Print

Roche

Taq DNA Polymerase, 1 U/µl

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

Cat. No. 11 647 679 001 250 U

Cat. No. 11 647 687 001 1,000 U (4 × 250 U)

Version 13
Content version: December 2011

Store at −15 to −25°C

1. What this Product Does

If 1.25 U are used per 50 µl reaction, Taq DNA Polymerase is designed for approximately:

- 200 reactions (Cat. No. 11 647 679 001)
- 800 reactions (Cat. No. 11 647 687 001)

Contents

Label	Contents
Taq DNA Polymerase (1 U/µl)	▪ 250 µl (250 U pack size) ▪ 4 × 250 µl (1,000 U pack size) Enzyme storage buffer: 20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1 M KCl, 0.5% Nonidet P40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v), pH 8.0 (+4°C).
PCR reaction buffer with $MgCl_2$,10 × conc.	▪ 1 ml ▪ 100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C)
$MgCl_2$ stock solution	▪ 1 ml ▪ 25 mM $MgCl_2$
PCR reaction buffer without $MgCl_2$, 10 × conc.	▪ 1 ml ▪ 100 mM Tris-HCl, 500 mM KCl, pH 8.3 (+20°C)

Storage and Stability

The undiluted enzyme solution is stable when stored at –15 to –25°C until the expiration date printed on the label.

Additional Equipment and Reagents Required

- Template DNA, gene-specific PCR primer pair
- dNTPs, PCR Grade*; Water, PCR Grade*
- Thermal block cycler (*e.g.,* Applied Biosystems GeneAmp PCR System 9600)
- 0.2 ml thin-walled PCR tubes
- Sterile reaction tubes for preparing master mixes and dilutions

** available from Roche Applied Science*

Application

Polymerase Chain Reaction (PCR): Taq DNA Polymerase activity is stable during prolonged incubation at high temperatures (+95°C) and can therefore be used to amplify DNA fragments by PCR.

- DNA labeling reactions (6, 7)
- Sequencing/ cycle sequencing (6, 8).

Enzyme Properties

Volume activity	1 U/µl
Optimal enzyme concentration	varies between 0,5 and 2.5 U per 50 µl reaction
Standard enzyme concentration	1.25 U per 50 µl reaction
Optimal Mg^{2+} concentration	varies between 1 and 10 mM (as $MgCl_2$)
Standard Mg^{2+} concentration	1.5 mM (as $MgCl_2$) when using 200 µM dNTP each

2. How To Use this Product

2.1 Before You Begin

General considerations

The optimal conditions of Taq DNA polymerase (incubation, times and temperatures, concentrations of the enzyme, template DNA, primers, Mg^{2+}) depend on the system used and have to be determined individually.

Especially the Mg^{2+} concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis.

Optimization of the $MgCl_2$ concentration

The optimal $MgCl_2$ concentration must be determined empirically. In most cases a concentration of 1.5 mM will produce satisfactory results. For individual optimization of the Mg^{2+} concentration a buffer without $MgCl_2$ and a $MgCl_2$ stock solution are supplied separately.

The table below gives the volumes of the $MgCl_2$ stock solution which give the designated $MgCl_2$ concentrations when added to a 50 µl PCR mixture. All other steps for preparing of the reaction mix are the same as described above.

$MgCl_2$	1 mM	1.25 mM	1.5 mM	1.75 mM	2 mM	2.5 mM	5 mM
Volume	2 µl	2.5 µl	3 µl	3.5 µl	4 µl	5 µl	10 µl

2.2 Preparation of Reaction Mixes

For multiple reactions, we recommend that you prepare two reaction mixes. This eliminates the need for a hot start and keeps the enzyme from interacting with primers and template during preparation of the reaction mixes. If you are setting up multiple reactions, we also recommend preparing a Master Mix that contains all reaction components that are present in each reaction. The volume of each Master Mix typically should be 110% of the volume needed for all the samples. *E.g.*, to prepare Master Mix 2 below for 20 reactions, make 550 µl of the mix (the extra volume allows for losses during pipetting).

Preparation of Master Mix 1

❶ ▪ Thaw the reagents and store on ice.
▪ Briefly vortex and centrifuge all reagents before setting up the reactions.

