

## Microbial DNA Enrichment

### *MolYsis™ Basic5*

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

- **Small Size Sample Volumes ( $\leq 1$  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 –**



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## Kit Information

### Kit Contents – *MolYsis™ Basic5*

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

## Symbols

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



Content of the package

**LOT**

Batch code



Corrosive



Manufactured by

**REF**

Catalogue number



Toxicity



Use by

**Cont.**

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  *$\beta$ -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

### *$\beta$ -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** ( $\leq 1$  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.

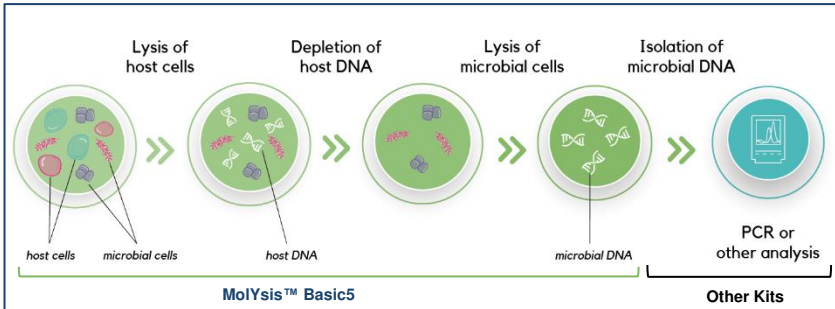
**MolYsis™ Basic5** allows for the pre-treatment of human and animal fluid samples:

- i) Samples of  $\leq 1$  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 ( $\leq 5 \times 10^8$  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.molzvm.com](http://www.molzvm.com).

<b>Gram-negative bacteria</b>	<i>Helicobacter pylori</i>	<i>Alloicoccus otitis</i>	<i>Nocardia</i> spp.
<i>Achromobacter xylooxidans</i>	<i>Kingella</i> spp.	<i>Anaerococcus</i> spp.	<i>Paenibacillus</i> spp.
<i>Acidovorax</i> spp.	<i>Klebsiella</i> spp.	<i>Atopobium</i> spp.	<i>Parvimonas micra</i>
<i>Acinetobacter</i> spp.	<i>Kerstersia</i> spp.	<i>Bacillus</i> spp.	<i>Peptoniphilus</i> spp.
<i>Aeromonas veronii</i>	<i>Kluyvera cryocrescens</i>	<i>Bifidobacterium</i> spp.	<i>Peptostreptococcus</i> spp.
<i>Afpia broomeae</i>	<i>Lautropia mirabilis</i>	<i>Brevibacterium</i> spp.	<i>Propionibacterium</i> spp.
<i>Aggregatibacter aphrophilus</i>	<i>Legionella pneumophila</i>	<i>Carnobacterium</i> spp.	<i>Rhodococcus</i> spp.
<i>Anaerotruncus coilhominis</i>	<i>Leptotrichia</i> spp.	<i>Clostridium</i> spp.	<i>Rothia</i> spp.
<i>Bacteroides</i> spp.	<i>Massilia</i> spp.	<i>Coprococcus catus</i>	<i>Staphylococcus</i> spp.
<i>Bartonella quintana</i>	<i>Methylobacterium</i> spp.	<i>Corynebacterium</i> spp.	<i>Streptococcus</i> spp.
<i>Bordetella</i> spp.	<i>Moraxella</i> spp.	<i>Dermabacter hominis</i>	<i>Tropheryma whippleii</i>
<i>Borrelia garinii</i>	<i>Morganella morganii</i>	<i>Dietzia</i> spp.	<i>Tsakumurella</i> spp.
<i>Bosea</i> spp.	<i>Neisseria</i> spp.	<i>Dolosigranulum pigrum</i>	<i>Ureaplasma urealyticum</i>
<i>Brucella</i> spp.	<i>Pantoea agglomerans</i>	<i>Eggerthella lenta</i>	<i>Vagococcus</i> spp.
<i>Burkholderia</i> spp.	<i>Paracoccus</i> spp.	<i>Enterococcus</i> spp.	<i>Wolbachia</i> spp.
<i>Campylobacter</i> spp.	<i>Pasteurella</i> spp.	<i>Eremococcus coleocola</i>	
<i>Candidatus Neoehrlichia mikurensis</i>	<i>Porphyromonas</i> spp.	<i>Eubacterium</i> spp.	<b>Fungi</b>
<i>Capnocytophaga</i> spp.	<i>Prevotella</i> spp.	<i>Facklamia</i> spp.	<i>Aspergillus</i> spp.
<i>Chryseobacterium indologenes</i>	<i>Proteus</i> spp.	<i>Finegoldia magna</i>	<i>Candida</i> spp.
<i>Citrobacter freundii</i>	<i>Providencia stuartii</i>	<i>Gardnerella vaginalis</i>	<i>Cladosporium cladosporioides</i>
<i>Cloacibacterium normanense</i>	<i>Pseudomonas</i> spp.	<i>Gemella</i> spp.	<i>Cryptococcus</i> spp.
<i>Comamonas testasteroni</i>	<i>Ralstonia</i> spp.	<i>Gordonia</i> spp.	<i>Dictyella exitiale</i>
<i>Coxiella burnetii</i>	<i>Raoultella planticola</i>	<i>Granulicatella adiacens</i>	<i>Davidiella tassiana</i>
<i>Cronobacter sakazakii</i>	<i>Rickettsia typhi</i>	<i>Janibacter</i> spp.	<i>Fusarium</i> spp.
<i>Curvibacter</i> spp.	<i>Serratia marcescens</i>	<i>Kocuria</i> spp.	<i>Issatchenkia orientalis</i>
<i>Delftia</i> spp.	<i>Shigella</i> spp.	<i>Lactobacillus</i> spp.	<i>Malassezia</i> spp.
<i>Dialister</i> spp.	<i>Stenotrophomonas maltophilia</i>	<i>Lactococcus</i> spp.	<i>Pseudallescheria boydii</i>
<i>Elizabethkingia meningoseptica</i>	<i>Veillonella</i> spp.	<i>Leifsonia</i> spp.	<i>Saccharomyces cerevisiae</i>
<i>Enhydrobacter aerosaccus</i>	<i>Weeksella</i> spp.	<i>Listeria monocytogenes</i>	<i>Schizophyllum radiatum</i>
<i>Enterobacter</i> spp.	<i>Yersinia</i> spp.	<i>Microbacterium</i> spp.	<i>Sporobolomyces</i> spp.
<i>Escherichia</i> spp.		<i>Micrococcus</i> spp.	
<i>Fusobacterium</i> spp.	<b>Gram-positive bacteria</b>	<i>Mogibacterium timidum</i>	<b>Protist</b>
<i>Haemophilus</i> spp.	<i>Abiotrophia</i> spp.	<i>Mycobacterium</i> spp.	<i>Plasmodium</i> spp.
<i>Hafnia alvei</i>	<i>Actinomyces</i> spp.	<i>Mycoplasma</i> spp.	
	<i>Aerococcus</i> 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 ( $\geq 12,000 \times g$ )
- 1x high speed centrifuge and fixed angle rotor for 50 ml tubes (9,500xg; 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  $\mu\text{l}$ , up to 200  $\mu\text{l}$  and up to 1000  $\mu\text{l}$
- 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  $\beta$ -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 ( $\leq 1$ 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  $\mu$ l 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  $\mu$ l buffer *DB1* and 10  $\mu$ l *MoIDNase 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  $\geq 12,000\times g$  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 ( $\geq 12,000\times g$ ) for 5 min. Carefully remove the supernatant by pipetting and discard.**

