Microbial DNA Enrichment

MolYsis™ Basic5

Sample pre-treatment kit for background-free PCR analysis of whole blood and other body fluids

- **Small Size Sample Volumes (≤1 ml)**
- **Medium Size Sample Volumes (5 ml)**

Kit includes all ingredients for the following steps of selective lysis of host cells and the degradation of released DNA:

- Lysis of human/animal cells
- Degradation of human/animal DNA
- Degradation of cell walls of Gram-positive and Gram-negative bacteria and fungi

To be used with other DNA isolation kits

– For research use only –

– Not for use in diagnostic procedures –

molzym
reveal DNA

Version 09

Date of first release: 01/2007
Last update: 11/2023

Contents

Kit Information

Kit Contents – *MolYsis™ Basic5*

	50 reactions (D-301-050)	100 reactions (D-301-100)
Extraction Buffers (store at +18 to +25°C)		
SU	1x 50 ml	2x 50 ml
CM	1x 100 ml	2x 100 ml
DB1	1x 100 ml	2x 100 ml
RS	1x 50 ml	2x 50 ml
RL	1x 15 ml	2x 15 ml
Enzymes & Reagents (store at -15 to -25°C)		
MolDNase B, solution	1x 0.5 ml	2x 0.5 ml
BugLysis, solution	1x 1.0 ml	2x 1.0 ml
β-mercaptoethanol, solution	1x 0.08 ml	2x 0.08 ml
Manual		
Manual	1x	1x

Symbols

Symbols used in labelling and in section 'Hazard and Precautionary Statements' (page 5).


Content of the package

Batch code


Corrosive


Manufactured by

Catalogue number


Toxicity


Use by

Consult instructions for use


Health hazard


Temperature limitation (store at)


Irritant


Environmentally Hazardous

Storage and Stability

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

Please take care that *MolDNase B, BugLysis* and *β-mercaptoethanol* are handled and stored at -15 to -25°C.

Store buffers at room temperature (+18 to +25°C).

Guarantee for full performance of reagents and buffers is given through the expiration date printed on the label at the outer box if the packed material is undamaged upon arrival and the reagents are unopened.

Product Use Limitations

This product is for **research use only** and not for use in diagnostic procedures.
Cells must be intact for reliable results. This requires specimens not to be stored in solutions, which induce cell lysis.
The kit is not suitable for frozen samples, if stored without cryoprotectant, as freeze-thaw can cause lysis of microbial cells.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable sleeve covers, sterile disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS) which are available on request.

CAUTION: Never add hypochlorite (bleach) or acidic solutions directly to the sample-preparation waste.

Buffer *CM* contains guanidine hydrochloride, which can form highly reactive compounds and toxic gases when combined with hypochlorite or other acidic solutions. 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 70% (v/v) ethanol. This kit is to be used only by skilled personnel trained for handling infectious material and molecular biological methods. To avoid false analytic results by DNA contamination of reagents and infection of the user by infectious agents during handling, always wear sterile protective gloves, disposable sleeve covers, a lab coat and protective goggles. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instruction manual of the manufacturer. The UV lamp must be switched off during working. Follow the instructions of the manufacturer for maintenance of the workstation. Dispose potentially infectious material and the waste of the sample preparation according to the national directive of the health organisation (e.g., in Germany: Vollzugshilfe zur Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes, 2021).
Separate material safety data sheets for chemicals used are available on request. Molzym reserves the right to alter or modify the product to improve the performance of the kit.

Hazard and Precautionary Statements

Buffer *CM*

Contains guanidine hydrochloride (> 10 %):
Acute toxicity (oral) and irritating (eyes and skin).

Warning

Hazard and precautionary statements*:
H302-H315-H319;
P301+P312-P302+P352-P305+P351+P338

ß-mercaptoethanol

Contains 2-mercaptoethanol (100 %, CAS no. 60-24-2):
Acute toxicity (oral, inhalation, skin), irritation (skin), eye damage, skin sensitization, reproductive toxicity, specific target organ toxicity and hazardous to aquatic environment (acute and chronic).

Danger

Hazard and precautionary statements*:
H310-H315-H317-H318-H361d-H373-H301+H331-H410;
P273-P280-P301+P310-P302+P352+P310-P304+P340+P310-P305+P351+P338

Emergency Information (24-hours service)

For emergency medical information, please contact the regional poison center in your country.