❷ Prepare a 10× conc. solution of each respective PCR primer.

🔍 If you are using, *e.g.,* a final concentration of 0.5 µM for each primer, the 10× conc. solution would contain a 5 µM concentration of the respective primer.

❸ Add the following reagents in a sterile 1.5 ml reaction tube on ice, in the following order:

Component	Volume	Final conc.
Water, PCR Grade	to make a final vol. up to 25 µl	
PCR Grade Nucleotide Mix (10 mM of each dNTP)	1 µl	200 µM (of each dNTP)
Downstream primer	5 µl	0.1 to 1 µM
Upstream primer	5 µl	0.1 to 1 µM
template DNA	variable	10 to 250 ng gDNA 0.1 to 15 ng cDNA
Final volume	**25 µl**	

❹ Mix and centrifuge briefly.

Preparation of Master Mix 2

❶ ▪ Thaw the reagents and store on ice.
▪ Briefly vortex and centrifuge all reagents before setting up the reactions.

❷ Add the following reagents in a 1.5 ml reaction tube on ice, in the following order:

Component	Volume	Final conc.
Water, PCR Grade	to make a final vol. up to 25 µl	
PCR reaction buffer, 10 ×	5 µl	
Taq DNA Polymerase, (1 U/µl)	0.5 to 2.5 µl	0.5 to 2.5 U/50 µl
Final volume	**25 µl**	

❸ Mix and centrifuge briefly.

PCR

❶ ▪ For each reaction, combine 25 µl Master Mix 1 and 25 µl Master Mix 2 in a thin-walled PCR tube on ice.
▪ Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.

⚠ Start thermal cycling immediately. Do not store complete reaction mixes on ice.

❷ Place your sample in a thermal block cycler and perform PCR.

❸ An example of a cycle profile is given for the Perkin Elmer GeneAmp 9600 Thermal Cycler.

⚠ When using other thermal block cyclers the cycle conditions have to be adjusted.

Run 30 cycles for DNA and 35 cycles for cDNA.

	Cycles	Time	Temp
Initial Denaturation	1	2 min	94°C*
Denaturation Annealing Elongation	25 to 30	15 to 30 sec 30 to 60 sec 45 sec to 3 min[2]	94°C 45 to 68°C[1] 72°C
Final Elongation	1	7 min	72°C
Cooling		indefinitely	4°C

[1] Annealing temperature depends on the melting temperature of the primers used.
[2] Elongation time depends on the length of the fragment to be amplified. Use 45 sec for targets up to 1 kb, use 1 min for fragments up to 1.5 kb and 2 min for fragments up to 3 kb.

❹ Samples can be stored at +2 to +8°C or -15 to -25°C.
After cycling, if the samples are not used immediately, store them frozen for later use.

🔍 For best results, do the following:
– Check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker.
– Purify the PCR product with the High Pure PCR Product Purification Kit* (*e.g.,* before performing nested PCR)

2.3 DIG DNA Labeling

Digoxigenin 11-dUTP* is incorporated into DNA by Taq DNA Polymerase. Please refer to Roche Applied Science DIG Kits, DIG Product Selection Guide or DIG Manuals for detailled protocols. For direct access please visit http://www.roche-applied-science.com/DIG.

3. Troubleshooting

	Possible Cause	Recommendation
Little or no PCR product	Difficult template, *e.g.,* GC-rich templates	▪ Perform PCR with GC-RICH PCR System*. ▪ Add DMSO (final concentration, 8%) and reduce enzyme concentration (*e.g.*, use as little as 0.5 U per reaction).
	DNA template problems	Check quality and concentration of template: ▪ Analyze an aliquot on an agarose gel to check for possible degradation. ▪ Test the template with an established primer pair or PCR system. ▪ Check or repeat template purification.
	Enzyme concentration too low	▪ Increase enzyme concentration to 2 U Taq DNA Polymerase per 50 µl reaction. ▪ If necessary, increase the amount of polymerase in 0.5 U steps.
	$MgCl_2$ concentration too low	Increase the $MgCl_2$ concentration in 0.25 mM steps. (The minimal acceptable concentration is 1.5 mM $MgCl_2$).
	Cycle conditions not optimal	▪ Decrease annealing temperature. ▪ Increase cycle number. ▪ Make sure that the final elongation step is included in the program.

