For general laboratory use.
FOR *IN VITRO* USE ONLY.

Taq DNA Polymerase

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

Cat. No. 1 146 165	100 U
Cat. No. 1 146 173	500 U
Cat. No. 1 418 432	4 × 250 U
Cat. No. 1 596 594	10 × 250 U
Cat. No. 1 435 094	20 × 250 U

Version 11, Jan. 2003

Store at −15 to −25° C

Product overview

Pack content

Vial	Content
Taq DNA Polymerase (5 U/µl)	• 20 µl (100 U pack size) • 100 µl (500 U pack size) • 4 × 50 µl (4 x 250 U pack size) • 10 × 50 µl (10 x 250 U pack size) • 20 × 50 µl (20 x 250 U pack size) Enzyme storage buffer: 20 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1 M KCl, 0.5% Nonidet P40 (v/v), 0.5% Tween20 (V/V), 50% glycerol (v/v), pH 8.0 (4°C)
PCR reaction buffer, 10× conc., (with $MgCl_2$)	• 1 ml (100 U pack size) • 3 × 1 ml (500 U pack size) • 6 × 1 ml (4 x 250 U pack size) • 15 × 1 ml (10 x 250 U pack size) • 30 × 1 ml (20 x 250 U pack size) Buffer composition (10 x conc.): 100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (20°C)

Product Description

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 approx. 95 kD.

Taq DNA Polymerase was originally isolated from the thermophilic eubacterium *Thermus aquaticus* BM, a strain lacking Taq I restriction endonuclease. The enzyme was cloned in *E. coli* and is isolated to be free of unspecific endo-or exonucleases.

Enzyme properties

Volume activity	5 U/µl
Error rate*	approx. 1.3×10^{-5}
Optimal enzyme concentration	varies from 0.5-2.5 U per 50 µl reaction
Standard enzyme concentration	1.25 U per 50 µl reaction
Optimal pH	around 9 (adjusted at 20°C)
Optimal elongation temperature	around 72°C
Optimal Mg^{2+} concentration	varies form 1.5 - 5 mM (as $MgCl_2$)
Standard Mg^{2+} concentration	1.5 mM (as $MgCl_2$) when using 200 µM dNTP each
PCR product size	optimized for up to 3 kb. PCR possible up to 10 kb with yield diminishing as DNA fragment length increases
PCR Cloning	TA-cloning; addition of A-overhangs
Incorporation of modified nucleotides	accepts modified nucleotides like radiolabeled nucleotides, DIG-dUTP, biotin-dUTP
Prevention of carry-over contamination	yes
Thermostability	Over 80% activity after 30 cycles (1 min 95°C, 1 min 37°C, 3 min 72°C)

* Relative fidelity determined by the lacI assay (4).

Storage and stability

Stable at −15 to −25°C until the control date printed on the label.

Applications

- Polymerase Chain Reaction (PCR)
- DNA labeling reactions (5-6)
- Sequencing/ cycle sequencing (5,7)

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 (8). 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 (2-3) when using 200 µM dNTP (each).
- 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 buffer for the template DNA is either simply sterile double-distilled water or 5-10 mM Tris (pH 7-8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.

Preparation of reaction mixes

For a larger number of reactions, we recommend that you prepare two reaction mixes. This circumvents the need of hot start and avoids that the enzyme interacts with primers or template during the reaction set-up.

It is also recommended to prepare a Master Mix for setting up multiple reactions. The Master Mix typically contains all of the components needed for all PCR tests to be performed at a volume 10% greater than that required for the total number of PCR assays.

