Isolation of Microbial DNA

MolYsis™ Complete5

Sample pre-treatment and bacterial/fungal DNA isolation kit for background-free PCR analysis of whole blood and other body fluids

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

Kit includes all ingredients for the following steps of the microbial DNA purification:

- Lysis of human/animal cells
- Degradation of human/animal DNA
- Degradation of cell walls of Gram-positive and Gram-negative bacteria and fungi
- Removal of PCR inhibitors
- Microbial DNA purification

- For research use only -

Molzym

Version 08

Date of first release: 05/2007
Last update: 10/2020

Contents

Kit Information

Kit Contents – *MolYsis™ Complete5*

	50 reactions	100 reactions
Extraction Buffers (store at +18 to +25°C)		
SU	1x 50ml	2x 50ml
CM	1x 100ml	2x 100ml
DB1	1x 100ml	2x 100ml
RS	1x 50ml	2x 50ml
RL	1x 5ml	2x 5ml
RP	1x 7.5ml	2x 7.5ml
CS	1x 12.5ml	2x 12.5ml
AB	1x 12.5ml	2x 12.5ml
WB	1x 20ml	2x 20ml
70% Ethanol	1x 20ml	2x 20ml
Deionized water	1x 5ml	2x 5ml
Enzymes & Reagents (store at -15 to -25°C)		
MolDNase B, solution	1x 0.5ml	2x 0.5ml
BugLysis, solution	1x 1.0ml	2x 1.0ml
β-mercaptoethanol, solution	1x 0.08ml	2x 0.08ml
Proteinase K, solution	1x 1.0ml	2x 1.0ml
Consumables (store at +18 to +25°C)		
Spin columns in 2.0ml *Collection tubes*	1x 50	2x 50
Collection tubes, 2.0ml	2x 50	4x 50
Elution tubes, 1.5ml	1x 50	2x 50
Manual		
Manual	1x	1x

Symbols

Symbols used in labelling and in section 'Hazard and Precautionary Statements' (pages 5 to 6).


Content of the package

Catalogue number


Manufactured by

Consult instructions for use


Irritant


Use by


Flammable


Health hazard


Temperature limitation (store at)


Corrosive


Environmentally Hazardous

Batch code


Toxicity

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*, *β-mercaptoethanol* and *Proteinase K* are handled and stored at -15 to -25°C.

Buffers and consumables should be stored at room temperature (+18 to +25°C).

Stable for 24 months from the date of manufacturing under proper storage condition. 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.

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.

Buffers *CM* and *CS* contain guanidine hydrochloride and guanidinium thiocyanate, respectively, 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. Richtlinie über die ordnungsgemäße Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes, 2002). 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*(page 6):
H302-H315-H319; P301+P312-P302+P352-P305+P351+P338

ß-mercaptoethanol

Contains 2-mercaptoethanol (100%): **Poisonous, irritating, environmental hazardous**

Danger

Hazard and precautionary statements*(page 6):
H227-H301-H310+H330-H315-H318-H410; P273-P301+P310-P302+P352-P304+P340-P305+P351+P338

Proteinase K

Contains *Proteinase K* (≥1%):
Respiratory sensitization, irritating (eyes and skin) and Specific target organ toxicity – single exposure

Danger

Hazard and precautionary statements*(page 6):
H315-H319-H334-H335; P302+P352-P304+P341-P305+P351+P338

Buffer *CS*

Contains guanidinium thiocyanate (>10%):
Acute toxicity (oral, dermal, inhalation) and chronic aquatic toxicity.

Warning

Hazard and precautionary statements*(page 6):
H302-H312-H332-H412-EUH032; P260-P273-P301+P312-P302+P350-P304+P340

Buffer *AB*

Contains 2-propanol (>40%): **Flammable liquids and irritating (eyes)**.

Danger

Hazard and precautionary statements*(page 6):
H225-H319-H336; P210-P233-P304+P340-P305+P351+P338

Buffer *WB*
Contains isopropanol (≥40%): **Flammable liquids and irritating (eyes)**.

