MolTaq 16S (Taq DNA Polymerase) (DNA-free)

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

Product overview

Kit/Component

Mol*Taq* 16S – thermostable DNA polymerase , DNA-free		
	100 units	**500 units**
Mol*Taq* 16S DNA polymerase	1 x 100 U	1 x 500 U
10 x PCR buffer (1.5 mM Mg^{2+} final concentration)	1 x 1.5 ml	2 x 1.5 ml
ddH_2O	2 x 1.25 ml	10 x 1.25 ml

Product description

Mol*Taq* 16S (EC 2.7.7.7.) is a genetically engineered, DNA-free thermostable DNA polymerase originally isolated from *Thermus* sp. **Mol*Taq* 16S** is especially useful for 16S + 23S rDNA gene amplification and cloning, because the enzyme preparation is free of genomic and plasmid DNA from the production strain. 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). **Mol*Taq* 16S** incorporates modified nucleotides (dNTPαS, c^7GTP, 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 30 min at 72 °C under standard assay conditions, using a DNA template. Specific activity is 50.000 U/mg protein.

Enzyme properties

Volume activity	5 U/µl
Error rate*	approx. $1.3x10^{-5}$
Optimal enzyme concentration	0.5-2.5 U per 50 µl reaction
Standard enzyme concentration	1.25 U per 50 µl reaction
Optimal pH	approx. 9 (adjusted at 20°C)
Optimal polymerization temperature	around 72°C
Optimal Mg^{2+} concentration	varies from 1.5 – 5 mM
Standard Mg^{2+} concentration	1.5 mM when using 200 µM dNTP each
PCR product size	optimized for up to 5 kb
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 (1 min 95°C, 1 min 37°C, 3 min 72°C)

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

Storage and stability

Store at –15 to –25°C upon delivery.

Stable for 24 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.

Applications

· Polymerase chain reaction (PCR)
. 16S and 23S rRNA gene amplification (without background)
. detection of bacteria in samples (e.g. blood)
· DNA labeling reactions
· Sequencing/ cycle sequencing

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.

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Recommendations for PCR analysis of bacteria:

Important: The enzyme must be stored at-15°C to -20°C and always kept on ice when working with it.

Avoidence 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 master mixes, pipetting into PCR tubes and performance of PCR runs. Wear protective gloves and lab coats at any handling step, also during DNA preparation. Use only sterilized disposables and glass ware. In particular, use only PCR strips or tubes and pipette filter tips from manufacturers guaranteeing DNA-free products. Generally, for each analysis, run positive and negative controls to check for proper performance of the reaction and sterility of reagents and buffers used. 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 template preparation, make sure to use kits guaranteeing bacterial DNA-free reagents, buffers and matrices like MolYsis products of Molzym for the sensitive detection of pathogens in blood and other body fluids.

PCR and Real-Time-PCR amplification of the 16S rDNA. Generally, keep all PCR reagents and template DNA chilled during handling. Follow the instructions for avoidance of DNA contamination as indicated above. When using Sybr-Green I as fluorescent dye KCl PCR buffer (please inquire: 10x KCl PCR buffer basic & 100 mM $MgSO_4$) is recommended for better performance. For detection of bacteria in whole blood and other samples using MolYsis kits, we have made good experience following the protocol described below. The primers recommended are well suited for detection purposes. Identification by sequencing is most suitable using R1n-U2 for amplification and using R1n for sequencing.

Place bacterial DNA-free PCR tubes with reaction mixes in a thermal cycler heated to 94 °C. Cycle parameter: 94 °C for 4 min, 35 cycles with 94 °C for 0.5 min, x °C (see primers) for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. Thereafter, keep tubes at 4 °C until analysis by gel electrophoresis.

Primers:

1) **R1n** (forward): 5'-GCTCAGATTGAACGCTGGCG-3';

   u2(reverse): 5'-ACATTTCACAACACGAGCTG-3'; annealing temperature (x): 46 °C; size of amplification product: 1064 bp; reference: Appl Environ Microbiol 62 (1996), 766-71.

