Hot MolTaq 16S/18S
DNA-free Taq DNA polymerase

From *Thermus aquaticus* BM, recombinant (*E.coli*)
Deoxynucleoside-triphosphate:
DNA deoxynucleotidyltransferase, *EC 2.7.7.7*

- For research use only -

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Product Information

Contents

Hot MolTaq 16S/18S (Taq DNA polymerase), microbial DNA-free		
	100 units	**500 units**
Hot MolTaq 16S/18S (Taq DNA polymerase, 5U/µl)	1x 100U	1x 500U
10x PCR buffer (1.5mM Mg^{2+} final concentration)	1x 1.5ml	2x 1.5ml
10x KCl buffer basic (without Mg^{2+})	1x 1.5ml	2x 1.5ml
100mM $MgCl_2$	1x 1.5ml	1x 1.5ml
DNA-free water (PCR grade)	2x 1.25ml	10x 1.25ml

Catalog Numbers (Cat. No.):

P-080-0100 **100 units**
P-080-0500 **500 units**

Buffers and Reagents

PCR buffer: 10 x concentrated; contains ammonium sulfate $(NH_4)_2SO_4$; contains 1.5 mM Mg^{2+} final concentration, microbial DNA-free

KCl buffer basic: 10 x concentrated, contains potassium chloride (KCl); without Mg^{2+} ; microbial DNA-free

$MgCl_2$: 100 mM, microbial DNA-free

DNA-free water: PCR grade, microbial DNA-free

Application of the Product:

- 16S, 18S and 23S rRNA gene amplification (without background)
- Routine PCR
- Real-Time PCR
- GC-Rich PCR
- Nested PCR
- MultiPlex PCR
- Colony PCR
- DNA labeling reactions

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Additional Equipment and Reagents Required:

- Template DNA, gene-specific PCR primer pair
- dNTPs, PCR grade
- PCR tubes, DNA-free
- DNA-free polypropylene tubes for preparation of mastermixes and dilutions (e.g., Biosphere®, Sarstedt, Germany)
- PCR cycler (e.g., Mastercycler Gradient, Eppendorf, Germany)
- UV irradiated workstation (e.g., Airclean 600, StarLab, Germany)

Product Description

Hot MolTaq 16S/18S is a mixture of a genetically engineered, thermostable, DNA-dependent DNA polymerase (EC 2.7.7.7.) originally isolated from Thermus sp. and an aptamer-based inhibitor that reversibly binds to the enzyme. *Hot MolTaq 16S/18S* is DNA-free and thus especially useful for 16S, 18S and 23S rDNA gene amplification and cloning, because the enzyme preparation is free of genomic and plasmid DNA from the production strain. The polymerase activity is inhibited at ambient temperatures. This allows reaction set-up at room temperature without loss of specificity or sensitivity.

The enzyme has the following activities:
5’-3’ polymerase (60 to 150 nucleotides/s; approx. 1 kb/min), 5’-3’ exonuclease (strand displacement) and 3’ terminal deoxynucleotidyl transferase (addition of single dATP to the duplex DNA). Hot MolTaq 16S/18S incorporates modified nucleotides (dNTPαS, c7GTP, biotin-11-dUTP, digoxigenin-11-dUTP and fluorescein-12-dUTP but not biotin-16-dUTP) at high rates. The error frequencies for misincorporation and frameshift mutations are 10-4 and 2 x 10-5, respectively. Only the last three bases adjacent to the 3’ end of the primer need to be correctly base-paired in order to initiate polymerization. The 5’ region of the primer is less sensitive to base mismatches. Thus, new restriction sites can be easily introduced into an amplification product. Up to 9 kb can be amplified from lambda DNA and up to 5 kb from genomic DNA.

Unit definition:
One unit of enzyme incorporates 10 nmoles of dNTPs into an acid-insoluble form in 30min at 72°C under standard assay conditions, using a DNA template. Specific activity is 50.000U/mg protein.

Heat activation:
The enzyme is activated during normal cycling conditions. An extra activation step is not required.

Stability

Stable for 20 months from the date of manufacturing under proper storage condition. Guarantee for full performance of reagents and buffers is given through the expiration date printed on the label at the outer box, if the packed material is undamaged upon arrival and the reagents are unopened.

