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Product Information

MTP™ *Taq* DNA Polymerase

Catalog Number **D7442**

TECHNICAL BULLETIN

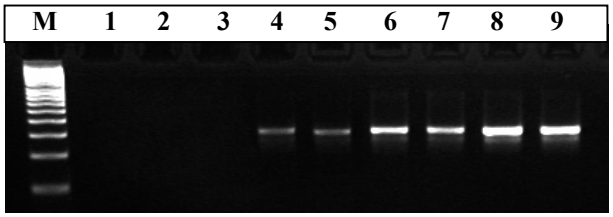
Product Description

MTP™ *Taq* DNA Polymerase is a recombinant thermostable enzyme from *Thermus aquaticus* expressed in *E. coli* and purified using a proprietary process to minimize levels of contaminating DNA. The enzyme has both 5′→3′ DNA polymerase and exonuclease activities, is ~95 kDa by SDS-PAGE, and has no detectable endonuclease or 3′→5′ exonuclease activities. Each lot of MTP *Taq* undergoes strict quality control testing to ensure the absence of detectable levels of contaminating DNA.

Contaminating DNA present in most other polymerase preparations often preclude or obscure the accurate interpretation of results, especially when targeting conserved sequences, e.g., bacterial 16S rRNA region.[1] Through Sigma's proprietary DNA removal methods and strict quality control standards, we can ensure the absence of the most commonly found contaminant DNA. Each lot of MTP *Taq* is assayed using PCR and primers specific to (1) the conserved region of bacterial 16S rRNA, (2) the *Taq* expression vector, and (3) the human β-actin gene.

While MTP *Taq* ensures a high-quality, low contaminant DNA polymerase for reliable PCR amplification, DNA contaminants can be introduced into PCR through a number of other reagents.[2] To further minimize the risk of contaminant DNA during PCR, we include 10× MTP *Taq* Buffer with each tube of MTP *Taq* DNA Polymerase. Each lot of 10× MTP *Taq* Buffer undergoes the same strict quality control testing as MTP *Taq* DNA polymerase to ensure the absence of contaminating DNA. To prevent false positive PCR results, only DNA-free reagents should be used in PCR reactions with MTP *Taq* DNA polymerase.

Figure 1.



Representative QC gel assaying for the presence of conserved 16S rRNA sequence. PCR products run in lanes 1-3 are from "no-template" reactions; PCR products in lanes 4-5, 6-7, and 8-9 are from reactions with 37 fg, 370 fg, and 3.7 pg of E. coli genomic DNA added, Catalog Number D4889.

Unit definition: One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Components

- MTP *Taq* DNA Polymerase,
  Catalog Number D7067.
  5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% TWEEN® 20, 50% glycerol.
- 10× MTP *Taq* Buffer, Catalog Number M9943.
  100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin.

Reagents and equipment required but not provided

- Deoxynucleotide Mix, Catalog Number D7295. Contains 10 mM of each dATP, dCTP, dGTP, and TTP.
- Water, PCR Reagent, Catalog Number W1754.
- Specific oligonucleotide primers (available from Sigma-Genosys at www.sigmagenosys.com)
- Template DNA to be amplified
- Dedicated pipettes
- Sterile, aerosol barrier pipette tips
- Any one of the following PCR tube products:
  a. 0.2 ml or 0.5 ml Thin-Walled PCR Tubes; Catalog Numbers P3114 and P3364
  b. 96-well PCR plates with Sterile thermal sealing film; Catalog Numbers Z374903 and Z369683
  c. PCR tube strips; Catalog Number T0322
- Mineral Oil, Catalog Number M8662 (optional)
- Thermal cycler

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. For convenience, 10× MTP *Taq* Buffer can be stored at room temperature.

Procedure

Note: Every precaution should be taken to avoid contamination of reagents with unknown/unwanted DNA. This includes the following:

- Use a "clean area" for the setup of PCR reactions.[2] A clean area is a separate lab space (such as a hood) preferably in a separate lab, both of which are free from PCR products. The clean area should contain dedicated lab coats and pipettes (see below) and should be cleansed (either with 10% bleach or UV lamp) after each use. PCR products should never enter a clean area.
- Use sterile, aerosol barrier pipette tips to minimize the risk of aerosol contamination. Change tips after each single use.
- Change gloves frequently, especially after handling DNA.