This washing removes residual *MoIDNase 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 *β*-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: *β*-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 9,500xg 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 ( $\geq 12,000xg$ ) 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
<b>Strong human/animal DNA background in gel electrophoresis or Real-Time PCR</b>	<ul style="list-style-type: none"> <li>• Buffer <i>CM</i> not added</li> <li>• Buffer <i>DB1</i> not added</li> <li>• <i>MolDNase B</i> not added</li> <li>• Solutions not mixed properly</li> </ul>	<p>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 <i>CM</i> has been added to lyse human/animal cells. Accordingly, addition of buffer <i>DB1</i> and <i>MolDNase B</i> is obligate. Keep the <i>MolDNase B</i> 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.</p>
<b>No microbial DNA detectable (spiking test with negative blood)</b>	<ul style="list-style-type: none"> <li>• Insufficient lysis</li> <li>• Insufficient homogenisation</li> <li>• Microbial titre too low</li> <li>• Loss of nucleic acids during purification</li> <li>• Wrong elution conditions</li> </ul>	<p>Make sure that <i>BugLysis</i> and <math>\beta</math>-mercaptoethanol treatments have been performed. Be aware that DNA is visible in a gelelectrophoresis only at amounts approx. &gt;10ng (approx.&gt;2x 10<sup>7</sup> <i>E. coli</i> cells). Use PCR based procedures for detection and quantitation of bacteria &lt;10<sup>7</sup> cells.</p> <p>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.</p> <p>Check the titre of the bacteria and fungi by plating and increase the titre for inoculation.</p> <p>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.</p> <p>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.</p>

	<ul style="list-style-type: none"> <li>• Loss of nucleic acids during the storage of the eluate</li> </ul>	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).
<b>False positive PCR result</b>	<ul style="list-style-type: none"> <li>• Cross contamination</li> <li>• Contamination during handling</li> </ul>	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

