Microbial DNA Enrichment

MolYsis™ Basic

Sample pre-treatment kit for background-free PCR analysis of whole blood and other liquid samples

Small Size Sample Volumes (0.2ml)

Kit includes all ingredients for the following steps of selective lysis of host cells and the degradation of released DNA:

- Lysis of human/animal cells
- Degradation of human/animal DNA
- Degradation of cell walls of Gram-positive and Gram-negative bacteria and fungi

To be used with other DNA isolation kits

- For research use only -

Molzym

Version 3.0

Date of first release: 03/2006
Last update: 10/2014

Contents

Kit Information

Kit Contents – *MolYsis™ Basic*

	50 reactions	100 reactions
Extraction Buffers (store at +18 to +25°C)		
CM	1x 2.5ml	2x 2.5ml
DB1	1x 2.5ml	2x 2.5ml
RS	1x 50ml	2x 50ml
RL	1x 15ml	2x 15ml
Enzymes (store at -15 to -25°C)		
MolDNase A, solution	1x 0.5ml	2x 0.5ml
BugLysis, solution	1x 1.0ml	2x 1.0ml
β-mercaptoethanol, solution	1x 0.08ml	2x 0.08ml
Manual		
Manual	1x	1x

Symbols

Symbols used in labelling and in section 'Risk and Safety Phrases' (pages 4 to 5).


Content of the package

REF Catalogue number


GHS05 – Corrosive


Manufactured by

Cont. Consult instructions for use


GHS06 – Toxicity


Use by


Harmful, Xn / Irritant, Xi


GHS09 – Environmentally Hazardous


Temperature limitation (store at)


Toxic; T

LOT Batch code


Hazardous to the environment; N

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 A, BugLysis* and *β-mercaptoethanol* are handled and stored at -15 to -25°C. Store buffers 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.

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

Risk and Safety Phrases

Buffer *CM*
Contains guanidine hydrochloride (>25%): **Harmful, irritant**.
Risk and safety phrases*(page 5): **R22-36/38**, **S26**

Buffer *RL*
Contains sodium azide (<1%): **Harmful**.
Risk and safety phrases*(page 5): **R22-32**, **S28-45-60-61**

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

Directive 67/548/EWG and 1999/45/EG
Risk and safety phrases*(page 5): **R23/24/25-38-41-50/53, S26-36/37/39-45-61**

Regulation (EC) No. 1272/2008

Danger

Hazard and precautionary statements**(page 5): **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.

* **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; **R36/38:** Irritating to eyes and skin; **R38:** Irritating to skin; **R41:** Risk of serious damage to eyes **R50/53:** Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment;

S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Refer to special instructions/safety data sheet, **S28**: After contact with skin, wash immediately with plenty of water, **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 DNA. ***MolYsis™ Basic*** removes this background of host DNA and thereby increases the reliability of the molecular analysis of pathogens in clinical and other samples. The kit contains all ingredients for the selective lysis of host cells and the degradation of released nucleic acids (DNA and RNA) in samples.

Patented ***MolYsis™ Basic*** is a sample pre-treatment tool for the removal of host as well as dead cell DNA. The kit can be used as a module in conjunction with any other nucleic acid extraction kit designed for handling in the mini bind-wash-elute format (e.g., mini spin columns, automated systems). Molzym also supplies kits, manual *MolYsis™ Complete5* (≤1 and 5ml; D-321-050) and *MolYsis™ Complete10* (5 to 10ml; D-325-050) and automated *SelectNA Blood Pathogen Kit* (1ml; D-340-048), for the complete process of microbial DNA isolation from clinical and other samples, including sample pre-treatment, enrichment and lysis of bacterial and fungal cells, DNA extraction and DNA purification. Whereas other systems result in a mixture of host and microbial DNA, sample pre-treatment tool, ***MolYsis™ Basic***, enables the preparation of microbial DNA from samples. Only two steps are needed to obtain a sample that is depleted of host and dead 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 A*. Thereafter, pathogen cells are sedimented, treated with *BugLysis* reagent to degrade cell walls of Gram-negative and Gram-positive bacteria and fungi and then further processed by protocols for the extraction and purification of nucleic acids.