Danger

Hazard and precautionary statements*:
H225-H319-H336; P210-P233-P304+P340-P305+P351+P338

70% Ethanol, DNA-free
Contains ethanol (>50%): **Flammable liquids**.

Danger

Hazard and precautionary statements*:
H225; P210-P233

Emergency Information (24-hours service)
Emergency medical information in English, French, and German can be obtained
24 hours a day from: Poison Information Centre Mainz, Germany; Tel: +49(0)6131 19240
Outside of Germany: Please contact the regional company representation in your country.

* **H225**: Highly flammable liquid and vapour; **H227**: Combustible liquids; **H301**: Toxic if swallowed; **H302:** Harmful if swallowed; **H302+312+332:** Harmful if swallowed, in contact with skin or if inhaled; **H310+H330**: Fatal if swallowed or in contact with skin; **H311+H331**: Toxic in contact with skin or if inhaled; **H312**: Harmful in contact with skin; **H315**: Causes skin irritation; **H318**: Causes serious eye damage; **H319**: Causes serious eye irritation; **H332**: Harmful if inhaled; **H334**: May cause allergy or asthma symptoms or breathing difficulties if inhaled; **H335**: May cause respiratory irritation; **H336**: May cause drowsiness or dizziness; **H410**: Very toxic to aquatic life with long lasting effects; **H412**: Harmful to aquatic life with long lasting effects; **EUH032**: Contact with acids liberates very toxic gas

P210: Keep away from heat/sparks/open flames/hot surfaces. – No-smoking; **P233**: Keep container tightly closed; **P260**: Do not breath fume/gas/mist/vapours; **P273:** Avoid release to the environment; **P301+P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician; **P301+P312:** IF SWALLOWED: Rinse mouth. Do NOT induce vomiting; **P302+P350**: IF ON SKIN: Gently wash with plenty of soap and water; **P302+P352:** IF ON SKIN: Wash with plenty of soap and water; **P304+P340:** IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing;**P304+P341**: IF INHALED: If breathing difficult, remove to fresh air and keep at rest in a position comfortable for breathing; **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 pathogenic 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 and dead cell DNA. Besides PCR inhibitors, unspecific binding of bacterial and fungal sequence-specific primers to host sequences can negatively interfere with pathogen analysis. With ***MolYsis™ Complete5*** Molzym has developed a tool for the enrichment of bacterial and fungal cells and isolation of enriched microbial DNA from blood and other fluids. ***MolYsis™ Complete5*** is the complete solution for the removal of PCR inhibitors and host and dead cell DNA from samples, allowing the reliable and sensitive detection of microbial pathogens through PCR or Real-Time PCR.

MolYsis™ Complete5 allows for the microbial DNA isolation from human and animal fluid samples:

i) Samples of ≤1ml from, e.g. pediatric patients or animals.

ii) Samples of 5ml from adult patients.

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, and monkey, hamster ovary cell culture (≤$5*10^8$ cells per sample), monkey renal cell culture, mammalian cell culture.

The *MolYsis™ Complete5* Technology

MolYsis™ Complete5 is Molzym's proprietary, patented technology enabling the enrichment and purification of microbial DNA from fluid clinical samples and animal model material for molecular analysis. The procedure includes protocols for: **i)** Human/animal DNA removal. **ii)** Universal lysis of Gram-negative and Gram-positive bacteria, and fungi. **iii)** Isolation of the microbial DNA.

Only three steps are needed to obtain microbial DNA preparations that are depleted of host DNA (Fig. 1):
I) The addition of a chaotropic buffer to a fluid sample lyses the host cells, whereas microbial cells are unaffected. The DNA released from host cells as well as dead cells is degraded by Molzym's proprietary, chaotrope-resistant *MolDNase B*.
II) Microbial cells are sedimented, treated with *BugLysis* reagents to degrade cell walls of Gram-negative bacteria, Gram-positive bacteria and fungi and then digested by *Proteinase K* treatment.
III) The microbial DNA is extracted and then isolated by a quick bind-wash-elute procedure, using Molzym's CCT technology with quantitative DNA binding to the filter matrix and high recovery of microbial DNA from the column.