Enzyme Properties

Volume activity	5U/µl
Error rate*	approx. $1.3x10^{-5}$
Optimal enzyme concentration	1 to 5U per 25µl reaction
Optimal pH	approx. 9 (adjusted at 20°C)
Optimal polymerization temperature	around 72°C
Optimal Mg^{2+} concentration	varies from 1.5 to 5mM
Standard Mg^{2+} concentration	1.5mM when using 200µM dNTP each
PCR product size	optimized for up to 5kb
PCR cloning	TA-cloning; addition of A-overhangs (“extendase activity”)
Incorporation of modified nucleotides	accepts modified nucleotides like radiolabeled nucleotides, DIG-dUTP, biotin-dUTP
Thermostability	Over 80% activity after 30 cycles (1min 95°C, 1min 37°C, 3min 72°C)

* According to the *lacI* assay (Frey, B. and Suppmann, B. (1995) *Biochemica* 2, 8-9.)

Packaging, Storage and Handling

The purification of the *Hot MolTaq 16S/18S* and its confectioning are done under standard precautions for the avoidance of air-borne and handling-based DNA contaminations. The *Hot MolTaq 16S/18S* is supplied in a DNA-free screw cap vial. Store all vials in the kit at -15 to -25°C upon receipt.

<u>Important:</u> Replace *Hot MolTaq 16S/18S* to the freezer (-15 to -25°C) after handling.

For usage, the *10x PCR buffer*, *10x KCl buffer basic*, *100mM* $MgCl_2$ and the *DNA-free water* of the kit are thawed at room temperature (+18 to +25°C). After use, these components can be stored in the refrigerator (+4 to +12°C) for further use at the same day, but should be replaced to -15 to -25°C for longer storage.

Avoidance of DNA Contamination:

PCR analysis demands special care with respect to avoidance of contamination from exogenous sources. Take care to separate places of DNA preparation from places where PCR reagents are handled for runs, in particular preparation of mastermixes, pipetting into PCR tubes and performance of PCR runs. Wear a protective lab coat, sterile protective gloves and sleeve covers, protective goggles and a disposable mask at any handling step, also during DNA preparation. Use only DNA-free disposables. In particular, use only PCR strips or tubes and pipette filter tips from manufacturers guaranteeing DNA-free products. Please contact Molzym for further information regarding our products and other suppliers of DNA-free plastic consumables.

Controls and DNA sample template:

Generally, for each analysis, run positive and negative controls to check for proper performance of the reaction and sterility of reagents and buffers used.

PCR negative controls using *DNA-free water* instead of template DNA are used for analysis of contamination of microbial DNA in the purified final mastermix. Ensure that negative controls are prepared first and the tube is sealed before positive controls and samples are pipetted. Take care to avoid cross contamination by aerosols.

For DNA sample template preparation, make sure to use kits guaranteeing microbial DNA-free reagents, buffers and matrices like Molzym´s products (e.g. MolYsis™ kits) for the sensitive detection of pathogens in blood, other body fluids and other matrices.

PCR positive controls are run using known amounts of genomic DNA. This can be extracted and purified by Molzym´s *PrestoSpin D Bug* or *PrestoSpin D Fungi* (see page 11) from a microorganism for the specific assay. Alternatively, use Molzym's Positive Control DNA (see page 11) for gam-positive bacteria and yeasts.

Quality Control and Specifications

Each lot of *Hot MolTaq 16S/18S* Taq DNA polymerase is produced and quality-controlled according to Molzym's recorded quality management system.

The *Hot MolTaq 16S/18S* DNA polymerase is tested for performance in PCR runs, using different primer pairs for amplification of sequences between 0.2 and 1 kb.

Guarantee of the DNA-free product is given for the absence of signals in PCR negative controls at a rate of ≥97% for up to 40 PCR cycles (provided the avoidance of contamination). DNA-free *Hot MolTaq 16S/18S* is defined as giving no bacterial and fungal DNA-specific signal. In negative control runs, the absence of banding in gel electrophoretic analysis is demonstrated.

Positive controls are done using known amounts of genomic DNA from *Staphylococcus aureus* or other bacteria. Alternatively, Molzym's Positive Control DNA (P1) is used as PCR positive control (cat. no. S-200-050).