MolYsis™ Basic allows for the pre-treatment of 0.2ml samples from pediatric 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 mouse, rat, and monkey, hamster ovary cell culture ($\leq 5*10^8$ cells per sample), monkey renal cell culture.

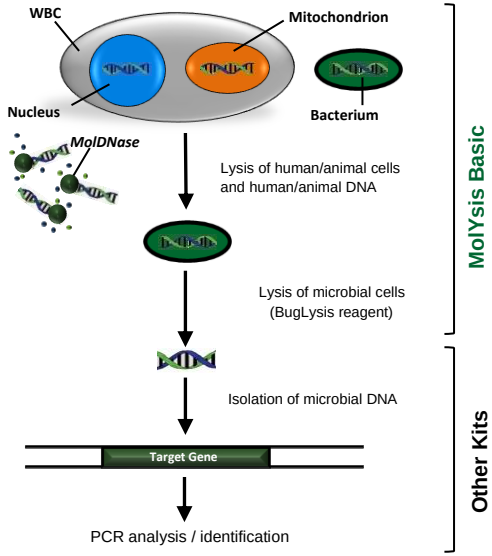

Fig. 1: The principle of testing for bacterial and fungal pathogens in liquid samples by ***MolYsis™ Basic*** and other Kits for the purification of pathogen DNA.

The *MolYsis™ Basic* Technology

MolYsis™ Basic is Molzym's proprietary, patented technology enabling the pre-treatment of whole blood and other liquid 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* A. Pathogen cells are subsequently centrifuged and treated by the *BugLysis* reagent for the degradation of cell walls. At the end, pathogen DNA is purified by other DNA isolation protocols including a proteinase digestion step. The preparation can then be used in broad-range and other PCR assays for analysis of pathogens.

List of Strains detected

The ***MolYsis™ Basic*** technology has been evaluated with a variety of clinical samples (below). *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 product, 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 reaction and sterility of reagents and buffers used.

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

Protocol

Small Size Sample Pre-treatment for DNA Isolation (0.2ml Liquid)

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. 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 thermomixer (2.0ml tubes)
- 1x vortexer
- 1x cooling rack for 1.5ml tubes (-15 to -25°C)
- Sample racks
- Precision pipettes and sterile filter pipette tips allowing pipetting volumes of up to 20µl, up to 200µl and up to 1000µl
- 2.0ml micro tubes, Biosphere®, Sarstedt, Germany (72.695.200) for bacterial and fungal cell lysis and DNA extraction
- 1x other Mini kit for nucleic acid extraction and purification

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

! Adjust a thermomixer to 37°C

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

Approximate time for 4 parallel pre-treatments of samples:
80min *(including 15min hands on time)*

Procedure

1. **Pipette 200µl sample into a sterile 2.0ml tube (not supplied; specification, page 9) and add 50µl buffer *CM*. Vortex at full speed for 15s to mix and 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 50µl buffer *DB1* and 10µl *MolDNase A* to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

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

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

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

   Depending on the sample, the sediment may be rigid and resuspension may take some time. In this case stir the sediment with the pipette tip and pipette in and out until resuspended.

5. **Centrifuge tube in a bench top microcentrifuge (≥12,000x*g*) for 5min. Carefully remove the supernatant by pipetting and discard.**

   This washing removes residual *MolDNase A* 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.**

   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 (page 7).

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

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

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

Further processing for nucleic acid extraction and purification (other Mini kits):

After step 7, the microbial lysat 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 lysat (step 7, above) up to the sample volume of this kit with buffer *RL* (e.g., for 200µl sample volume add 100µl *RL* to the microbial lysat). 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.