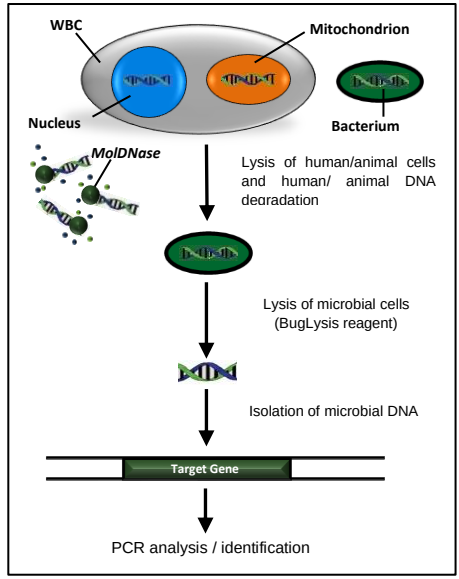

Fig. 1: The principle of testing for bacterial and fungal DNA in samples by ***MolYsis™ Complete5***.

List of Strains detected

The ***MolYsis™ Complete5*** technology has been evaluated with a variety of clinical samples (page 7). *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: Microorganisms identified in clinical evaluations.

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.

Call us for further information at +49(0)421 69 61 62 0.

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 pathogen 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 50ml tubes (9,500x*g*; only for protocol 2)
- 1x thermomixer (2.0ml tubes)
- 1x vortexer
- 1x cooling rack for 1.5ml tubes (-15 to -25°C)
- Sample racks
- Precision pipettes and sterile filter pipette tips allowing pipetting volumes of up to 20µl, up to 200µl and up to 1000µl
- 2.0ml micro tubes, Biosphere®, Sarstedt, Germany (72.695.200) for bacterial and fungal cell lysis and DNA extraction
- 1.5ml micro tubes, Biosphere®, Sarstedt, Germany (72.706.200) for Deionized water, DNA-free

- Only for protocol 2:
  - Sterile, disposable 5ml pipette equipped with aerosol filter, or a 5ml tip of a precision pipette.
  - Sterile 50ml tubes (Cellstar tubes, order no. 227261, Greiner Bio-One / 50ml Centrifuge Tubes, Cat. no. 21008-242, 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*, *β-mercaptoethanol* and *Proteinase K* 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 the thermomixer to 37°C. Pipette an aliquot of *Deionized water* (100µl for each sample) into a sterile 1.5ml Biosphere® tube (not supplied) and place into the thermomixer (needed for step 15).

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

Approximate time for 4 parallel DNA preparations: ***120min***

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

Please read section 'How to Start' before starting the procedure (page 10)!

Procedure

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

Samples less than 1ml are filled up using buffer *SU*. Transfer the sample by pipetting into a sterile 2ml polypropylene tube (not supplied**;** specification, page 10). Then add buffer *SU* until reaching the 1ml mark of the tube. Discard the pipette tip with residual buffer *SU*. Continue with protocol1 part B (below).

B) Sample pre-treatment and DNA isolation procedure

1. **Pipette 1ml sample into a sterile 2.0ml tube (not supplied; specification, page 10) or use filled-up sample (protocol1 part A, above). Then add 250µl buffer *CM* and vortex at full speed for 15s 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 250µl buffer *DB1* and 10µl *MolDNase B* to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

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

3. **Centrifuge tube in a bench top microcentrifuge at ≥12,000x*g* for 10min. Thereafter, carefully remove the supernatant by pipetting and discard.**

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

   Depending on the sample, the pellet 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 the tube in a bench top microcentrifuge ≥12,000x*g* for 5min. 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 12).