* **H302:** Harmful if swallowed; **H310:** Fatal in contact with skin; **H315:** Causes skin irritation; **H317:** May cause an allergic skin reaction; **H318:** Causes serious eye damage; **H319:** Causes serious eye irritation; **H361d:** Suspected of damaging the unborn child; **H373:** May cause damage to organs (liver, heart) through prolonged or repeated exposure if swallowed; **H301+H331:** Toxic if swallowed or if inhaled; **H410:** Very toxic to aquatic life with long lasting effects.

P273: Avoid release to the environment; **P280:** Wear protective gloves/protective clothing/eye protection/face protection; **P301+P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor; **P301+P312:** IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.; **P302+P352:** IF ON SKIN: Wash with plenty of water; **P302+P352+P310:** IF ON SKIN: Wash with plenty of water. Immediately call a POISON CENTER/doctor; **P304+P340+P310:** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor; **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Introduction

Kit Description

Molecular analysis of bacteria and fungi in fluid samples from clinical materials and animal systems (e.g., blood and other body fluids) can be severely disturbed by a high background of host DNA. ***MolYsis™ Basic5*** removes this background of host DNA and thereby increases the reliability of the molecular analysis of bacteria and fungi in clinical and other samples. The kit contains all ingredients for the selective lysis of host cells and the degradation of released nucleic acids (DNA) in samples.

Patented ***MolYsis™ Basic5*** is a sample pre-treatment tool for the removal of host as well as free cell DNA. The kit can be used as a module in conjunction with any other nucleic acid extraction kit designed for handling in the mini bind-wash-elute format (e.g., mini spin columns, automated systems). Molzym also supplies kits, manual *MolYsis™ Complete5* (≤ 1 and 5 ml; D-321-050) and automated MolYsis-SelectNA™*plus* (1 ml D-450-048), for the complete process of microbial DNA isolation from clinical and other samples, including sample pre-treatment, depletion of host DNA, enrichment and lysis of bacterial and fungal cells, DNA extraction and DNA purification.

Whereas other commercial DNA extraction systems result in a mixture of host and microbial DNA, sample pre-treatment tool, ***MolYsis™ Basic5,*** enables the selective preparation of microbial DNA from samples. Only two steps are needed to obtain a sample that is depleted of host and free cell DNA (Fig. 1, page 7):
i) The addition of a chaotropic buffer to a sample lyses the host cells, whereas microbial cells are unaffected.
ii) The DNA released from host cells is degraded by Molzym's proprietary, chaotrope-resistant *MolDNase B*. Thereafter, microbial cells are sedimented, treated with *BugLysis* reagent to degrade cell walls of Gram-negative and Gram-positive bacteria and fungi and then further processed by protocols for the extraction and purification of nucleic acids.

MolYsis™ Basic5 allows for the pre-treatment of human and animal fluid samples:
i) Samples of ≤ 1 ml from pediatric patients or animal systems.
ii) Samples of 5 ml from adult patients or animal systems.

Samples evaluated:

Human origin: Whole blood (with anti-coagulants), synovial fluid, pleural fluid, cerebrospinal fluid, ascites fluid, pus, broncho-alveolar lavage, nasal douche fluid, urine

Animal origin: Whole blood (with anti-coagulants) from bird, mouse, rat, canine and monkey, hamster ovary cell culture (≤5*10^8 cells per sample), monkey renal cell culture, mammalian cell culture.

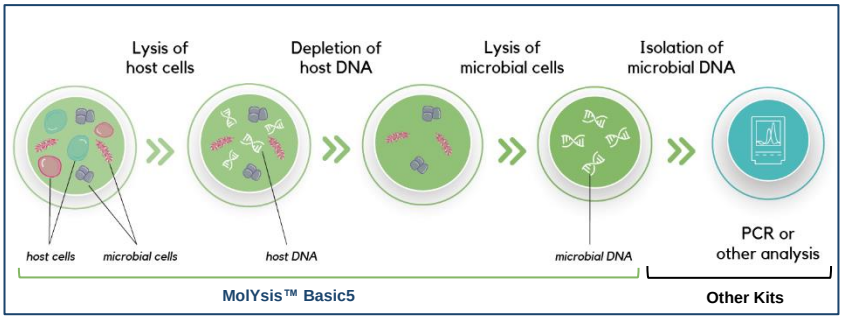

Fig. 1: The principle of testing for bacterial and fungal pathogens in fluid samples by ***MolYsis™ Basic5*** and other kits for the purification of microbial DNA.