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

## 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.
- Bueno J, Virto L, Toledano-Osorio M, Figuero E, Toledano M, Medina-Castillo AL, Osorio R, Sanz M, Herrera D.** (2022) Antibacterial Effect of Functionalized Polymeric Nanoparticles on Titanium Surfaces Using an In Vitro Subgingival Biofilm Model. *Polymers*; 14(3):358
- Buhmann MT, Abt D, Nolte O, Neu TR, Stempel S, Albrich WC, Betschart P, Zumstein V, Neels A, Maniura-Weber K, Ren Q** (2019) Encrustations on ureteral stents from patients without urinary tract infection reveal distinct urotypes and a low bacterial load. *Microbiome*, 7(1), 60
- 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.
- Dada N, Sheth M, Liebman K, Pinto J, Lenhart A** (2018) Whole metagenome sequencing reveals links between mosquito microbiota and insecticide resistance in malaria vectors. *Scientific Reports*, 8(1)
- Dekker JP** (2018) Metagenomics for clinical infectious disease diagnostics steps closer to reality. *J Clin Microbiol* 56(9): e00850-18
- Disqué C** (2007) Einfluss der DNA-Extraktion auf die PCR-Detektion von Sepsiserregern. *BIOspektrum* 06, 627-629.
- Downey LC, Smith BP, Benjamin DK, Cohen-Wolkowicz** (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-Rodríguez N, del-Prado G, Ortiz-Pérez A, Molina-Manso D, Cordero-Ampuero J, Sandoval E, Fernández-Roblas R, Gómez-Barrena E** (2012) PCR-hybridization after sonication improves diagnosis of implant-related infection. *Acta Orthopaedica* 3, 299-304.
- Freeman CN, Herman EK, Abi Younes J, Ramsay DE, Erikson N, Stothard P, Links MG, Otto SJG, Waldner C.** (2022) Evaluating the potential of third generation metagenomic sequencing for the detection of BRD pathogens and genetic determinants of antimicrobial resistance in chronically ill feedlot cattle. *BMC Veterinary Research*, 18(1), 211
- 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.
- Gündoğdu A, Ulu-Kilic A, Kilic H, Nalbantoglu OU** (2019) Rapid detection of difficult-to-culture bacterial pathogens using real-time nanopore sequencing. *Infectious Diseases and Clinical Microbiology*, 1(3), 128–133
- 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.
- Ivy MI, Thoendel MJ, Jeraldo PR, Greenwood-Quaintance KE, Hanssen AD, Abdel MP, Chia N, Yao JZ, Tande AJ, Mandrekar JN, Patel R** (2018) Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing. *Journal of Clinical Microbiology*, 56(9), 10.1128/jcm.00402-18
- 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.
- Krohn S, Zeller K, Böhm S, Chatzinotas A, Harms H, Hartmann J, Heidtmann A, Herber A, Kaiser T, Treuheit M, Hoffmeister A, Berg T, Engelmann C** (2018) Molecular quantification and differentiation of *Candida* species in biological specimens of patients with liver cirrhosis. *PLOS One* **13**(6), e0197319
- 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, 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.
- 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.
- 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, Kelliher 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.
- Miller HB, Fisher SL, Simner PJ** (2019) Comparison of Host Depletion Methods for Metagenomic Next-Generation Sequencing in Cerebral Spinal Fluid, Poster CPHM-935, ASM Microbe, [www.abstractsonline.com/pp8/#!/7859/presentation/15428](http://www.abstractsonline.com/pp8/#!/7859/presentation/15428)
- Peterson SW, Demczuk W, Martin I, Adam H, Bharat A, Mulvey MR** (2023) Identification of bacterial and fungal pathogens directly from clinical blood cultures using whole genome sequencing. *Genomics*, **115**(2), 110580
- Rajar P, Dhariwal A, Salvadori G, Junges R, Åmdal HA, Berild D, Fugelseth D, Saugstad OD, Lausten-Thomsen U, Greisen G, Haaland K and Petersen FC** (2022) Microbial DNA extraction of high-host content and low biomass samples: Optimized protocol for nasopharynx metagenomic studies. *Front. Microbiol.* **13**:1038120
- Richardson LJ, Kaestli M, Mayo 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.
- 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.
- Schmidt K, Mwaigwisya S, Crossman LC, Doumith M, Munroe D, Pires C, Khan AM, Woodford N, Saunders NJ, Wain J, O’Grady J, Livermore DM** (2017) Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing. *J Antimicrob Chemother* **2017**; **72**: 104–114
- 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 MJ, Jeraldo PR, Greenwood-Quaintance KE, Yao JZ, Chia N, Hanssen AD, Abdel MP, Patel R** (2018) Identification of prosthetic joint infection pathogens using a shotgun metagenomics approach. *Clin Inf Dis* **15**;67(9):1333-1338
- 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.