6. **Add 80µl 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 *Proteinase K* digestion (step 8, below).

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

   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.

8. **Adjust the temperature of the thermomixer to 56°C. Add 150µl buffer RP and 20µl *Proteinase K* (do not premix) to the tube. Vortex at full speed for 15s and incubate at 56°C and 1,000rpm for 10min. Thereafter, adjust the temperature of the thermomixer to 70°C (make sure that the tube containing *Deionized water, DNA-free* is placed in the mixer, needed at step 15).**

9. **Briefly centrifuge to remove lysate from the lid. Add 250µl buffer *CS* and vortex at full speed for 15s.**

   Cells are lysed and protein is denatured.

10. **Briefly centrifuge and add 250µl binding buffer *AB*, vortex at full speed for 15s.**

11. **Briefly centrifuge and transfer the lysate to a *Spin column*. Close lid and centrifuge loaded column at ≥12,000x*g* for 30s (or minimum time of the centrifuge). Remove the *Spin column*, discard the *Collection tube* with flow-through and replace the column into a new 2.0ml *Collection tube*.**

    At this point DNA binds to the matrix.

12. **Add 400µl buffer *WB* to the Spin column. Close lid and centrifuge at ≥12,000x*g* for 30s (or minimum time of the centrifuge). Remove the *Spin column*, discard the *Collection tube* with flow-through and replace the column into a new 2.0ml *Collection tube*.**

13. **Wash the Spin column with 400µl of *70% Ethanol* by centrifugation at ≥12,000x*g* for 3min.**

    This step removes salts and dries the column matrix.

14. **Carefully remove the column from the centrifuge. Avoid splashing of the flow-through to the column. Transfer the Spin column to a 1.5ml *Elution tube*.**

15. **Place 100µl *Deionized water* (tube in the thermomixer is already preheated to 70°C), in the centre of the column, close lid and incubate for 1min at room temperature (+18 to +25°C). Thereafter, centrifuge at ≥12,000x*g* for 1min to elute the DNA.**

    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).

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

Please read section 'How to Start' before starting the procedure (page 10)!

Procedure

1. **Pipette 5ml sample into a sterile 50ml tube (not supplied, specification page 10) and add 2ml buffer *CM*. Vortex at full speed for 15s. Let stand on the bench at room temperature (+18 to +25°C) for 5min.**

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

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

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

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

   The pellet 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 2.0ml tube (not supplied; specification page 10). Centrifuge tube in a bench top microcentrifuge (≥12,000x*g*) for 5min. 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 12).

Continue with step 6 of Protocol 1 'Small Size Sample DNA Isolation' (page 12).

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 microbial 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 pathogen DNA detectable (spiking test with negative blood)	• Insufficient lysis	Make sure that *BugLysis*, *β-mercaptoethanol* and *Proteinase K* 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 and 6 (pages 11 12 and 13) are not totally homogenized, microbial cells may be included in the debris and not reached by lytic enzymes. See comments at page 12.
	• Pathogen titre too low	Check the titre of the pathogen by plating and increase the titre for inoculation.
	• Loss of nucleic acids during purification	Ensure that buffer *AB* has been added to and mixed with the lysate (step 10, page 12). Accordingly, make sure that the column has been washed with buffer *WB* (step 12, page 12).
	• Wrong elution conditions	Make sure to elute with supplied heated *Deionized water* (70°C; step 15, page 12). 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 9). Use DNA-free pipette tips and other plastics.
False negative PCR result	• PCR inhibitors co-eluted	Check whether *Proteinase K* treatment has been performed during DNA preparation. Make sure that all washing steps of the procedure have been followed. Optionally, after *70% Ethanol* washing (step 13, page 12), discard flow-through and centrifuge for another 1min to avoid ethanol carryover to the eluate

References

Aghamollaei H, Moghaddam MM, Kooshki H, Heiat M, Mirnejad R, Barzi NS (2015) Detection of Pseudomonas aeruginosa by a triplex polymerase chain reaction assay based on lasI/R and gyrB genes. J Infect Pub Health 418; doi.org/10.1016/j.jiph.2015.03.003.

