properly after thawing	Thaw frozen samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.

		Comments and suggestions
f)	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.
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)	Buffers AW1 and AW2 used in the wrong order	Ensure that Buffers AW1 and AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
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.
DNeasy Mastitis Mini Kit (192)	For 192 preps: DNeasy Mini Columns, Pathogen Lysis Microtubes S (racked), Buffers, Collection Tubes (2 ml)	69805
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
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

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

† 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

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Limited License Agreement for DNeasy Mastitis Mini Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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