Amplification Procedure

The optimal conditions for the concentration of MTP *Taq* DNA Polymerase, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. It is recommended that the enzyme and the $MgCl_2$ be titrated to determine the optimal efficiency if the below protocol is shown to be less than satisfactory. Sigma offers a separate PCR Optimization Kit, Catalog Number OPT2, that contains a variety of buffers and adjuncts for optimizing the specificity, fidelity, and yield of a PCR product.

1. Add the following reagents to a suitable PCR tube/plate in the following order:

Volume	Reagent	Final Concentration
w µL	Water	-
5 µL	10× MTP *Taq* buffer	1×
1 µL	10 mM dNTP mix	200 µM each dNTP
x µL	Forward Primer (typically 15-30 bases in length)	0.1–0.5 µM
y µL	Reverse Primer (typically 15-30 bases in length)	0.1–0.5 µM
0.5 µL	MTP *Taq* DNA Polymerase	0.05 units/µL
z µL	Template DNA (typically 10 ng)	200 pg/µL
50 µL*	Total reaction volume	

* Maintain final concentrations when scaling reaction volumes. Preparing enzyme / nucleotide / buffer master mixes either with or without primers or template facilitates the setup of multiple reactions.

2. Vortex gently to mix. Briefly centrifuge to collect reaction on the bottom of the tube.
3. If using a thermal cycler without a heated lid, add 50 µL of mineral oil to the top of each tube to prevent evaporation.

4. Cycle using thermocycler of choice. The amplification parameters may require optimization for individual primers, templates, and thermal cyclers.

   Common cycling parameters are:

Step	Temperature	Time	Cycles
Denature	94 ºC	1 minute	1
Denature	94 ºC	1 minute	30
Anneal	5 ºC below primer Tm	15 - 60 seconds	
Extend	72 ºC	30 - 65 seconds/kb	
Cool	4 ºC	≥ 0 seconds	1

5. Amplified DNA can be evaluated by standard methods (e.g. agarose gel electrophoresis).[3]
6. If used, mineral oil may be removed by a single 50 µL chloroform extraction.

References

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2. Millar, B.C., et al., Risk assessment models and contamination management: implications for broad-range ribosomal DNA PCR as a diagnostic tool in medical bacteriology. *J. Clin. Microbiol.*, **40,** 1575-1580 (2002).
3. *Molecular Cloning: A Laboratory Manual*, Sambrook, J., and Russel, D. W., Third Edition, (Cold Spring Harbor Laboratory Press, New York, 2000), Catalog Number M8265.
4. Rychlik, W., and Rhoads, R. E., A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. *Nucleic Acids Res.*, **17,** 8543-8551 (1989).
5. *PCR: Essential Data*, Newton, C. R., (Ed.). (John Wiley & Sons, New York, 1995).
6. Choi, J.S., et al., Improved Cycle sequencing of GC-rich DNA template. *Exp. Mol. Med*. **31**, 20-24 (1999).
7. Rees, W. A., et al., Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry*. **32,** 137-144 (1993).
8. Don, R. H., et al., 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res*. **19,** 4008 (1991).