Benítez-Páez A, Álvarez M, Belda-Ferre P, Rubido S, Mira A, Tomás I (2013) Detection of transient bacteraemia following dental extractions by 16S rDNA pyrosequencing: A pilot study. PLoS ONE **8**, e57782. doi:10.1371/journal.pone.0057782.

Bille J (2010) New nonculture-based methods for the diagnosis of invasive candidiasis. Curr Opin Crit Care **16**, 460-464.

Czurda S, Lion T (2017) Broad-spectrum molecular detection of fungal nucleic acids by PCR-based amplification techniques. In: Lion T (ed.), Human Fungal Pathogen Identification: Methods and Protocols, Methods in Molecular Biology, vol. 1508, p. 257-266. DOI 10.1007/978-1-4939-6515-1_4, © Springer Science+Business Media New York.

Disqué C (2007) Einfluss der DNA-Extraktion auf die PCR-Detektion von Sepsiserregern. BIOspektrum **06**, 627-629.

Downey LC, Smith BP, Benjamin DK, Cohen-Wolkowiez (2010) Recent advances in the detection of neonatal candidiasis. Curr Fungal Infect Rep **4**, 17-22.

Duran-Pinedo AE, Chen T, Teles R, R Starr JR, Wang X, Krishnan K, Frias-Lopez J (2014) Community-wide transcriptome of the oral microbiome in subjects with and without periodontitis. The ISME Journal, doi:**10**.1038/ismej.2014.23.

Esteban J, Alonso-Rodriguez N, del-Prado G, Ortiz-Pérez A, Molina-Manso D, Cordero-Ampuero J, Sandova E, Fernández-Roblas R, Gómez-Barrena E (2012) PCR-hybridization after sonication improves diagnosis of implant-related infection. Acta Orthopaedica **3**, 299-304.

Fricke WF, Maddox C, Song Y, Bromberg JS (2014), Human microbiota characterization in the course of renal transplantation. Am J Transplant **14**, 416–427.

Gebert S, Siegel D, Wellinghausen N (2008) Rapid detection of pathogens in blood culture bottles by real-time PCR in conjunction with the pre-analytic tool MolYsis. J Infect **57**, 307-316.

Gyarmati P, Kjellander C, Aust C, Song Y, Öhrmalm L, Giske CG (2016) Metagenomic analysis of bloodstream infections in patients with acute leukemia and therapy-induced neutropenia. Sci Rep. 6, 23532. doi: 10.1038/srep 23532.

Handschur M, Karlic H, Hertl C, Pfeilstöcker M, Haslberger AG (2009) Preanalytic removal of human DNA eliminates false signals in general 16S rDNA PCR monitoring of bacterial pathogens in blood. Comp Immunol Microbiol Infect Dis **32**, 207-219.

Hansen WLJ, Bruggeman CA, Wolffs PFG (2009) Evaluation of new preanalysis sample treatment tools and DNA isolation protocols to improve bacterial pathogen detection in whole blood. J Clin Microbiol **47**, 2629-2631.

Hansen WLJ, Bruggeman CA, Wolffs PFG (2013) Pre-analytical sample treatment and DNA extraction protocols for the detection of bacterial pathogens from whole blood. Meth Mol Biol **943**, 81-90.

Horz HP, Scheer S, Huenger F, Vianna ME, Conrads G (2008) Selective isolation of bacterial DNA from human clinical specimens. J Microbiol Meth **72**, 98-102.

Horz HP, Scheer S, Vianna ME, Conrads G (2010) New methods for selective isolation of bacterial DNA from human clinical specimens. Anaerobe **16**, 47-53.

Kemp M, Jensen KH, Dargis R, Christensen JJ (2010) Routine ribosomal PCR and DNA sequencing for detection and identification of bacteria. Future Microbiol **5**, 1101-1107.