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 A* is obligate. Keep the *MolDNase A* vial chilled, because warming may reduce enzyme activity and hence increase human/animal DNA background. It is important that solutions are thoroughly mixed after addition of buffers. Follow instructions for vortexing.
No pathogen DNA detectable (spiking test with negative blood)	• Insufficient lysis	Make sure that *BugLysis* and *β-mercaptoethanol* treatments have been performed. Be aware that DNA is visble in a gelelectrophoresis only at amounts approx. >10ng (approx.>2x 10^7 *E. coli* cells). Use PCR based procedures for detection and quantitation of bacteria <10^7 cells.
	• Insufficient homogenisation	If the pellets from steps 4 and 6 (page 10) are not totally homogenized, microbial cells may be included in the debris and not reached by lytic enzymes. See comments at page 10.
	• Pathogen titre too low	Check the titre of the pathogen by plating and increase the titre for inoculation.
	• Loss of nucleic acids during purification	See troubleshooting guides of procedures in laboratory manuals or these kits. Alternatively, use Molzym´s complete DNA isolation kits which have been extensively evaluated for isolation of pico to femtogram amounts of pathogen DNA.

	• Wrong elution conditions	For elution of the DNA from the column matrix, good experience was made using DNA-free water, PCR grade (Molzym order no. P-020-0003) heated to 70°C. This increases the DNA yield significantly.
	• Loss of nucleic acids during the storage of the eluate	Store the eluted DNA at +4 to +12°C if analysed at the same day or freeze at -15 to -25°C until further use. Avoid frequent freeze-thaw cycles, because this may result in a decay of the eluted DNA (in particular at low DNA concentrations).
False positive PCR result	• Cross contamination • Contamination during handling	Principally, work under UV-irradiated workstations. Avoid the generation of aerosols by careful pipetting. Frequently change gloves. UV-irradiate the workstation at the end of handling a series of samples. Prepare mastermixes and handle DNA samples under different UV workstations (page 8). Use DNA-free pipette tips and other plastics. Make sure that the other Mini kit used for nucleic acid extraction and purification is DNA-free.

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Information *MolYsis™ Complete* Kits and DNA-Free PCR Reagents

MolYsis™ Complete

The ***MolYsis™ Complete*** **Kits** (***MolYsis™ Complete 5*** and ***MolYsis™ Complete 10***) are the complete solution for the human DNA removal and the microbial DNA isolation.
The kits provide reagents and materials for human DNA removal, bacteria and fungi enrichment and microbial DNA isolation. ***MolYsis™ Complete5*** enables midi size preparations from ≤1ml and 5ml volumes. Maxi size isolation of enriched microbial DNA from 5-10ml volumes is possible with ***MolYsis™ Complete10***. The kits guarantee exclusion of false signals by unspecific background amplification thus constituting your standard for extremely high PCR detection sensitivity: Enhancemant over total DNA extraction can account for up to 40,000-fold.

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.
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
MolTaq 16S Taq DNA polymerase, DNA-free	100 units 500 units	P-019-0100 P-019-0500
DNA-free water, *PCR grade*	10x 1.7ml	P-020-0003

Technical Support

If you have questions please contact us.

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

Material safety data sheets are available on request.

Order Information

Product	Contents	Cat. No.
MolYsis™ Basic	**50** sample pre-treatments: Human/animal DNA removal, pathogen cell wall degradation	D-300-050
	100 sample pre-treatments: Human/animal DNA removal, pathogen cell wall degradation	D-300-100

Related Products for Whole Blood and other Liquid Samples

Product	Contents	Cat. No.
Pre-treatment of samples of medium and large sizes (used with other DNA isolation kits)		
MolYsis™ Basic5 ≤1ml and 5ml sample volumes	**50** reactions **100** reactions	D-301-050 D-301-100
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
MolYsis™ Complete10 5 to 10ml sample volumes	**50** reactions **100** reactions	D-325-050 D-325-100

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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