The *MolYsis™ Basic5* Technology

MolYsis™ Basic5 is Molzym's proprietary, patented technology enabling the pre-treatment of whole blood and other fluid clinical samples for depletion of human/animal DNA and enrichment of bacterial and fungal organisms. The procedure includes protocols for human/animal DNA removal and universal lysis of Gram-negative and Gram-positive bacteria and fungi. A chaotropic buffer is added to a sample which lyses the human/animal cells (pathogens are unaffected) and the nucleic acids released are degraded by *MolDNase B*. Microbial cells are subsequently centrifuged and treated by the *BugLysis* reagent for the degradation of cell walls. At the end, microbial DNA is purified by other DNA isolation protocols including a proteinase digestion step. The preparation can then be used in broad-range and other PCR assays or NGS-based methods for analysis of bacteria and fungi.

List of Strains detected

The ***MolYsis™ Basic5*** technology has been evaluated with a variety of clinical samples. *BugLysis* reagent is a component of all kits and designed to lyse Gram-positive and Gram-negative bacteria and fungi with high efficiency. Strains from the following genera have been identified in clinical material so far (universal 16S PCR for bacteria, universal 18S PCR for fungi, plus sequencing), showing the broad range of lysing capability of *BugLysis*.

Tab. 1: Extract of microorganisms identified in clinical evaluations. Full list available at www.molzym.com.

Gram-negative bacteria	*Helicobacter pylori*	*Alloiococcus otitis*	*Nocardia* spp.
Achromobacter xylosoxidans	*Kingella* spp.	*Anaerococcus* spp.	*Paenibacillus* spp.
Acidovorax spp.	*Klebsiella* spp.	*Atopobium* spp.	*Parvimonas micra*
Acinetobacter spp.	*Kerstersia* spp.	*Bacillus* spp.	*Peptoniphilus* spp.
Aeromonas veronii	*Kluyvera cryocrescens*	*Bifidobacterium* spp.	*Peptostreptococcus* spp.
Afipia broomeae	*Lautropia mirabilis*	*Brevibacterium* spp.	*Propionibacterium* spp.
Aggregatibacter aphorophilus	*Legionella pneumophila*	*Carnobacterium* spp.	*Rhodococcus* spp.
Anaerotruncus colihominis	*Leptotrichia* spp.	*Clostridium* spp.	*Rothia* spp.
Bacteroides spp.	*Massilia* spp.	*Coprococcus catus*	*Staphylococcus* spp.
Bartonella quintana	*Methylobacterium* spp.	*Corynebacterium* spp.	*Streptococcus* spp.
Bordetella spp.	*Moraxella* spp.	*Dermabacter hominis*	*Tropheryma whippleii*
Borrelia garinii	*Morganella morganii*	*Dietzia* spp.	*Tsukamurella* spp.
Bosea spp.	*Neisseria* spp.	*Dolosigranulum pigrum*	*Ureaplasma urealyticum*
Brucella spp.	*Pantoea agglomerans*	*Eggerthella lenta*	*Vagococcus* spp.
Burkholderia spp.	*Paracoccus* spp.	*Enterococcus* spp.	*Wolbachia* spp.
Campylobacter spp.	*Pasteurella* spp.	*Eremococcus coleocola*	
Candidatus Neoehrlichia mikurensis	*Porphyromonas* spp.	*Eubacterium* spp.	**Fungi**
Capnocytophaga spp.	*Prevotella* spp.	*Facklamia* spp.	*Aspergillus* spp.
Chryseobacterium indologenes	*Proteus* spp.	*Finegoldia magna*	*Candida* spp.
Citrobacter freundii	*Providencia stuartii*	Gardnerella vaginalis	*Cladosporium cladosporioides*
Cloacibacterium normanense	*Pseudomonas* spp.	*Gemella* spp.	*Cryptococcus* spp.
Comamonas testosteroni	*Ralstonia* spp.	*Gordonia* spp.	*Didymella exitialis*
Coxiella burnetii	*Raoultella planticola*	*Granulicatella adiacens*	*Davidiella tassiana*
Cronobacter sakazakii	*Rickettsia typhi*	*Janibacter* spp.	*Fusarium* spp.
Curvibacter spp.	*Serratia marcescens*	*Kocuria* spp.	*Issatchenkia orientalis*
Delftia spp.	*Shigella* spp.	*Lactobacillus* spp.	*Malassezia* spp.
Dialister spp.	*Stenotrophomonas maltophilia*	*Lactococcus* spp.	*Pseudallescheria boydii*
Elizabethkingia meningoseptica	*Veillonella* spp.	*Leifsonia* spp.	*Saccharomyces cerevisiae*
Enhydrobacter aerosaccus	*Weeksella* spp.	*Listeria monocytogenes*	*Schizophyllum radiatum*
Enterobacter spp.	*Yersinia* spp.	*Microbacterium* spp.	*Sporobolomyces* spp.
Escherichia spp.	**Gram-positive bacteria**	*Micrococcus* spp.	
Fusobacterium spp.	*Abiotrophia* spp.	*Mogibacterium timidum*	**Protist**
Haemophilus spp.	*Actinomyces* spp.	*Mycobacterium* spp.	*Plasmodium* spp.
Hafnia alvei	*Aerococcus* spp.	*Mycoplasma* spp.	