Krohn S, Böhm S, Engelmann C, Hartmann J, Brodzinski A, Chatzinotas A, Zeller K, Prywerek D, Fetzer I, Berg T (2014) Application of qualitative and quantitative real-time PCR, direct sequencing, and terminal restriction fragment length polymorphism analysis for detection and identification of polymicrobial 16S rRNA genes in ascites. J Clin Microbiol **52**, 1754-1757.

Laakso S, Mäki M (2013) Assessment of a semi-automated protocol for multiplex analysis of sepsis-causing bacteria with spiked whole blood samples. MicrobiologyOpen **2**, 284–292.

Leggieri N (2010) Molecular diagnosis of bloodstream infections: planning to (physically) reach the bedside. Curr Opin Infect Dis **23**, 311-319.

Leitner E, Kessler HH (2015) Broad-range PCR for the identification of bacterial and fungal pathogens from blood: a sequencing approach. Sepsis: diagnostic methods and protocols. Methods in Molecular Biology (Mancini N, ed.), p. 129-138. Springer New York.

Loonen AJM, Jansz AR, Stalpers J, Wolffs PFG, van den Brule AJC (2012) An evaluation of three processing methods and the effect of reduced culture times for faster direct identification of pathogens from BacT/ALERT blood cultures by MALDI-TOF MS. Eur J Clin Microbiol Infect Dis **31**, 1575-1583.

Loonen AJM, Jansz AR, Kreeftenberg H, Bruggeman CA, Wolffs PFG, van den Brule AJC (2011) Acceleration of the direct identification of Staphylococcus aureus versus coagulase-negative staphylococci from blood culture material: a comparison of six bacterial DNA extraction methods. Eur J Clin Microbiol Infect Dis **30**, 337-342.

Lorenz MG, Mühl H, Disqué C (2015) Bacterial and fungal DNA extraction from blood samples: manual protocols. Sepsis: diagnostic methods and protocols. Methods in Molecular Biology (Mancini N, ed.), p. 109-119. Springer New York.

Lorenz MG, Disqué C, Mühl H (2015) Bacterial and fungal DNA extraction from blood samples: automated protocols. Sepsis: diagnostic methods and protocols. Methods in Molecular Biology, vol. 1237 (Mancini N, ed.), p. 121-128. Springer New York.

Masek BJ, Hardick J, Won H, Yang S, Hsieh YH, Rothman RE, Gaydos CA (2014) Sensitive detection and serovar differentiation of typhoidal and nontyphoidal Salmonella enterica species using 16S rRNA Gene PCR coupled with high-resolution melt analysis. J Mol Diagn **16**, 261–266.

Mayr A, Lass-Flörl C (2011) Non–culture-based methods for the diagnosis of invasive candidiasis. Curr Fungal Infect Reports **5**, 151-156.

McCann CD, Jordan JJ (2014) Evaluation of MolYsis™Complete5 DNA extraction method for detecting *Staphylococcus aureus* DNA from whole blood in a sepsis model using PCR/pyrosequencing. J Microbiol Meth **99**, 1-7.

Meurs KM, Heaney AM, Atkins CE, DeFrancesco TC, Fox PR, Keene BW, Kellihan HB, Miller MW, Oyama MA, Oaks JL (2011) Comparison of polymerase chain reaction with bacterial 16S primers to blood culture to identify bacteremia in dogs with suspected bacterial endocarditis. J Vet Inter Med **25**, 959–962.

Richardson LJ, Kaestlia M, Mayoa M, Bowers JR, Tuanyok A, Schupp J, Engelthaler D, Wagner DM, Keim PS, Currie BJ (2012) Towards a rapid molecular diagnostic for melioidosis: Comparison of DNA extraction methods from clinical specimens. J Microbiol Meth **88**, 179–181.