Material Safety Data Sheet (MSDS)

Please contact Molzym for an actual MSDS (according to Regulation (EC) No. 1272/2008) of this product:

Tel.: +49(0)421 69 61 62 0 • **E-Mail:** info@molzym.com

Patents/Disclaimer

Some applications in which this product can be used may be covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

PCR Protocol

Standard PCR Procedure

General Considerations

The optimal conditions (incubation times and temperatures, concentrations of enzyme, template DNA, Mg^{2+}) depend on the system used and have to be determined individually. In particular, the Mg^{2+} concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis. As a starting point, use the following guidelines:

- **Optimal enzyme concentration:** 1 to 5U/25µl
- **Optimal Mg^{2+}** concentration can vary from 1.5 to 5mM; in most cases Mg^{2+} concentration of 1.5mM will produce satisfactory results when using 200µM dNTPs (each).
  The 10x PCR buffer supplied contains 1.5mM Mg^{2+}. The *10x KCl buffer basic* supplied contains no Mg^{2+}.
- **dNTP concentration:** Always use balanced solutions of all four dNTPs. The final concentration of each dNTP should be between 50 and 500µM; the most commonly used concentration is 200µM. Increase concentrations of Mg^{2+} when increasing the concentration of dNTPs.
- **Template concentration:**
  - Genomic DNA template: 10 to 250ng (e.g. human genomic DNA template)
  - Plasmid DNA template: 0.1 to 15ng,
  - Bacterial DNA template: 10pg to 10ng (*10x KCl buffer basic*, 3mM Mg^{2+} is recommended)
- **Dilution buffer for template DNA:** The optimal dilution buffer is either simply sterile double distilled water or 5-10 mM Tris (pH 8-9).
  Storage of DNA should be in 10mM Tris pH 8.0 to 8.5 or TE buffer (10mM Tris pH 8.0; 1mM EDTA).

PCR and Real-Time PCR Amplification of the 16S/18S rDNA

Follow the instructions for avoidance of DNA contamination as indicated on page 4.

Fluorescent dye:

When using SYBR Green I as fluorescent dye the *10x KCl PCR buffer basic* and *100mM* $MgCl_2$ (3mM Mg^{2+} final concentration) are recommended for better performance.
For detection of bacteria in whole blood and other body liquids using Molzym´s kits MolYsis™ kits, we have made good experience following the protocol B described on page 8.

Preparation of the Reaction Mixtures

Take care that all handling is done in a DNA-free environment. ***Work under a UV irradiated workstation***. Make sure that plastic consumables (including PCR tubes, pipette tips, polypropylene tubes) are free of contaminating microbial DNA when used in combination with the amplification reaction mixture. With each series of PCR, run a negative control and a positive control. Work according to the sequence of steps of protocol A or protocol B below (25µl final reaction volume per assay):

Protocol A:
Hot MolTaq 16S/18S with *10x PCR buffer* (1.5mM Mg^{2+} final concentration)

1. **Reagents Pretreatment**

   Thaw *10x PCR buffer* and *DNA-free water* at room temperature (+18 to +25°C). Vortex for a few seconds to mix and briefly centrifuge vials to clear the lids. Place the *Hot MolTaq 16S/18S* in the rack of the other components. After handling, place the *Hot MolTaq 16S/18S* vial and other reagents back to the freezer (-15 to -25°C).

2. **Preparation of the Mastermix**

   Pipette the following components (table below) in a DNA-free 1.5ml polypropylene tube. In case of more reactions, prepare a mastermix containing all PCR reagents in proportions as indicated in the table below. Please note: The volume of the *DNA-free water* added is corrected for the variable volumes of the components.

   Vortex the prepared mastermix for few seconds to mix and briefly centrifuge to clear the lid of the tube.

Components	Volume for 1 reaction	Final concentration
DNA-free water (PCR-grade; supplied)	add up to 25µl	
10x PCR buffer (supplied)	2.5µl	1x (1.5mM Mg^{2+})
dNTP mix (10mM)	0.5µl	200µM (of each dNTP)
Forward primer	variable	0.1 to 0.6µM
Reverse primer	variable	0.1 to 0.6µM
Hot MolTaq 16S/18S (supplied)	0.2 to 1.0µl	1 to 5U/reaction
Template (sample DNA, DNA-free water or positive control DNA)	x* µl (variable)	variable
Final volume	25µl	

* x µl = volume of template DNA added

3. Pipette aliquots of the mastermix to each PCR tube and add the supplied *DNA-free water* for a PCR negative control.
4. Place the PCR tubes in another rack designated for transport to the place where DNA is handled.

