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

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 -

Molzym

Version 08

Date of first release: 01/2007
Last update: 10/2020

Contents

Kit Information

Kit Contents – *MolYsis™ Basic5*

	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 15ml	2x 15ml
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
Manual		
Manual	1x	1x

Symbols

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


Content of the package

Batch code


Irritant


Manufactured by

Catalogue number


Corrosive


Use by

Consult instructions for use


Toxicity


Temperature limitation (store at)


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).

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.

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. 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*:
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*:
H227-H301-H310+H330-H315-H318-H410; P273-P301+P310-P302+P352-P304+P340-P305+P351+P338

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.

* **H227**: Combustible liquids; **H301**: Toxic if swallowed; **H302:** Harmful if swallowed; **H310+H330**: Fatal if swallowed or in contact with skin; **H315**: Causes skin irritation; **H318**: Causes serious eye damage; **H319**: Causes serious eye irritation; **H410**: Very toxic to aquatic life with long lasting effects;

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+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; **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 DNA. ***MolYsis™ Basic5*** removes this background of host DNA and thereby increases the reliability of the molecular analysis of pathogens 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 5ml; D-321-050) and automated *SelectNA Blood Pathogen Kit* (1ml; D-340-048) and MolYsis-SelectNA™*plus* (1ml D-450-048), for the complete process of microbial DNA isolation from clinical and other samples, including sample pre-treatment, enrichment and lysis of bacterial and fungal cells, DNA extraction and DNA purification.

Whereas other 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, pathogen 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 ≤1ml from pediatric patients or animal systems.
ii) Samples of 5ml 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, and monkey, hamster ovary cell culture (≤5*10^8 cells per sample), monkey renal cell culture, mammalian cell culture.

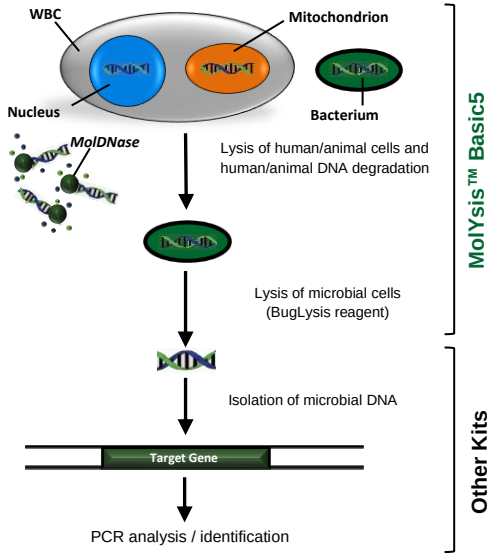

Fig. 1: The principle of testing for bacterial and fungal pathogens in fluid samples by ***MolYsis™ Basic5*** and other kits for the purification of pathogen DNA.

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*. Pathogen cells are subsequently centrifuged and treated by the *BugLysis* reagent for the degradation of cell walls. At the end, pathogen 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 for analysis of pathogens.

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: 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.
- 1x other Mini kit for nucleic acid extraction and purification

- 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* 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:
80min *(including 15min hands on time)*

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

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

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 9). Then add buffer *SU* until reaching the 1ml 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 1ml sample into a sterile 2.0ml tube (not supplied; specification, page 9) or use filled-up sample (part A). 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 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 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 are degraded.

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

4. **Add 1ml 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 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 11).

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 enzymatic treatments, in particular *Proteinase K* digestion with other kits (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.

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 200µl sample volume add 100µl *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 (5ml Fluid)

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

Procedure

1. **Pipette 5ml sample into a sterile 50ml tube (not supplied, specification, page 9) 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 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 2.0ml tube (not supplied; specification, page 9). 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 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 pathogen 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.
	• Pathogen titre too low	Check the titre of the pathogen 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.

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 *MolYsis™ Complete*5 and DNA-Free PCR Reagents

***MolYsis™ Complete*5:** The kit is the complete solution for the host DNA removal and microbial DNA isolation. The kit includes reagents and materials for host DNA removal, bacteria and fungi enrichment and microbial DNA isolation. The Kit enables small and medium size preparations from ≤1ml and 5ml volumes.

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
MolTaq 16S/18S Taq DNA Polymerase, DNA-free	100 units 500 units	P-019-0100 P-019-0500
Hot MolTaq 16S/18S Taq DNA Polymerase, DNA-free	100 units 500 units	P-080-0100 P-080-0500
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™ Basic5	**50** sample pre-treatments: Human/animal DNA removal, pathogen cell wall degradation; ≤1ml and 5ml sample volumes	D-301-050
	100 sample pre-treatments: Human/animal DNA removal, pathogen cell wall degradation; ≤1ml and 5ml sample volumes	D-301-100

Related Products for Whole Blood and other Body Fluids

Product	Contents	Cat. No.
Complete manual system of sample pre-treatment, extraction and DNA purification		
MolYsis™ Complete5 ≤1ml and 5ml sample volumes	**50** reactions **100** reactions	D-321-050 D-321-100

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