	Possible Cause	Recommendation
	Primer design not optimal	Design alternative primers.
	Primer concentration not optimal	▪ Both primers must have the same concentration. ▪ Titrate primer concentration (0.1 to 1 μM).
	Primer quality or storage problems	▪ If you use an established primer pair, check performance in an established PCR system (*e.g.,* with a control template). ▪ Make sure that the primers are not degraded. ▪ Always store primers at –15 to –25°C.
	Formation of primer dimers	▪ Use two Master Mixes, as directed in the protocol above. ▪ Use FastStart Taq DNA Polymerase* instead of Taq DNA Polymerase.
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature (Longer primers have higher annealing temperatures).
	Primer design or concentration not optimal	▪ Review primer design. ▪ Titrate primer concentration (0.1 to 0.6 μM). ▪ Both primers must have the same concentration. ▪ Perform nested PCR with nested primers.
	Difficult template (*e.g.,* GC-rich template)	Perform PCR with GC-RICH PCR System*.
	DNA template problems	Use serial dilution of template.
PCR products in negative control experiments	Carryover contamination	▪ Replace all reagents, especially water. ▪ Use aerosol-resistant pipette tips. ▪ Set up PCR reactions in an area separate from that used for PCR product analysis. ▪ To eliminate carryover contaminants: Use dUTP* (600 μM) instead of dTTP (200 μM) and thermolabile UNG* (1 U/50 μl reaction); also, increase Mg^{2+} concentration (to a maximum of 4 mM) to compensate for higher dNTP conc.
Problems specific to RT-PCR	No product, additional bands, background smear	▪ The volume of cDNA template (RT-reaction) should not exceed 10% of the final volume of the PCR reaction. ▪ Follow troubleshooting tips above. ▪ Increase $MgCl_2$ in 0.25 mM steps.

4. Additional Information on this Product

Product Description

The enzyme was cloned in *E. coli* and is isolated to be free of unspecific endo- or exonucleases according to the current quality control procedures. Taq DNA Polymerase (1, 2) is a highly processive 5'-3' DNA polymerase that lacks 3'-5' exonuclease activity (3). It consists of a single polypeptide chain with a molecular weight of approximately 95 kDa.The enzyme exhibits highest activity at a pH of around 9 (adjusted at +20°C) and temperatures around +75°C. Taq DNA Polymerase also accepts modified deoxyribonucleosidetriphosphates as substrates, and can be used to label DNA-fragments either with radionucleotides, digoxigenin (see above), fluorescein or biotin (6,7).

The high processivity, absence of exonuclease activity and temperature optima of Taq DNA Polymerase enable the use of this enzyme in DNA sequencing, especially where the resolution of secondary structures plays a major role (8, 9).

Volume activity

1 U/μl as determined in the assay on activated DNA.

Origin

Taq DNA Polymerase (2, 3, 4) was originally isolated from the thermophilic eubacterium *Thermus aquaticus* BM, a strain lacking Taq I restriction endonuclease.

Unit Assay on Activated DNA

Incubation buffer: 67 mM Tris/HCl; pH 8.3 (+25°C), 5 mM $MgCl_2$, 10 mM Mercaptoethanol, 0.2% Polydocanol, 0.2 mg/ml Gelatine, 0.2 mM each dATP, dGTP, dTTP and 0.1 mM dCTP.

Incubation procedure: M13mp9ss, M13 primer (17mer) and 1 μCi [α^{32}P] dCTP are incubated with suitable dilutions of Taq DNA Polymerase in 50 μl incubation buffer for 60 min at +65°C. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Unit Definition

One unit Taq DNA Polymerase is defined as the amount of enzyme that incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA within 60 min at +65°C under the assay conditions stated above.