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Preparation of reaction mixes

Step	Action
1	Briefly centrifuge all reagents before starting.
2	• Prepare two mixes of reagents in sterile microfuge tubes (on ice): • **Mix 1** (for one reaction)

Reagents	Volume	Final conc.
sterile double-dist. water	add up to 25 µl	
dNTP mix (10 mM)	1 µl	200 µM (of each dNTP)
Upstream primer	variable	0.1 – 0.6 µM
Down-stream primer	variable	0.1 – 0.6 µM
Template DNA	variable	0.1 – 250 ng[a]
Final vol.	**25 µl**	

• **Mix 2** (for one reaction)

Reagents	Volume	Final conc.
sterile double-distilled water	19.75 µl	
PCR reaction buffer, 10 x conc	5 µl	1 x (1.5 mM $MgCl_2$)
Taq DNA Polymerase, 5 U/µl	0.25 µl	1.25 U/ reaction
Final volume	**25 µl**	

3	• Combine Mix 1 and 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 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.

[a] E.g. human genomic DNA template: 10 ng-250 ng; plasmid DNA template: 0.1 ng-15 ng.

Thermal cycling

1. Place samples in the thermal cycler, and start cycling using the thermal profiles mentioned below.

• **Thermal profile A:** single extension time

	Temp.	Time	Cycle No.
Initial denaturation	94°C[b]	2 min	1 x
Denaturation Annealing Elongation	94°C[b] 55°C-65°C[c] 72°C[d]	15 s – 30 s 30 s – 60 s 45 s – 3 min	25 – 30 x
Final elongation	72°C[d]	7 min	1 x
Cooling	4°C	unlimited time	

• **Thermal profile B:** gradually increasing extension time; this procedure ensures a higher yield of amplification products.

	Temp.	Time	Cycle No.
Initial denaturation	94°C[b]	2 min	1 x
Denaturation Annealing Elongation	94°C [b](55°C-65°C[c] 72°C[d]	15 s – 30 s 30 s – 60 s 45 s – 3 min	10 x
Denaturation Annealing Elongation	94°C[b] 50°C-65°C[c] 72°C[d]	15 s – 30 s 30 s 45 s – 3 min + 5 s cycle elongation for each succ. cycle[e]	15 – 20 x
Final elongation	72°C[d]	7 min	1 x
Cooling	4°C	unlimited time	

[b] The denaturation temperature can vary from 92°C-95°C. 94°C is the standard denaturation temperature.

[c] Optimal annealing temperature depends on the melting temperature of the primers and on the system used.

[d] 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.

[e] For example, cycle no. 11 is 5 s longer than cycle 10, cycle no. 12 is 10 s longer than cycle 10, cycle no. 13 is 15 s longer than cycle 10, etc.

2. After cycling, the samples may be frozen for later use. Possible further procedures:

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

• Purify the PCR product with the High Pure PCR Product Purification Kit (Cat. No. 1 732 676), e.g. before performing nested PCR.

Trouble shooting

Little or no PCR product

Possible cause	Recommendation
Difficult template e.g. GC-rich templates	• Use the GC-RICH PCR System. • Add DMSO (at 8%) and titrate enzyme concentration down to 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. • 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 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 steps of 0.25 mM. (1.5 mM $MgCl_2$ is the minimal concentration.)
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.
Formation of primer dimers	• Use FastStart Taq DNA Polymerase. • Use two reaction mixes according to the protocol above.

Multiple bands or background smear

Possible cause	Recommendation
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 – 0.6 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 solution.
DNA template problems	Use serial dilution of template.

PCR products in negative control experiments

Possible cause	Recommendation
Carry-over contamination	• Exchange all reagents, especially water. • Use aerosol-resistant pipette tips. • Set up PCR reactions in an area separate from that used for PCR product analysis. • Use dUTP (600 µM) instead of dTTP (200 µM) in combination with thermolabile UNG (1 U/ 50 µl reaction) and increase Mg^{2+} concentration up to 4 mM at most.

Specific problems in RT-PCR application

Possible cause	Recommendation
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 trouble shooting above. • Increase $MgCl_2$ by titration in steps of 0.25 mM.

Unit assay

Unit definition

One unit Taq DNA polymerase is defined as the amount of enzyme that incorporates 20 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA within 60 min at 65°C under the assay conditions stated below.

Unit assay: assay on activated DNA

Incubation buffer for assay on activated DNA: 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, pH 8.3 (25°C).

