Isolation of Microbial DNA

MolYsis™ Complete10

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

Large Size Sample Volumes (5 to 10ml)

Kit includes all ingredients for the following steps of the bacterial 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
- Bacterial DNA purification

- For research use only -

Molzym

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Version 2.0

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Contents

Kit Information

Kit Contents – *MolYsis™ Complete10*

	50 reactions	100 reactions
Extraction Buffers (store at +18 to +25°C)		
SU	2x 125ml	4x 125ml
CM	2x 100ml	4x 100ml
DB1	2x 100ml	4x 100ml
RS	1x 50ml	2x 50ml
RL	1x 5ml	2x 5ml
RP	1x 7.5ml	2x 7.5ml
CS	1x 12.5ml	2x 12.5ml
AB	1x 12.5ml	2x 12.5ml
WB	1x 20ml	2x 20ml
70% Ethanol	1x 20ml	2x 20ml
Deionized water	1x 5ml	2x 5ml
Enzymes (store at -15 to -25°C)		
MolDNase B, solution	1x 0.5ml	2x 0.5ml
BugLysis, solution	1x 1.0ml	2x 1.0ml
β-mercaptoethanol, solution	1x 0.08ml	2x 0.08ml
Proteinase K, solution	1x 1.0ml	2x 1.0ml
Consumables (store at +18 to +25°C)		
Spin columns in 2.0ml *Collection tubes*	1x 50	2x 50
Collection tubes, 2.0ml	2x 50	4x 50
Elution tubes, 1.5ml	1x 50	2x 50
Manual		
Manual	1x	1x

Symbols

Symbols used in labelling and in section 'Risk and Safety Phrases' (pages 5 to 6).


Content of the package


Manufactured by


Use by


Temperature limitation (store at)

Batch code

Catalogue number

Consult instructions for use


Harmful, Xn / Irritant, Xi


Highly flammable, F


Toxic; T


Hazardous to the environment; N


GHS05 – Corrosive


GHS06 – Toxicity


GHS09 – 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, 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 the kit is given for up to ***24 months*** at the conditions specified.

Product Use Limitations

This product is for **research use only** and not for use in diagnostic procedures.

Safety Information

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

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

Buffers *CM* and *CS* contain guanidine hydrochloride and guanidinium thiocyanate, respectively, which can form highly reactive compounds and toxic gases when combined with hypochlorite or other acidic solutions. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 70% (v/v) ethanol. This kit is to be used only by skilled personnel trained for handling infectious material and molecular biological methods. To avoid false analytic results by DNA contamination of reagents and infection of the user by infectious agents during handling, always wear sterile protective gloves, disposable sleeve covers, a lab coat and protective goggles. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instruction manual of the manufacturer. The UV lamp must be switched off during working. Follow the instructions of the manufacturer for maintenance of the workstation. Dispose potentially infectious material and the waste of the sample preparation according to the national directive of the health organisation (e.g. Richtlinie über die ordnungsgemäße Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes, 2002). Separate Material Safety Data Sheets for chemicals used are available on request. Molzym reserves the right to alter or modify the product to improve the performance of the kit.

Risk and Safety Phrases

Buffers *SU* and *RL*
Contains sodium azide (<1%): **Harmful**.
Risk and safety phrases*(page 6): **R22-32**, **S28-45-60-61**

Buffer *CM*
Contains guanidine hydrochloride (>25%): **Harmful, irritant**.
Risk and safety phrases*(page 6): **R22-36/38**, **S26**

Proteinase K
Contains *Proteinase K* (≥1%): **Harmful, irritant**.
Risk and safety phrases*(page 6): **R36/37/38-42, S24-26-36/37**

Buffer *CS*
Contains guanidinium thiocyanate (>25%): **Harmful**.
Risk and safety phrases*(page 6): **R20/21/22-32-52/53, S13-61**

Buffer *AB*
Contains 2-propanol (<80%): **Highly flammable and irritant**.
Risk and safety phrases*(page 6): **R11-36-67, S7-16-24/25-26**

Buffer *WB*
Contains 2-propanol (≤40%): **Irritant and flammable**.
Risk and safety phrases*(page 6): **R10-36-67, S7-16-24/25-26**

70% Ethanol, DNA-free
Contains ethanol (<70%): **Flammable**.
Risk and safety phrases*(page 6): **R10, S7-16**

***2-mercaptoethanol* (*ß-mercaptoethanol*):**
Poisonous, irritating, environmental hazardous

Directive 67/548/EWG and 1999/45/EG
Risk and safety phrases*(page 6): **R23/24/25-38-41-50/53, S26-36/37/39-45-61**

Regulation (EC) No. 1272/2008

Danger

Hazard and precautionary statements**(page 6): **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.