Richtlinie über die ordnungsgemäße Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes vom 01.01.2002, Robert-Koch-Institut

Rudkjøbing VB, Aanaes K, Wolff TY, von Buchwald C, Johansen HK, Thomsen TR (2014) An exploratory study of microbial diversity in sinus infections of cystic fibrosis patients by molecular methods. J Cys Fibrosis doi:10.1016/j.jcf.2014.02.008.

Sánchez MC, Llama-Palacios A, Fernández E, Figuero E, Marín MJ, León R, Blanc V, Herrera D, Sanz M (2014) An in vitro biofilm model associated to dental implants: Structural and quantitative analysis of in vitro biofilm formation on different dental implant surfaces, Dental Materials doi.org/10.1016/j.dental.2014.07.008.

Thoendel M, Jeraldo P, Greenwood-Quaintance KE, Yao J, Chia N, Hanssen AD, Abdel MP, Patel R (2017) Impact of Contaminating DNA in Whole Genome Amplification Kits Used for Metagenomic Shotgun Sequencing for Infection Diagnosis: J. Clin. Microbiol. JCM.02402-16

Thoendel M, Jeraldo PR, Greenwood-Quaintance KE, Yao JZ, Chia N, Hanssen AD, Abdel MP, Patel R (2016) Comparison of microbial DNA enrichment tools for metagenomic whole genome sequencing. J Microbiol Meth 21, 141-145.

Thomsen TR, Xu Y, Lorenzen J, Nielsen PH, Schønheyder HC (2012) Improved diagnosis of biofilm infections using various molecular methods. Culture negative orthopedic biofilm infections. Springer Series on Biofilms **7**, 29-41.

Vitrenko Y, Kostenko I, Kulebyakina K, Duda A, Klunnyk M, Sorochynska K (2017) Fetal Tissues Tested for Microbial Sterility by Culture- and PCR-Based Methods Can be Safely Used in Clinics: Cell Transplantation, Volume 26, Number 2, 2017, pp. 339-350(12)

Wellinghausen N, Siegel D, Gebert S, Winter J (2009) Rapid detection of *Staphylococcus aureus* bacteremia and methicillin resistance by real-time PCR in whole blood samples. Eur J Clin Microbiol Infect Dis **28**, 1001-1005.

Wellinghausen N, Siegel D, Winter J, Gebert S (2009) Rapid diagnosis of candidaemia by real-time PCR detection of *Candida* DNA in blood samples. J Med Microbiol **58**, 1106-1111.

Wiesinger-Mayr H, Jordana-Lluch E, Martró E, Schoenthaler S, Noehammer C (2011) Establishment of a semi-automated pathogen DNA isolation from whole blood and comparison with commercially available kits. J Microbiol Meth **85**, 206–213.

Wolff TY, Moser C, Bundgaard H, Høiby N, Nielsen PH, Thomsen TR (2011) Detection of microbial diversity in endocarditis using cultivation-independent molecular techniques. Scand J Infect Dis **43**, 857-869.

Xu Y, Børsholt Rudkjøbing V, Simonsen O, Pedersen C, Lorenzen J, Schønheyder HC, Nielsen PH, Rolighed Thomsen T (2012) Bacterial diversity in suspected prosthetic joint infections: an exploratory study using 16S rRNA gene analysis. FEMS Immunol Med Microbiol **65**, 291–304.

Zhao Y, Armeanu E, DiVerniero T, Lewis TA, Dobson RC, Kontoyiannis DP, Roilides E, Walsh TJ, Perlin DS (2014) Fungal DNA detected in blood samples of patients who received contaminated methylprednisolone injections reveals increased complexity of causative agents. J Clin Microbiol **52**, 2212-2215.

Zhou L, Pollard AJ (2012) A novel method of selective removal of human DNA improves PCR sensitivity for detection of Salmonella Typhi in blood samples. BMC Inf Dis 12, 164 doi:10.1186/1471-2334-12-164

Information DNA-Free PCR Reagents

A common drawback of PCR assays targeting microbial sequences is the contamination of amplification reagents by microbial DNA. This problem becomes even more evident when the assay is directed to a broad range of microbial targets. Consequences of DNA contamination may be false-positive results and loss in analytical sensitivity.