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 at 65°C for 60 min. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Quality control

Each lot of Taq DNA polymerase is tested for contaminating activities as 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 at 65°C for 16 h. The amount of enzyme showing no degradation of the lambda DNA is stated under "Endo 1".

Absence of endonucleases

1 µg *Eco* RI/*Hind* III-fragments of lambda DNA is incubated with Taq DNA polymerase in 50 µl test buffer at 65°C for 16 h. The amount of enzyme showing no alteration of the banding pattern is stated under "Endo 2".

Absence of "nicking activity"

1 µg supercoiled pBR322 DNA is incubated with Taq DNA polymerase in 50 µl test buffer at 65°C for 16 h. The amount of enzyme showing no alteration of the no relaxation of supercoiled DNA is stated under "Nick.Act.".

Absence of exonucleases

Different amounts of Taq DNA polymerase are incubated in 100 µl test buffer containing (^{3}H)-labeled DNA at 65°C for 4 h. The amount of enzyme showing no exonuclease-activity is stated under "Exo".

References

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5 Innis, M.A. et al. (1988) *Proc. Natl. Acad. Sci USA* **85**, 9436-9440.
6 Lo, Y.-M.D., Mehal, W.Z. and Fleming, K.A. (1988) *Nucleic Acids Res.* **16**, 8719.
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Related Products

Roche Applied Science offers a large selection of enzymes, reagents, and systems for PCR and RT-PCR assays. For a complete overview of our products and for more detailed information on PCR and RT-PCR please visit and bookmark our Amplification Special Interest Site at http://www.roche-applied-science.com/PCR.

PCR Product Selection Guide:

Application	Product Size [kb]	Recommended Product	Pack size	Cat. No.
Standard PCR	≤ 3	Taq DNA Polymerase	100 U 500 U 4x250 U 10x250 U 20x250 U	1 146 165 1 146 173 1 418 432 1 596 594 1 435 094
	≤ 5	Expand High Fidelity PCR System	100 U 2x250 U 10x250 U	1 732 641 1 732 650 1 759 078
High Specificity PCR	≤ 3	FastStart Taq DNA Polymerase (for hot start PCR)	50 U 100 U 500 U 4x250 U 10x250 U 20x250 U	2 158 264 2 032 902 2 032 929 2 032 937 2 032 945 2 032 953
High Fidelity PCR	≤ 3	Tgo DNA Polymerase	50 react. 100 react. 250 react.	3 186 172 3 186 202 3 186 199
	≤ 5	Expand High Fidelity PCR System	100 U 2x250 U 10x250 U	1 732 641 1 732 650 1 759 078
Long Template PCR	5 – 20	Expand Long Template PCR System	100 U 2x250 U 10x250 U	1 681 834 1 681 842 1 759 060
	20 – 35	Expand 20 kb ^{PLUS} PCR System	100 U	1 811 002
Difficult DNA Template PCR	≤ 5	GC RICH PCR System	100 U	2 140 306
	5 – 20	Expand Long Template PCR System	100 U 2x250 U 10x250 U	1 681 834 1 681 842 1 759 060

PCR Nucleotide Selection:

Product	Description	Pack size	Cat. No.
Set of Deoxy-Nucleotides, PCR Grade	Separate vials of dATP, dCTP, dGTP, and dTTP. 100 mM each	4 x 25 µmol (250 µl)	1 969 064
PCR Nucleotide Mix	Premixed ready-to-use solution of PCR Grade dATP, dCTP, dGTP, and dTTP. 10 mM.	200 µl 10 x 200 µl	1 581 295 1 814 362
PCR Nucleotide Mix^{PLUS}	Premixed ready-to-use solution of PCR Grade dATP, dGTP, dTTP (each 10 mM), and dUTP (30 mM)	2 x 100 µl	1 888 412 1 814 362

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Roche Diagnostics GmbH
Roche Applied Science
Nonnenwald 2
82372 Penzberg
Germany