* **R10**: Flammable; **R11**: Highly flammable; **R20/21/22:** Harmful by inhalation, in contact with skin and if swallowed; **R22:** Harmful if swallowed, **R23/24/25:** Toxic by inhalation, in contact with skin and if swallowed; **R32**: Contact with acids liberates very toxic gas; **R36:** Irritating to eyes; **R36/37/38**: Irritating to eyes, respiratory system and skin; **R36/38:** Irritating to eyes and skin; **R38:** Irritating to skin; **R41:** Risk of serious damage to eyes **R42**: May cause sensitization by inhalation, **R50/53:** Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; **R52/53**: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment; **R67:** Vapours may cause drowsiness and dizziness

S7: Keep container tightly closed; **S13**: Keep away from food, drink and animal feed; **S16:** Keep away from sources of ignition - No smoking; **S24**: Avoid contact with skin; **S24/25:** Avoid contact with skin and eyes; **S26**: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; **S28**: After contact with skin, wash immediately with plenty of water, **S36/37:** Wear suitable protective clothing and gloves; **S36/37/39:** Wear suitable protective clothing, gloves and eye/face protection; **S45**: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible); **S60:** This material and its container must be disposed of as hazardous waste; **S61**: Avoid release to the environment. Refer to special instructions/safety data sheet.

** **H227**: Combustible liquids; **H301**: Toxic if swallowed; **H310+H330**: Fatal if swallowed or in contact with skin; **H315**: Causes skin irritation; **H318**: Causes serious eye damage; **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; **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 liquid samples from clinical materials and animal systems (e.g., blood and other body liquids) can be severely disturbed by a high background of host and dead cell DNA. Besides PCR inhibitors, unspecific binding of bacterial and fungal sequence-specific primers to host sequences can negatively interfere with pathogen analysis. With ***MolYsis™ Complete10*** Molzym has developed a tool for the enrichment of bacterial and fungal cells and isolation of pure microbial DNA from blood and other liquids. ***MolYsis™ Complete10*** 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 bacterial and fungal pathogens through PCR or Real-Time PCR.

MolYsis™ Complete10 allows for the microbial DNA isolation from 5 to 10ml liquid samples.

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

The *MolYsis™ Complete10* Technology

MolYsis™ Complete10 is Molzym's proprietary, patented technology enabling the enrichment and purification of microbial DNA from liquid clinical samples and animal model material for molecular analysis. The procedure includes protocols for **i)** human/animal DNA removal, **ii)** universal lysis of Gram-negative and Gram-positive bacteria, and fungi and **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 liquid sample lyses the host cells, whereas microbial cells are unaffected. The DNA released from host cells as well as dead cells is degraded by Molzym's proprietary, chaotrope-resistant *MolDNase B*.
II) Microbial cells are sedimented, treated with *BugLysis* reagents to degrade cell walls of Gram-negative bacteria, Gram-positive bacteria and fungi and then digested by *Proteinase K* treatment.
III) The microbial DNA is extracted and then isolated by a quick bind-wash-elute procedure, using Molzym's CCT technology with quantitative DNA binding to the filter matrix and high recovery of microbial DNA from the column.

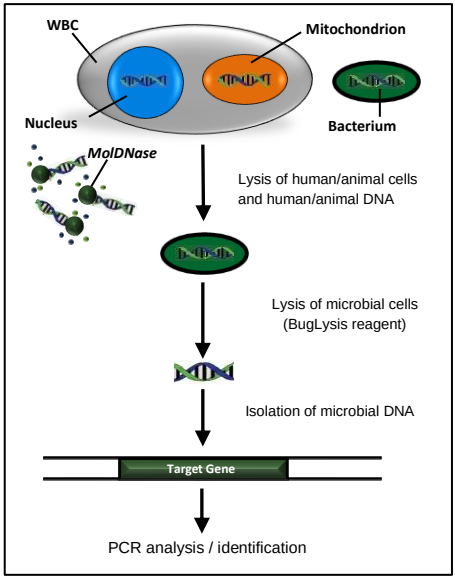

Fig. 1: The principle of testing for bacterial and fungal pathogens in samples by ***MolYsis™ Complete10***.