Label License Statement

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Troubleshooting Guide		
Problem	**Possible Causes**	**Solution**
No PCR product	Missing or degraded PCR component(s)	Repeat reaction, including positive controls to ensure all components are functioning. Use a checklist to mark off each reaction component as it is added.
	Insufficient PCR cycles	Increase the number of PCR cycles (3-5 additional cycles at a time)
	Annealing temperature too high	Decrease annealing temperature in 2-4 °C increments. Use a temperature gradient thermocycler.
	Sub-optimal primer design	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primer to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%. We recommend the use of a primer selection program such as Oligo[4], or primer3 available at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi.
	Insufficient template	First try increasing cycle number. If that fails, repeat reaction with ≥5-fold higher concentration of template.
	Poor-quality template	Evaluate the template integrity by agarose gel electrophoresis. It may be necessary to repurify template using methods that minimize nicking and shearing.[3] For amplification of damaged template unable to be amplified with standard enzymes, try Sigma's DNA repair and amplification enzyme blend Restorase® , Catalog Number R1028.
	Insufficient enzyme	0.5 µL (2.5 units) is sufficient for most applications. It is recommended that the cycling parameters be optimized before the enzyme concentration is increased. In rare cases, yields can be improved by increasing the enzyme concentration. However, if the enzyme amount is above 1.0 µL (5.0 units), higher background levels may be seen.
	Inappropriate denaturation temperature	Optimize denaturation temperature by increasing or decreasing the temperature in 1 °C increments or on a temperature gradient thermocycler.
	Denaturation time may be too long or too short	Optimize the denaturation time by increasing or decreasing it in 10-second increments
	Insufficient extension	Increase extension time. Generally, increasing the extension time 1 minute per 1 kb is sufficient; however, longer extension times may be beneficial.
	Sub-optimal [Mg^{++}]	This is unlikely if the 10x reaction buffer (provided) is used and the deoxynucleotides do not exceed a concentration of 0.2 mM each. However, if the concentration of EDTA in the sample is greater than 5 mM, this can reduce the effective concentration of magnesium.[5]
	Sub-optimal [dNTPs]	This is unlikely if the final concentration of each deoxynucleotide is 0.2 mM. This concentration of dNTPs is suitable for a wide range of applications. If the dNTPs are being prepared in the laboratory, be sure that the final concentration of each deoxynucleotide is 0.2 mM. If the concentration of dNTPs is increased, the Mg^{2+} concentration will need to be increased proportionately.[5]
	Inherently difficult target template	In many cases, targets that are difficult to amplify contain unusually high GC content and/or secondary structure.[6] 2-Betaine has been reported to help amplification of high GC content templates at a concentration of 0.8-1.3 M.[7] In some cases, the addition of 1-4% DMSO may help.[6]

Troubleshooting Guide (continued)

Problem	Possible Causes	Solution
Multiple products	Too many cycles	Nonspecific bands may be eliminated with reduced cycling.
	Annealing temperature too low	Increase the annealing/extension temperature in increments of 2-3 °C.
	Sub-optimal primer design	See recommendations under "No PCR product"
	Contaminant DNA in PCR	If reagents other than Sigma MTP reagents were used in PCR reactions, it is likely that those unqualified reagents contained contaminating DNA. Otherwise, if Sigma MTP reagents were used, check your methodology. All PCR reactions should be assembled in a hood in a clean area that is free from PCR products. Clean areas should be wiped down with 10% bleach after each use. All reagents should be portioned into small aliquots upon receipt to minimize the risk of contamination. Once contaminated, all suspect reagents should be discarded, and PCR repeated with fresh reagents.
Products are smeared	Too many cycles	Reduce the cycle number in 3-5 cycle increments.
	Denaturation temperature too low	Increase the denaturation temperature in 1 °C increments.
	Extension time too long	Decrease the extension time in 1-2 minute increments
	Multiple PCR products generated	Perform Touchdown PCR.[8]
	Too much enzyme	0.5 µL (2.5 units) is sufficient for most applications. However, this concentration may be too high for some applications. It is recommended the cycling parameters be optimized first, as described above, then, reduce the enzyme concentration to 0.5-0.2x.
	$[Mg^{++}]$ too high	In general magnesium should be 0.7 mM above the dNTP concentration. Some reactions may be benefited by higher concentrations. Titrate Mg^{+2} in 0.2 mM increments.[5]
	Template concentration too high	Reduce template concentration in a series of 10 fold dilutions.
Product is wrong size	Sub-optimal primer design	See recommendations under "No PCR product"
	Extension time too short	Increase the extension times or use touchdown PCR.[8]
	Contaminant DNA in PCR	See recommendations under "Multiple products"
Faint Product	[Template] too low	Add additional template in 50 ng increments for genomic or 1-2 ng for viral or plasmid DNA
	Insufficient PCR cycles	Increase the cycle number in 3-5 cycle increments
	Extension time too short	Increase the extension times in 2 minute increments
	Sub-optimum reaction conditions	Add PCR enhancers, e.g. 1-4% dimethyl sulfoxide (DMSO) or 0.8-1.3 M betaine, final concentration.[5]