Recommendations for PCR Analysis of Bacteria and Fungi

Avoidance of DNA contamination: PCR analysis demands special care with respect to the avoidance of contamination from exogenous sources. Take care to separate places of DNA preparation from places where PCR reagents are handled, in particular preparation of mastermixes, pipetting into PCR tubes and performance of PCR runs. Wear sterile protective gloves at any handling step, also during DNA preparation. Frequently change sterile protective gloves during handling. Use only sterilized or, optimally, guaranteed DNA-free disposables. If analysis of microorganisms is desired, e.g., bacteria identification by sequencing of broad-range 16S amplification products, it is important to make sure that only polymerases (e.g., Taq polymerase) free of DNA contamination are used. For this purpose, Molzym offers guaranteed DNA-free MolTaq 16S/18S (P-019-0100) and Hot MolTaq 16S/18S (P-080-0100). Also, Molzym offers a DNA-free mastermix (Mastermix 16S Complete; S-020-0100) containing primers for universal 16S rDNA amplification of bacterial sequences. For the analysis of fungal DNA sequences, Molzym offers the DNA-free mastermix (Mastermix 18S Complete; S-070-0100) containing universal 18S primers. Generally, for each analysis, run positive and negative controls to check for proper performance of the reaction and sterility of reagents and buffers used.

Protocols

How to Start

Caution:

Work in a UV Class II biological safety cabinet. The UV lamp must be switched off during working. Use protective gloves and a disposable lab coat when handling infectious material!

! **Body fluid specimens:** Sampled under aseptic conditions and transferred to a sterile sample container (not supplied).

Whole blood samples: Use only EDTA or citrate-stabilized blood

! For optimal results, use only fresh samples. ***Do not freeze samples*** to avoid loss of microbial DNA due to cell disruption. For longer storage, use Molzym's *UMD-Tubes* (order no. Z-801-020).

! To be supplied by the user:

- 1x UV Class II biological safety cabinet
- 1x bench top microcentrifuge (≥12,000x*g*)
- 1x high speed centrifuge and fixed angle rotor for 50 ml tubes (9,500x*g*; only for protocol 2)
- 1x thermomixer (2.0 ml tubes)
- 1x vortexer
- 1x cooling rack for 1.5 ml tubes (-15 to -25°C)
- Sample racks
- Precision pipettes and sterile filter pipette tips allowing pipetting volumes of up to 20 µl, up to 200 µl and up to 1000 µl
- 2.0 ml micro tubes, Biosphere®, Sarstedt, Germany (72.695.200) for microbial cell lysis and DNA extraction.
- 1x other Mini kit for nucleic acid extraction and purification

- Only for protocol 2:
  - Sterile, disposable 5 ml pipette equipped with aerosol filter, or a 5 ml tip of a precision pipette.
  - Sterile 50 ml tubes (VWR® Ultra-High Performance Centrifuge Tubes, Cat. no. 525-1109, VWR) for preparation of sample lysates by high-speed centrifugation. If using other brands, **make sure that tubes can be used at RCF of 9,500xg**

! Take care that *MolDNase B, BugLysis* and *β-mercaptoethanol* solutions are placed in a cooling rack adjusted to -15 to -25°C. Replace enzymes and reagent to the freezer (-15 to -25°C) immediately after handling.

! Adjust a thermomixer to 37°C

! To avoid contamination, close caps of bottles after removal of solution.

Approximate time for 4 parallel pre-treatments of samples:
80 min *(including 15 min hands on time)*

Protocol 1: Small Size Sample DNA Isolation (≤ 1 ml Fluid)

Please read section 'How to Start' before starting the procedure (page 9)!