List of Strains detected

The ***MolYsis™ Complete10*** technology has been evaluated with a variety of clinical samples (page 7). *BugLysis* reagent is a component of all kits and designed to lyse Gram-positive and Gram-negative bacteria, and fungi with high efficiency. Strains from the following genera have been identified in clinical material so far (universal 16S PCR for bacteria, universal 18S PCR for fungi, plus sequencing), showing the broad range of lysing capability of *BugLysis*:

Gram-negative bacteria: *Acinetobacter, Aeromonas, Bacteroides, Bartonella, Bordetella, Borrelia, Bradyrhizobium, Brevibacterium, Candidatus Neoehrlichia, Citrobacter, Cloacibacterium, Coxiella, Dialister, Edwardsiella, Enterobacter, Escherichia, Fusobacterium, Haemophilus, Klebsiella, Leptotrichia, Methylobacterium, Moraxella, Morganella, Neisseria, Parabacteroides, Paracoccus, Petrobacter, Proteus, Providencia, Pseudomonas, Ralstonia, Raoultella, Serratia, Sphingomonas, Stenotrophomonas, Veillonella, Weeksella, Zoogloea*.

Gram-positive bacteria: *Actinomyces, Anaerococcus, Bacillus, Clostridium, Corynebacterium, Dolosigranulum, Enterococcus, Facklamia, Finegoldia, Gemella, Granulicatella, Lactobacillus, Lactococcus, Leifsonia, Listeria, Micrococcus, Mycobacterium, Nocardia, Parvimonas, Peptostreptococcus, Propionibacterium, Rothia, Ruminococcus, Staphylococcus, Streptococcus, Tropheryma, Vagococcus*.

Fungi: *Aspergillus, Candida, Cladosporium, Cryptococcus, Didymella, Davidiella, Malassezia, Peniophora, Saccharomyces, Shizophyllum, Sistotrema, Sporobolomyces, Udeniomyces*

Recommendations for PCR Analysis of Bacteria

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 bacteria is desired, e.g., 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* (P-019-0100). Also, Molzym offers a DNA-free mastermix (*Mastermix 16S Complete*; S-020-0100) containing primers for universal 16S rDNA amplification of bacterial sequences. Generally, for each analysis, run positive and negative controls to check for proper performance of the amplification reaction and sterility of reagents and buffers used.

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

Protocol

Large size sample DNA isolation (5 to 10ml liquid samples)

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 liquid 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.

! To be supplied by the user:

- 1x UV Class II biological safety cabinet
- 1x high speed centrifuge and fixed angle rotor for 50ml tubes (9,500x*g*)
- 1x bench top microcentrifuge (≥12,000x*g*)
- 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
- 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**
- 2.0ml micro tubes, Biosphere®, Sarstedt, Germany (72.695.200) for bacterial and fungal cell lysis and DNA extraction
- 1.5ml micro tubes, Biosphere®, Sarstedt, Germany (72.706.200) for Deionized water, DNA-free

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

! Adjust the thermomixer to 37°C. Pipette an aliquot of *Deionized water* (100µl for each sample) into a sterile 1.5ml Biosphere® tube (not supplied) and place into the thermomixer (needed for step 15).

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

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

Procedure

A) Fill up procedure for samples ≥5 and less than 10ml volume

Samples ≥5 and less than 10ml are filled up using buffer *SU*. Transfer the sample by pipetting into a sterile 50ml tube (not supplied; specification page 8). Then add buffer *SU* using a disposable 5ml pipette until reaching the 10ml mark of the tube. Discard pipette/pipette tip with excess buffer *SU*. Continue with part B (below).

B) Sample pre-treatment and DNA isolation procedure

1. **Pipette 10ml sample into a sterile 50ml tube (not supplied; specification page 8) or use filled-up sample (part A, above). Then add 4ml 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 4ml buffer *DB1* and 10µl *MolDNase B* to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

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

3. **Centrifuge 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 full speed centrifugation 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.