References

1 Baril, E. (1977) *Nucleic Acids Res.* **4**, 2641–2653.
2 Chien, A., D. B. & Trela, J. M. (1976) Deoxyribonucleic acid polymerase from the extreme Edgarthermophile Thermus aquaticus. *J. Bacteriol.* **127,** 1550-1557.
3 Kaledin, A. S., Slyusarenko, A. G. & Gorodetskii, S. I. (1980) *Biokhymia* **45**, 644– 651.
4 Lawyer, F. C. *et al.* (1989) Isolation, characterization and expression in Escherichia coli of the DNA polymerase gene from the extreme thermophile Thermus aquaticus. *J. Biol. Chem.* **264,** 6427-6437.
5 Tindall, K. R. & Kunkel, T. A. (1988) Fidelity of DNA synthesis by the Thermus aquaticus DNA polymerase. *Biochemistry* **27**, 6008- 6013.
6 Innis, M. A., *et al.* (1988) DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. *Proc. Natl. Acad. Sci. USA* **85,** 9436-9440.
7 Lo, Y.-M. D., Mehal, W. Z. & Fleming, K. A. (1988) Rapid production of vector-free biotinylated probes using the polymerase chain reaction. *Nucleic Acids Res.* **16**, 8719.
8 Taq polymerase: increased enzyme versatility in DNA sequencing (1988) Applied Biosystems.
9 Innis, M.A. *et al.* (1988) DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. *Proc. Natl. Acad. Sci. U S A.* **85,** 9436-9440.
10 Erlich, H. A. (ed.) (1989) PCR Technology: Principles and Application for DNA Amplification, Stockton Press, New York.

Quality Control

Each lot of Taq DNA Polymerase is tested for contaminating activities described in the following:

Test Buffer

10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3 (+20°C).

Absence of Endonucleases

1 µg lambda DNA is incubated with Taq DNA Polymerase in 50 µl test buffer for 16 hours at +37°C. The amount of enzyme showing no degradation of the lambda DNA is >20 U.

Absence of Nicking Activity

1 µg supercoiled pBR322 DNA is incubated with Taq DNA Polymerase in 50 µl test buffer for 4 hours at +37°C. The amount of enzyme showing no relaxation of supercoiled DNA is >20 U.

Absence of Exonucleases

Different amounts of Taq DNA Polymerase are incubated in 100 µl test buffer containing [^{3}H]-labeled DNA with a paraffin oil overlay for 4 hours at +65°C. The amount of enzyme that shows no exonuclease activity is >3 U.

5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Numbered Instructions labeled ①, ②, *etc.*	Steps in a process that usually occur in the order listed
Numbered Instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- Lot specific information is now included in the text and no longer shown in the label on the upper left-hand side of the Instructions for Use. Please refer to the Certificate of Analysis for more information.
- Update of License Disclaimer
- Editorial changes

5.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com and our Special Interest Sites including: http://www.roche-applied-science.com/PCR/

	Product	Pack Size	Cat. No.
Amplification ***e.g.,***	Taq DNA Polymerase (1 U/µl), dNTPack	250 U 1,000 U	04 738 225 001 04 738 241 001
	Taq DNA Polymerase, dNTPack	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	04 728 866 001 04 728 874 001 04 728 882 001 04 728 904 001 04 728 858 001
DNA Purification	High Pure PCR Template Purification Kit	100 purifications	11 796 828 001
	High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001
Additional Reagents	Digoxigenin-11-dUTP (alkali-labile)	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
	Digoxigenin-11-dUTP (alkali-stable)	25 nmol (25 µl)	11 093 088 910
	Biotin-16-dUTP	50 nmol (50 µl)	11 093 070 910
	Fluorescein-12-dUTP	25 nmol (25 µl)	11 373 242 910
	Water, PCR Grade	25 ml (25 × 1 ml) 25 ml (1 × 25 ml) 100 ml (4 × 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
	DNA, MB-grade (from fish sperm)	500 mg (50 ml)	11 467 140 001

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Trademarks

FASTSTART and HIGH PURE are Trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

License Disclaimer

NOTICE: This product may be subject to certain use restrictions. Before using this product please refer to the Online Technical Support page (http://technical-support.roche.com) and search under the product number or the product name, whether this product is subject to a license disclaimer containing use restrictions.

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Instructions for Use and Material Safety Data Sheets.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany