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

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 –

– Not for use in diagnostic procedures –

molzym
reveal DNA

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Contents

Kit Information **3**
Kit Contents – *MolYsis™ Complete5* 3
Symbols 4
Storage and Stability 4
Product Use Limitations 4
Safety Information 5
Hazard and Precautionary Statements 5
Introduction **8**
Kit Description 8
The *MolYsis™ Complete5* Technology 9
List of Strains detected 10
Recommendations for PCR Analysis of Bacteria and Fungi 11
Protocols **12**
How to Start 12
Protocol 1: Small Size Sample DNA Isolation (≤ 1 ml Fluid) 13
Procedure 13
Protocol 2: Medium Size Sample DNA Isolation (5 ml Fluid) 15
Procedure 15
Supplementary Information **16**
Troubleshooting 16
Tradenames 17
References 18
Information DNA-Free PCR Reagents 22
Technical Support 22
Order Information 23
Contact 24

Kit Information

Kit Contents – *MolYsis™ Complete5*

	50 reactions (D-321-050)	100 reactions (D-321-100)
Extraction Buffers (store at +18 to +25°C)		
SU	1x 50 ml	2x 50 ml
CM	1x 100 ml	2x 100 ml
DB1	1x 100 ml	2x 100 ml
RS	1x 50 ml	2x 50 ml
RL	1x 5 ml	2x 5 ml
RP	1x 7.5 ml	2x 7.5 ml
CS	1x 12.5 ml	2x 12.5 ml
AB	1x 12.5 ml	2x 12.5 ml
WB	1x 20 ml	2x 20 ml
70% Ethanol	1x 20 ml	2x 20 ml
Deionized water	1x 5 ml	2x 5 ml
Enzymes & Reagents (store at -15 to -25°C)		
MolDNase B, solution	1x 0.5 ml	2x 0.5 ml
BugLysis, solution	1x 1.0 ml	2x 1.0 ml
β-mercaptoethanol, solution	1x 0.08 ml	2x 0.08 ml
Proteinase K, solution	1x 1.0 ml	2x 1.0 ml
Consumables (store at +18 to +25°C)		
Spin columns in 2.0 ml *Collection tubes*	1x 50	2x 50
Collection tubes, 2.0 ml	2x 50	4x 50
Elution tubes, 1.5 ml	1x 50	2x 50
Manual		
Manual	1x	1x

Symbols

Symbols used in labelling and in section 'Hazard and Precautionary Statements' (pages 5 to 7).


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).

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.

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., 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*(page 7):
H302-H315-H319; P301+P312-P302+P352-P305+P351+P338

ß-mercaptoethanol

Contains 2-mercaptoethanol (100 %, CAS no. 60-24-2):
Acute toxicity (oral, inhalation, dermal), 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*(page 7):
H301+H331-H310-H315-H317-H318-H361d-H373-H410;
P273-P280-P301+P310-P302+P352+P310-P304+P340+P310-P305+P351+P338

Proteinase K

Contains *Proteinase K* (≥ 1 %):
Respiratory sensitization and skin sensitization

Danger

Hazard and precautionary statements*(page 7):
H317-H334; P280-P302+P352-P333+P313-P363

Buffer RP

Contains sodium dodecyl sulfate (< 10 %):
Acute toxicity (oral, inhalation), irritation (skin and eye)

Warning

Hazard and precautionary statements*(page 7):
H302-H315-H319-H332; P280-P301+312-P304+P340+P312-P305+P351+P338

Buffer *CS*

Contains guanidinium thiocyanate (> 10 %):
Acute toxicity (oral), skin sensitization, eye damage and hazardous to aquatic environment (chronic).

Danger

Hazard and precautionary statements*(page 7):
H302-H312-H314-H318-H412-EUH032; P280-P303+P361+P353-P305+P351+P338-P310-P362+P364

Buffer *AB*

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

Danger

Hazard and precautionary statements*(page 7):
H225-H319-H336; P210-P233-P305+P351+P338

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

Danger

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

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

Danger

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

Emergency Information (24-hours service)
For emergency medical information, please contact the regional poison center in your country.