2) **515f** (forward): 5'-GTGCCAGCAGCCGCGGTAAT -3'; **u2** (reverse): s.a; annealing temperature (x): 52 °C; size of amplification product: 570 bp; reference: modified after Appl Environ Microbiol 59 (1993), 695-700.

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: 0.5 – 2.5 U/50 µl ; a concentration of 1.25 U will usually produce satisfactory results.
• Optimal Mg^{2+} concentration can vary from 1.5 mM to 5 mM; in most cases a Mg^{2+} concentration of 1.5 mM will produce satisfactory results when using 200 µM dNTP (each). The 10x PCR buffer supplied contains 1.5 mM Mg^{2+}.
• dNTP concentration: always use balanced solutions of all four dNTP. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. Increase concentrations of Mg^{2+} when increasing the concentration of dNTP.
• Template concentration: e.g. human genomic DNA template: 10 ng-250 ng; plasmid DNA template: 0.1 ng-15 ng.
• The optimal dilution buffer for the template DNA is either simply sterile double-distilled water or 5-10 mM Tris (pH 8-9). Storage of DNA should be in 10 mM Tris pH 8.0-8.5 or TE buffer (10 mM Tris pH 8.0; 1 mM EDTA).

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Preparation of reaction mixtures

1. Briefly centrifuge all reagents before starting. Keep the MolTaq 16S vial chilled. After handling, immediately place the MolTaq 16S vial back to the freezer.
2. Prepare a mix of reagents in sterile thin-walled PCR tubes **on ice**:

Reagents	Volume	Final concentration
Sterile ddH_20	add up to 50 µl	
10x PCR reaction buffer	5 µl	1x (1.5 mM Mg^{2+})
dNTP mix (10 mM)	1 µl	200 µM (of each dNTP)
Primer 1	variable	0.1-0.6 µM
Primer 2	variable	0.1-0.6 µM
Template DNA[§]	variable	0.1-1000 ng
Mol*Taq 16S* (5 U/µl)	0.4 µl	2 U/ reaction
Final volume	**50 µl**	

In case of more reactions, prepare a master mix containing sterile ddH_2O, 10 x PCR reaction buffer, dNTP mix (10 mM) and **Mol*Taq* 16S** (5 U/µl) in proportions as indicated in the table above. Please note that the volume of ddH_2O added is corrected for the volumes of primer 1 and 2 and template DNA. Pipette aliquots of the master mix into PCR tubes and add primers and template DNA.
Gently vortex the mixture to produce a homogenous reaction, then centrifuge briefly to collect the sample at the bottom of the tube. Continue to thermal cycling immediately.
Note: Carefully overlay the reaction with mineral oil if required by your type of thermal cycler.

[§] For genomic DNA template 10 ng – 1000 ng; for plasmid DNA 0.1 ng – 15 ng

Thermal cycling

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

	Temperature	Time	Cycle No.
Initial denaturation*	95°C	1 min	1x
Denaturation*	95°C	15-30 sec	25-40x
Annealing[#]	45-65°C	30-60 sec	
Elongation[+]	72°C	45 sec - 3min	
Final elongation	72°C	10 min	1x
Cooling	4°C	unlimited time	-

* The denaturation temperature can vary from 92°C-95°C.
[#] Optimal annealing temperature depends on the melting temperature of the primers and on the system used.
[+] For PCR products up to 1 kb elongation temperature should be around 72°C; for PCR products larger than 1 kb elongation temperature should be around 68°C.

After cycling, the samples may be frozen for storage.
Possible further procedures:

- Check the PCR product on an agarose gel for size and specificity using an appropriate size marker

Quality control

Each lot of Mol*Taq* 16S DNA polymerase is tested for performance in PCR runs, using different primer pairs for amplification of sequences between 0.5 and 3 kb.

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