- Vijayvargiya P, Jeraldo PR, Thoendel MJ, Greenwood-Quaintance KE, Garrigos ZE, Rizwan Sohail M, Chia N, Pritt BS, Patel R** (2019) Application of metagenomic shotgun sequencing to detect vector-borne pathogens in clinical blood samples. *PLoS ONE*, 14(10)
- Vitrenko Y, Kostenko I, Kulebyakina K, Duda A, Klunnyk M, Sorochnytska 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)
- Vollzugshilfe zur Entsorgung** von Abfällen aus Einrichtungen des Gesundheitsdienstes vom **01.01.2021**, Robert-Koch-Institut
- 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, Højby 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, Schönheyder 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, Røilides 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
- Zumstein V, Betschart P, Buhmann MT, Albrich WC, Nolte O, Güsewell S, Engeler DS, Schmid HP, Ren Q, Abt D.** (2019) Detection of microbial colonization of the urinary tract of patients prior to secondary ureterorenoscopy is highly variable between different types of assessment: results of a prospective observational study. *Biofouling*.35(10):1083-1092

## 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 bacterial and fungal targets. Consequences of DNA contamination may be false-positive results and loss in analytical sensitivity.

**Molzym's Mastermix 16S/18S products are guaranteed free of contaminating DNA thus generating reliable results.**

### DNA-Free PCR Product order information

Product	Contents	Cat. No.
<b>Mastermixes, DNA-free (2.5x concentrated)</b>		
<b><i>Mastermix 16S Complete</i></b>	100 reactions	S-020-0100
Universal 16S rDNA PCR and Real-Time PCR assay for detection of bacterial DNA.	250 reactions	S-020-0250
	1000 reactions	S-020-1000
<b><i>Mastermix 18S Complete</i></b>	100 reactions	S-070-0100
Universal 18S rDNA PCR and Real-Time PCR assay for detection of fungal DNA.	250 reactions	S-070-0250
	1000 reactions	S-070-1000
<b><i>Mastermix 16S/18S Dye</i></b>	100 reactions	S-030-0100
Premixed reagents and fluorescent dye for Real-Time PCR with custom primers.	250 reactions	S-030-0250
	1000 reactions	S-030-1000
<b><i>Mastermix 16S/18S Basic</i></b>	100 reactions	S-040-0100
Premixed reagents for PCR analysis with custom primers.	250 reactions	S-040-0250
	1000 reactions	S-040-1000
<b><i>MolTaq 16S/18S</i></b>	100 units	P-019-0100
Taq DNA Polymerase, DNA-free	500 units	P-019-0500
<b><i>Hot MolTaq 16S/18S</i></b>	100 units	P-080-0100
Taq DNA Polymerase, DNA-free	500 units	P-080-0500
<b><i>DNA-free water, PCR grade</i></b>	10x 1.7 ml	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.
<b><i>MolYsis™ Basic5</i></b>	50 reactions	D-301-050
Flexible solution for host DNA depletion from body fluids – to be used with other DNA isolation kits. ≤1ml and 5ml fluid samples	100 reactions	D-301-100

## Related Products

Product	Contents	Cat. No.
<b><i>MolYsis™ Complete5</i></b>	50 reactions	D-321-050
Kit includes reagents for host DNA depletion, microbial DNA extraction and purification. ≤1 ml and 5 ml fluid samples	100 reactions	D-321-100
<b><i>Ultra-Deep Microbiome Prep</i></b>	25 reactions	G-020-025
Kit includes reagents for tissue pre-treatment, host DNA depletion, microbial DNA extraction and purification. ≤1 ml fluid samples ≤0.5 cm <sup>3</sup> tissue samples	50 reactions	G-020-050
<b><i>Ultra-Deep Microbiome Prep10</i></b>	25 reactions	G-030-025
Kit includes reagents for tissue pre-treatment, host DNA depletion, microbial DNA extraction and purification. 1-10 ml fluid samples ≤0.5 cm <sup>3</sup> tissue samples	50 reactions	G-030-050
<b>Automated solution to be used with the SelectNA™ plus benchtop instrument:</b>		
<b><i>MolYsis-SelectNA™ plus</i></b>	48 reactions	D-450-048
Kit includes reagents for tissue pre-treatment, host DNA depletion, microbial DNA extraction and purification. ≤1 ml fluid samples & swabs ≤0.5 cm <sup>3</sup> tissue samples		

See also Molzym's homepage ([www.molzym.com](http://www.molzym.com)) for more information.

### Order Hotline:

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