* **H225:** Highly flammable liquid and vapour; **H302:** Harmful if swallowed; **H310:** Fatal in contact with skin; **H312:** Harmful in contact with skin; **H314:** Causes severe skin burns and eye damage; **H315:** Causes skin irritation; **H317:** May cause an allergic skin reaction; **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; **H336:** May cause drowsiness or dizziness; **H361d:** Suspected of damaging fertility. 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; **H412**: Harmful to aquatic life with long lasting effects; **EUH032**: Contact with acids liberates very toxic gas.

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.; **P233:** Keep container tightly closed; **P273:** Avoid release to the environment; **P280:** Wear protective gloves/protective clothing/eye protection/face protection; **P310:** Immediately call a POISON CENTER/doctor; **P363:** Wash contaminated clothing before reuse; **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; **P303+P361+P353:** IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]; **P304+P340+P310:** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor; **P304+P340+P312:** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell; **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; **P362+P364:** Take off contaminated clothing and wash it before reuse.

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. Besides PCR inhibitors, unspecific binding of bacterial and fungal sequence-specific primers to host sequences can negatively interfere with microbial analysis. With ***MolYsis™ Complete5*** Molzym has developed a tool for the depletion of human and animal DNA 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 DNA through PCR or Real-Time PCR.

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

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

ii) Samples of 5 ml 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, canine 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 depletion.

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 lysed 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.

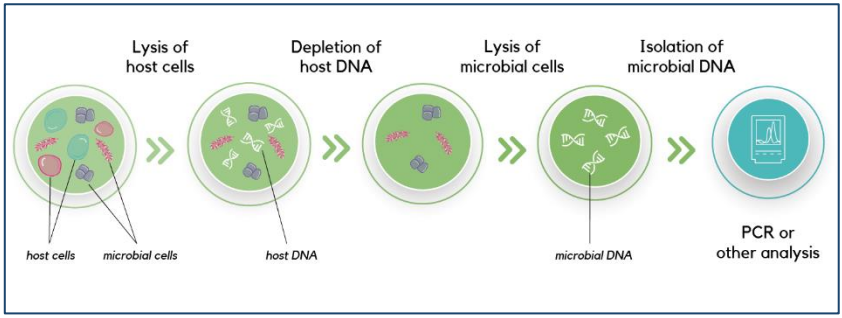

Fig. 1: The principle of testing for bacterial and fungal DNA in samples by ***MolYsis™ Complete5***.

List of Strains detected

The ***MolYsis™ Complete5*** technology has been evaluated with a variety of clinical samples (page 8). *BugLysis* reagent is a component of all kits and designed to lyse Gram-positive and Gram-negative bacteria, and fungi with high efficiency. Strains from the following genera have been identified in clinical material so far (universal 16S PCR for bacteria, universal 18S PCR for fungi, plus sequencing), showing the broad range of lysing capability of *BugLysis*:

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

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

Recommendations for PCR Analysis of Bacteria and Fungi

Avoidance of DNA contamination:
PCR analysis demands special care with respect to the avoidance of contamination from exogenous sources. Take care to separate places of DNA preparation from places where PCR reagents are handled, in particular preparation of mastermixes, pipetting into PCR tubes and performance of PCR runs.

Wear sterile protective gloves at any handling step, also during DNA preparation. Frequently change sterile protective gloves during handling. Use only sterilized or, optimally, guaranteed DNA-free disposables.

If analysis of microorganisms is desired, e.g., bacteria identification by sequencing of broad-range 16S amplification products, it is important to make sure that only polymerases (e.g., Taq polymerase) free of DNA contamination are used.

For this purpose, Molzym offers guaranteed DNA-free MolTaq 16S/18S (P-019-0100) and Hot MolTaq 16S/18S (P-080-0100). Also, Molzym offers a DNA-free mastermix (Mastermix 16S Complete; S-020-0100) containing primers for universal 16S rDNA amplification of bacterial sequences. For the analysis of fungal DNA sequences, Molzym offers the DNA-free mastermix (Mastermix 18S Complete; S-070-0100) containing universal 18S primers. Generally, for each analysis, run positive and negative controls to check for proper performance of the reaction and sterility of reagents and buffers used.

Protocols

How to Start

Caution:

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

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

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

! For optimal results, use only fresh samples. ***Do not freeze samples*** to avoid loss of 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 50 ml tubes (9,500x*g*; only for protocol 2)
- 1x thermomixer (2.0 ml tubes)
- 1x vortexer
- 1x cooling rack for 1.5 ml tubes (-15 to -25°C)
- Sample racks
- Precision pipettes and sterile filter pipette tips allowing pipetting volumes of up to 20 µl, up to 200 µl and up to 1000 µl
- 2.0 ml micro tubes, Biosphere®, Sarstedt, Germany (72.695.200) for bacterial and fungal cell lysis and DNA extraction
- 1.5 ml micro tubes, Biosphere®, Sarstedt, Germany (72.706.200) for Deionized water, DNA-free

- 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*, *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 1.5 ml 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: ***120 min***

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

Please read section 'How to Start' before starting the procedure (page 12)!

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 12). Then add buffer *SU* until reaching the 1 ml 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 1 ml sample into a sterile 2.0 ml tube (not supplied; specification, page 12) or use filled-up sample (protocol1 part A, above). Then add 250 µl buffer *CM* and vortex at full speed for 15 s to mix. Let stand on the bench at room temperature (+18 to +25°C) for 5 min.**

   Buffer *CM* is a chaotropic buffer that lyses the human/animal cells. For optimal results it is important to mix thoroughly.

   Caution: buffer *CM* is an irritant. Avoid contact with skin and eyes.

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

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

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

4. **Add 1 ml 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 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 14).

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 *Proteinase K* digestion (step 8, below).

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

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

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

8. **Adjust the temperature of the thermomixer to 56°C. Add 150 µl buffer RP and 20 µl *Proteinase K* (do not premix) to the tube. Vortex at full speed for 15 s and incubate at 56°C and 1,000 rpm for 10 min. 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 250 µl buffer *CS* and vortex at full speed for 15 s.**

   Cells are lysed and protein is denatured.

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

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

    At this point DNA binds to the matrix.

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

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

    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 1.5 ml *Elution tube*.**

15. **Place 100 µl *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 1 min 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 (5 ml Fluid)

Please read section 'How to Start' before starting the procedure (page 12)!

Procedure

1. **Pipette 5 ml sample into a sterile 50 ml tube (not supplied, specification page 12) and add 2 ml buffer *CM*. Vortex at full speed for 15 s. Let stand on the bench at room temperature (+18 to +25°C) for 5 min.**

   Buffer *CM* is a chaotropic buffer that lyses the human/animal cells. For optimal results it is important to mix thoroughly.

   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

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

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

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

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

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

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

   Note: At this point the procedure can be interrupted by freezing the sample (-15 to -25°C). For further processing, thaw the sample to room temperature (+18 to +25°C) and proceed with step 6 (page 14).

Continue with step 6 of Protocol 1 'Small Size Sample DNA Isolation' (page 14).

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 microbial 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 13 14 and 15) are not totally homogenized, microbial cells may be included in the debris and not reached by lytic enzymes. See comments at page 14.
	• Microbial titre too low	Check the titre of the bacteria and fungi by plating and increase the titre for inoculation.
	• Loss of nucleic acids during purification	Ensure that buffer *AB* has been added to and mixed with the lysate (step 10, page 14). Accordingly, make sure that the column has been washed with buffer *WB* (step 12, page 14).
	• Wrong elution conditions	Make sure to elute with supplied heated *Deionized water* (70°C; step 15, page 14). 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 11). 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 14), discard flow-through and centrifuge for another 1min to avoid ethanol carryover to the eluate

Tradenames

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Mastermix 16S/18S Basic	Molyzm
Mastermix 16S/18S Dye	Molyzm
MolTaq 16S/18S	Molyzm
MolYsis™ Basic5	Molyzm
MolYsis™ Complete5	Molyzm
MolYsis-SelectNA™*plus*	Molyzm
SelectNA™*plus*	Molzym
Ultra-Deep Microbiome Prep	Molzym
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