Procedure

A) Fill up procedure for samples less than 1 ml volume

Samples less than 1 ml are filled up using buffer *SU*. Transfer the sample by pipetting into a sterile 2 ml polypropylene tube (not supplied; specification, page 9). Then add buffer *SU* until reaching the 1 ml mark of the tube. Discard pipette tip with excess buffer *SU*. Continue with the pre-treatment protocol 1 part B (below).

B) Sample pre-treatment procedure

1. **Pipette 1 ml sample into a sterile 2.0 ml tube (not supplied; specification, page 9) or use filled-up sample (part A). Then add 250 µl buffer *CM* and vortex at full speed for 15 s to mix. Let stand on the bench at room temperature (+18 to +25°C) for 5 min.**

   Buffer *CM* is a chaotropic buffer that lyses the human/animal cells. For optimal results it is important to mix thoroughly.
   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 250 µl buffer *DB1* and 10 µl *MolDNase B* to the lysate and immediately vortex for 15 s. Let stand on the bench for 15 min.**

   During this step the DNA released from human/animal cells are degraded.

3. **Centrifuge tube in a bench top microcentrifuge at ≥ 12,000x*g* for 10 min. Thereafter, carefully remove the supernatant by pipetting taking care to not disturb the sediment and discard.**

4. **Add 1 ml buffer *RS* and resuspend the sediment by vigorous vortexing.**

   Depending on the sample, the sediment may be rigid and resuspension may take some time. In this case stir the sediment with the pipette tip and pipette in and out until resuspended.

5. **Centrifuge tube in a bench top microcentrifuge (≥ 12,000x*g*) for 5 min. Carefully remove the supernatant by pipetting and discard.**

   This washing removes residual *MolDNase B* activity, chaotropic salts and most of the PCR inhibitors.

   Note: At this point the procedure can be interrupted by freezing the sample (-15 to -25°C). For further processing, thaw the sample to room temperature (+18 to +25°C) and proceed with step 6 (page 11).

6. **Add 80 µl buffer *RL* and resuspend the sediment by vigorous vortexing.**

   The pellet consists of cell debris and microbial cells. Resuspension may take some time. Take care that all visible material has been resuspended. Potential residual small particles in the suspension can be neglected, because they are dissolved during enzymatic treatments, in particular *Proteinase K* digestion with other kits (below).

7. **Add 20 µl *BugLysis* solution and 1.4 µl *ß-mercaptoethanol*, vortex for 15 s and incubate tube in a thermomixer at 37°C and 1,000rpm for 30 min.**

   The cell walls of potentially present bacteria and fungi are degraded.

   Caution: ß-mercaptoethanol is toxic. Take care not to inhale and otherwise come into contact with.

Further processing for DNA extraction and purification (other Mini kits):

After step 7, the microbial lysate can be processed by protocols for DNA isolation using manual or automated commercial kits or in-house extraction protocols. Protocols must include a protease or Proteinase K digestion after step 7 (above). Note that protease/ Proteinase K treatment is essential for optimal results. For this purpose, fill the microbial lysate (step 7, above) up to the sample volume of this kit with buffer *RL* (e.g., for 200 µl sample volume add 100 µl *RL* to the microbial lysate). For elution of the DNA from the column matrix, good experience was made by using DNA-free water, PCR grade (Molzym order no. P-020-0003) heated to 70°C.

Protocol 2: Medium Size Sample DNA Isolation (5 ml Fluid)

Please read section 'How to Start' before starting the procedure (page 9)!

Procedure

1. **Pipette 5 ml sample into a sterile 50 ml tube (not supplied, specification, page 9) and add 2 ml buffer *CM*. Vortex at full speed for 15 s. Let stand on the bench at room temperature (+18 to +25°C) for 5 min.**

   Buffer *CM* is a chaotropic buffer that lyses the human/animal cells. For optimal results it is important to mix thoroughly.
   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 2 ml buffer *DB1* and 10 µl *MolDNase B* to the lysate and immediately vortex for 15 s. Let stand on the bench for 15 min.**

   During this step the DNA released from human/animal cells are degraded.

3. **Centrifuge 50 ml tube in a high speed centrifuge at 9,500x*g* for 10 min. Thereafter, carefully decant the supernatant.**

4. **Add 1 ml buffer RS and resuspend the sediment by vigorous vortexing.**

   The sediment consists of cell debris and pathogen cells. Resuspension may take some time. Take care that all visible material has been resuspended.

5. **Transfer the suspension by pipetting to a sterile 2.0 ml tube (not supplied; specification, page 9). Centrifuge tube in a bench top microcentrifuge (≥ 12,000x*g*) for 5 min. Carefully remove the supernatant by pipetting and discard.**

   This washing removes residual *MolDNase B* activity, chaotropic salts and most of the PCR inhibitors.