   Add x µl of the DNA template (sample DNA or positive control DNA) into PCR tubes containing the mastermix.
5. Seal tubes and place in a PCR machine immediately.
6. Start the program of the specific assay (‘PCR Thermocycling Conditions’, page 9).

Protocol B:
Hot MolTaq 16S/18S with *10x KCl buffer basic* (without Mg^{2+})

1. **Reagents Pretreatment**

   Thaw *10x KCl buffer basic*, *100mM $MgCl_2$* and *DNA-free water* at room temperature (+18 to +25°C). Vortex for a few seconds to mix and briefly centrifuge vials to clear the lids. Place the *Hot MolTaq 16S/18S* in the rack of the other components. After handling, place the *Hot MolTaq 16S/18S* vial and other reagents back to the freezer (-15 to -25°C).

2. **Preparation of the Mastermix**

   Pipette the following components (table below) in a DNA-free 1.5ml polypropylene tube. In case of more reactions, prepare a mastermix containing all PCR reagents in proportions as indicated in the table below. Please note: The volume of the *DNA-free water* added is corrected for the variable volumes of the components.

   Vortex the prepared mastermix for few seconds to mix and briefly centrifuge to clear the lid of the tube.

Components	Volume for 1 reaction	Final concentration
DNA-free water (PCR-grade; supplied)	add up to 25µl	
10x KCl buffer basic (supplied, without Mg^{2+})	2.5µl	
100mM $MgCl_2$	0.375 to 1.25µl	1.5 to 5mM
dNTP mix (10mM)	0.5µl	200µM (of each dNTP)
Forward primer	variable	0.1 to 0.6µM
Reverse primer	variable	0.1 to 0.6µM
Hot MolTaq 16S/18S (supplied)	0.2 to 1.0µl	1 to 5U/reaction
Template** (sample DNA#, DNA-free water or positive control DNA)	x* µl (variable)	variable
Final volume	25µl	

* x µl = volume of template DNA added

The following table shows an overview of the final Mg^{2+} concentration and the corresponding volume of the *100mM $MgCl_2$* reagents:

Final Mg^{2+} concentration (25µl PCR volume)								
Final concentration (mM)	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**
Required volume (µl) of *100mM $MgCl_2$* per reaction	**0.375**	**0.5**	**0.625**	**0.75**	**0.875**	**1.0**	**1.125**	**1.25**

3. Pipette aliquots of the mastermix to each PCR tube and add the supplied *DNA-free water* for a PCR negative control.
4. Place the PCR tubes in another rack designated for transport to the place where DNA is handled.

   Add x µl of the DNA template (sample DNA or positive control DNA) into PCR tubes containing the mastermix.
5. Seal tubes and place in a PCR machine immediately.
6. Start the program of the specific assay ('PCR Thermocycling Conditions', page 9).

PCR Thermocycling Conditions

Place the samples in the thermal cycler, and start cycling. For single extension time use the protocol below:

Method		Target Temperature	Incubation time	Cycles
Initial Denaturation*		95°C	1 to 3min	1
Cycling	Denaturation*	95°C	15 to 30s	25 to 40
	Annealing#	50 to 68°C	30 to 60s	
	Elongation+	72°C	30s to 1min	
Final elongation		72°C	10min	1
Cooling		4°C	unlimited time	∞

* The denaturation temperature can vary from 92 to 95°C.
# Optimal annealing temperature depends on the melting temperature of the primers and on the system used.
+ For PCR products longer than 1kb, use an extension time of approximately 1min per kb DNA.

After amplification, the PCR products can be stored overnight at +4 to +12°C, or at -15 to -25°C for longer storage. Avoid frequent freeze-thaw cycles, because this may results in a decay of the DNA (in particular at low DNA concentrations).

Possible further procedures:
Check the PCR product on an agarose gel for size and specificity using an appropriate size marker.