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

   Potential residual small particles in the suspension can be neglected, because they are dissolved during *Proteinase K* digestion (step 8, below).

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

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

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

8. **Adjust the temperature of the thermomixer to 56°C. Add 150µl buffer *RP* and 20µl *Proteinase K* (can be premixed) to the tube. Vortex at full speed for 15s and incubate at 56°C and 1,000rpm for 10min. Thereafter, adjust the temperature of the thermomixer to 70°C (make sure that the tube containing *Deionized water*, DNA-free is placed in the mixer, needed at step 15).**

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

   Cells are lysed and protein is denatured.

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

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

    At this point DNA binds to the matrix.

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

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

    This step removes salts and dries the column matrix.

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

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

    Store the eluted DNA at +4 to +12°C if analysed at the same day or freeze at -15 to -25°C until further use. Avoid frequent freeze-thaw cycles, because this may result in a decay of the eluted DNA (in particular at low DNA concentrations).

Supplementary Information

Troubleshooting

This guide may help solve 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	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, 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 10 to 11) are not totally homogenized, microbial cells may be included in the debris and not reached by lytic enzymes. See comments at pages 10 to 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	Ensure that buffer *AB* has been added to and mixed with the lysate (step 10, page 11). Accordingly, make sure that the column has been washed with buffer *WB* (step 12, page 11).
	• Wrong elution conditions	Make sure to elute with supplied heated *Deionized water* (70°C; step 15, page 11). 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.
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 11), centrifuge for another 1min to avoid ethanol carryover to the eluate

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Information DNA-Free PCR Reagents

A common drawback of PCR assays targeting bacterial sequences is the contamination of amplification reagents by bacterial DNA. This problem becomes even more evident when the assay is directed to a broad range of bacteria. Consequences of DNA contamination may be false-positive results and loss in analytical sensitivity.
Molzym´s Mastermix 16S and other PCR reagents are guaranteed free of contaminating DNA thus generating reliable results.

Products offered include DNA-free Taq polymerase (*MolTaq 16S*) and various ready-to-use mastermixes for the detection of femtogram amounts of bacterial DNA. Amplification reactions can be performed over 40 cycles. DNA-free ***MolTaq 16S*** is a highly active Taq DNA polymerase for ultra-sensitive PCR analysis of bacterial DNA in samples.

The mastermixes contain all reagents for optimal amplification: dNTPs, buffer, magnesium ions (3mM final) and bovine serum albumin. If you want to run your specific assays, just add primers to ***Mastermix 16S Basic*** and ***Mastermix 16S Dye*** and start the PCR and Real-Time PCR (SYBR Green) reaction, respectively. Complete assays are available with ***Mastermix 16S Primer*** and ***Mastermix 16S Complete*** (V3/V4 region of the 16S rRNA gene). 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 bacteria	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 bacteria	100 reactions 250 reactions 1000 reactions	S-021-0100 S-021-0250 S-021-1000
Mastermix 16S 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 Basic Premixed reagents for PCR analysis with custom primers	100 reactions 250 reactions 1000 reactions	S-040-0100 S-040-0250 S-040-1000
Taq DNA Polymerase, DNA-free		
MolTaq 16S	100 units 500 units	P-019-0100 P-019-0500
PCR-Grade Water, DNA-free		
DNA-free water, *PCR grade*	10x 1.7ml	P-020-0003

Technical Support

If you have questions please contact us.

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

Material safety data sheets are available on request

Order Information

Product	Contents	Cat. No.
MolYsis™ Complete10	**50** microbial DNA isolations from samples	D-325-050
	100 microbial DNA isolations from samples	D-325-100

Related products for Whole Blood and other Liquid Samples

Product	Contents	Cat. No.
Pre-treatment of samples of small, medium and large sizes (used with other DNA isolation kits)		
MolYsis™ Basic 0.2ml sample volumes	**50** reactions **100** reactions	D-300-050 D-300-100
MolYsis™ Basic5 ≤1ml and 5ml sample volumes	**50** reactions **100** reactions	D-301-050 D-301-100
MolYsis™ Basic10 5 to 10ml sample volumes	**50** reactions **100** reactions	D-305-050 D-305-100
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 polymerase, mastermixes and 16S rRNA gene PCR assays.

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