Molzym´s Mastermix 16S /18S and other PCR reagents are guaranteed free of contaminating DNA thus generating reliable results.

Products offered include higly active DNA-free Taq polymerase ***MolTaq 16S/18S*** and ***Hot MolTaq 16S/18S*** and various ready-to-use mastermixes for the detection of femtogram amounts of bacterial and fungal DNA. Amplification reactions can be performed for 40 cycles. The DNA-free mastermixes contain all reagents for optimal amplification: dNTPs, buffer, magnesium ions (3mM final) and BSA. If you want to run your specific assays, just add primers to ***Mastermix 16S/18S Basic*** and ***Mastermix 16S/18S Dye***. Eubacterial assays are available with ***Mastermix 16S Primer*** and ***Mastermix 16S Complete*** (V3/V4 region of the 16S rRNA gene) for the detection of bacterial DNA. The panfungal assay ***Mastermix 18S Complete*** allows for the sensitive detection of fungal DNA in a sample. Further information see Molzym´s homepage (www.molzym.com).

DNA-Free PCR Product order information

Product	Contents	Cat. No.
Mastermixes, DNA-free (2.5x concentrated)		
Mastermix 16S Complete Universal 16S rDNA PCR and Real-Time PCR assay for detection of bacterial DNA.	100 reactions 250 reactions 1000 reactions	S-020-0100 S-020-0250 S-020-1000
Mastermix 16S Primer PCR assay for universal PCR detection of bacterial DNA.	100 reactions 250 reactions 1000 reactions	S-021-0100 S-021-0250 S-021-1000
Mastermix 18S Complete Universal 18S rDNA PCR and Real-Time PCR assay for detection of fungal DNA.	100 reactions 250 reactions 1000 reactions	S-070-0100 S-070-0250 S-070-1000
Mastermix 16S/18S Dye Premixed reagents and fluorescent dye for Real-Time PCR with custom primers.	100 reactions 250 reactions 1000 reactions	S-030-0100 S-030-0250 S-030-1000
Mastermix 16S/18S Basic Premixed reagents for PCR analysis with custom primers.	100 reactions 250 reactions 1000 reactions	S-040-0100 S-040-0250 S-040-1000
Taq DNA Polymerase, DNA-free		
MolTaq 16S/18S	100 units 500 units	P-019-0100 P-019-0500
Hot MolTaq 16S/18S	100 units 500 units	P-080-0100 P-080-0500
PCR-Grade Water, DNA-free		
DNA-free water, *PCR grade*	10x 1.7ml	P-020-0003

Technical Support

If you have questions please contact us.

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

Material safety data sheets are available on request.

Order Information

Product	Contents	Cat. No.
MolYsis™ Complete5	**50** microbial DNA isolations from samples	D-321-050
	100 microbial DNA isolations from samples	D-321-100

Related Products for Whole Blood and other Body Fluids

Product	Contents	Cat. No.
Pre-treatment of samples of small, medium and large sizes (used with other DNA isolation kits)		
MolYsis™ Basic5 ≤1ml and 5ml sample volumes	**50** reactions **100** reactions	D-301-050 D-301-100

See also Molzym's homepage (www.molzym.com) for automated pathogen DNA isolation products and highly active, DNA-free Taq polymerases, mastermixes and 16S and 18S rRNA gene PCR assays.

Order Hotline:

Tel.: +49(0)421 69 61 62 0 • **Fax:** +49(0)421 69 61 62 11 • **E-Mail:** order@molzym.com

Contact

Molzym GmbH & Co. KG
Mary-Astell Str. 10
28359 Bremen, Germany

Tel.: +49(0)421 69 61 62 0 • **Fax:** +49(0)421 69 61 62 11
E-Mail: info@molzym.com • **Web:** www.molzym.com