Please address any questions relating this product to the support hotline:

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

Troubleshooting

Possible cause	Recommendation
Little or no PCR product	
DNA template problems	Check quality and concentration of template: • Analyze an aliquot with photometric methods or on an agarose gel to check for possible degradation. • Make a control reaction on template with an established primer pair or PCR system. • Check or repeat purification of template.
Enzyme concentration too low	• Increase the amount of polymerase in 0.5U steps.
Mg^{2+} concentration too low	Increase the Mg^{2+} concentration in steps of 0.25mM. (1.5mM Mg^{2+} is the final concentration in the mastermix.)
Cycle conditions not optimal	• Decrease annealing temperature. • Increase cycle number. • Make sure that the final elongation step was carried out.
Primer design not optimal	Design alternative primers
Primer concentration not optimal	• Both primers must have the same concentration. • Titrate primer concentration (0.1 – 0.6 µM)
Primer quality or storage problems	• If you use an established primer pair, check performance on an established PCR system (control template). • Make sure that the primers are not degraded. • Always store primers at -15 to -25°C.
Multiple bands or background smear	
Annealing temperature too low	Increase annealing temperature according to the primer length.
Primer design or concentration not optimal	• Review primer design. • Titrate primer concentration (0.1 – 0.6µM). • Both primers must have the same concentration. • Perform nested PCR with nested primers.
Difficult template e.g. GC-rich template	Perform PCR with PCR enhancer.
DNA template problems	Use serial dilution of template.
PCR products in negative control experiments	
Carry-over contamination	• Set up PCR reactions in an area separate from that used for PCR product analysis (UV workstation e.g., Airclean 600, StarLab, Germany) and use sterile disposables (gloves, sleeves and bouffant covers). • Exchange all reagents, use DNA-free reagents (e.g., Molzym´s DNA-free PCR reagents). • Use aerosol-resistant and microbial DNA-free pipette tips. • Use DNA-free consumables (PCR tubes, polypropylene tubes (e.g., 1.5ml micro tubes, Biosphere®, Sarstedt, Germany) • Use dUTP (600µM) instead of dTTP (200µM) in combination with thermolabile UNG (1U/50µl reaction) and increase Mg^{2+} concentration up to 4mM at most. **Further information see part Product Information – Avoidance of DNA contamination, page 4**
Specific problems in RT-PCR application	
No product, additional bands, background	• The volume of cDNA template (RT-reaction) should not exceed 10% of the smear final volume of the PCR reaction. • Follow trouble shooting above. • Increase Mg^{2+} by titration in steps of 0.25mM.

Related Products

Product	Contents	Cat. No.
Isolation of microbial DNA from whole blood and other body liquids (DNA-free reagents)		
MolYsis™ Complete5 ≤1ml and 5ml sample volumes	**50** microbial DNA isolations from samples **100** microbial DNA isolations from samples	D-321-050 D-321-100
PCR Mastermix, DNA-free (incl. *MolTaq 16S/18S*)		
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 18S Complete Universal 18S rDNA PCR and Real-Time PCR assay for detection of fungi.	**100** reactions **250** reactions **1000** reactions	S-070-0100 S-070-0250 S-070-1000
Mastermix 16S/18S Dye Premixed reagents and fluorescent dye for Real-Time PCR with custom primers	**100** reactions **250** reactions **1000** reactions	S-030-0100 S-030-0250 S-030-1000
Mastermix 16S/18S Basic Premixed reagents for PCR analysis with custom primers	**100** reactions **250** reactions **1000** reactions	S-040-0100 S-040-0250 S-040-1000
PCR Reagents		
***DNA-free water* (PCR grade)**	Microbial DNA-free PCR grade water for molecular analysis **10x 1.7ml**	P-020-0003
Positive Control DNA (P1)	Positive run control in universal PCRs and Real-Time PCRs. **50** reactions	S-200-050
Panfungal Sequencing Primer	For sequencing analysis of PCR amplicons produced with *Mastermix 18S Complete* and *Mastermix 18S Primer*	S-785-100
Set of Eubacterial Sequencing Primers	For sequencing analysis of PCR amplicons produced with *Mastermix 16S Complete* and *Mastermix 16S Primer*. **100** reactions, each primer	S-775-100

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