   Note: At this point the procedure can be interrupted by freezing the sample (-15 to -25°C). For further processing, thaw the sample to room temperature (+18 to +25°C) and proceed with step 6 (page 11).

Continue with step 6 of Protocol 1 'Small Size Sample DNA Isolation' (page 11).

Supplementary Information

Troubleshooting

This guide may help solving problems that may arise. The Molzym team is always pleased to answer any of your questions about our products.

Phone: +49(0)421 69 61 62 0 • **E-Mail:** support@molzym.com

Observation	Possible cause	Comments/suggestions
Strong human/animal DNA background in gel electrophoresis or Real-Time PCR	• Buffer *CM* not added • Buffer *DB1* not added • *MolDNase B* not added • Solutions not mixed properly	Eluates usually contain traces of human/animal DNA co-eluted with bacterial/fungal DNA. If the extraction has not been performed according to the protocol, increased amounts of human/animal DNA can be the result, which negatively influences the PCR reaction. Ensure that buffer *CM* has been added to lyse human/animal cells. Accordingly, addition of buffer *DB1* and *MolDNase B* is obligate. Keep the *MolDNase B* vial chilled, because warming may reduce enzyme activity and hence increase human/animal DNA background. It is important that solutions are thoroughly mixed after addition of buffers. Follow instructions for vortexing.
No microbial DNA detectable (spiking test with negative blood)	• Insufficient lysis	Make sure that *BugLysis* and *β-mercaptoethanol* treatments have been performed. Be aware that DNA is visble in a gelelectrophoresis only at amounts approx. >10ng (approx.>2x 10^7 *E. coli* cells). Use PCR based procedures for detection and quantitation of bacteria <10^7 cells.
	• Insufficient homogenisation	If the pellets from steps 4 (pages 10 and 12) and 6 (page 11) are not totally homogenized, bacterial and fungal cells may be included in the debris and not reached by lytic enzymes. See comments at page 11.
	• Microbial titre too low	Check the titre of the bacteria and fungi by plating and increase the titre for inoculation.
	• Loss of nucleic acids during purification	See troubleshooting guides of procedures in laboratory manuals or these kits. Alternatively, use Molzym´s complete DNA isolation kits which have been extensively evaluated for isolation of pico to femtogram amounts of pathogen DNA.
	• Wrong elution conditions	For elution of the DNA from the column matrix, good experience was made using DNA-free water, PCR grade (Molzym order no. P-020-0003) heated to 70°C. This increases the DNA yield significantly.

	• Loss of nucleic acids during the storage of the eluate	Store the eluted DNA at +4 to +12°C if analysed at the same day or freeze at -15 to -25°C until further use. Avoid frequent freeze-thaw cycles, because this may result in a decay of the eluted DNA (in particular at low DNA concentrations).
False positive PCR result	• Cross contamination • Contamination during handling	Principally, work under UV-irradiated workstations. Avoid the generation of aerosols by careful pipetting. Frequently change gloves. UV-irradiate the workstation at the end of handling a series of samples. Prepare mastermixes and handle DNA samples under different UV workstations (page 8). Use DNA-free pipette tips and other plastics. Make sure that the other Mini kit used for nucleic acid extraction and purification is DNA-free.

Tradenames

Tradename	**Factory**
Biosphere®	Sarstedt
Hot MolTaq 16S/18S	Molyzm
Mastermix 16S Complete	Molyzm
Mastermix 18S Complete	Molyzm
Mastermix 16S/18S Basic	Molyzm
Mastermix 16S/18S Dye	Molyzm
MolTaq 16S/18S	Molyzm
MolYsis™ Basic5	Molyzm
MolYsis™ Complete5	Molyzm
MolYsis-SelectNA™*plus*	Molyzm
SelectNA™*plus*	Molzym
Ultra-Deep Microbiome Prep	Molzym
Ultra-Deep Microbiome Prep10	Molzym

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Kit includes reagents for tissue pre-treatment, host DNA depletion, microbial DNA extraction and purification.	50 reactions	G-020-050
≤1 ml fluid samples		
≤0.5 cm^3 tissue samples		
Ultra-Deep Microbiome Prep10	25 reactions	G-030-025
Kit includes reagents for tissue pre-treatment, host DNA depletion, microbial DNA extraction and purification.	50 reactions	G-030-050
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