Taq DNA Polymerase, GMP Grade

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

Cat. No. 03 734 927 001 1,000 U

Version 07
Content version: September 2019
Store at −15 to −25°C

1. What this Product Does

Contents

Vial	Label	Contents / Function Cat. No. 03 734 927 001
1	Taq DNA Polymerase, GMP Grade (5 U/µl)	• 200 µl (1,000 U)
2	PCR buffer with $MgCl_2$ (10× conc.)	• 2 × 5 ml (1,000 U) • [100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C)]

Storage and Stability

If stored at −15 to −25°C, the product is stable until the expiration date printed on the label.

The kit is shipped on dry ice.

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% Tween 20 (v/v), 50% glycerol (v/v), pH 8.0 (+4°C)

Application

Taq DNA Polymerase, GMP Grade is used for the amplification of DNA fragments by the polymerase chain reaction (PCR) or RNA fragments by RT-PCR. Taq DNA Polymerase also accepts modified deoxyribonucleoside triphosphates as substrates for highly efficient DNA labeling using radionucleotides, digoxigenin, fluorescein, or biotin. Taq DNA Polymerase, GMP Grade can further be applied for primer extension and cycle sequencing.

Additional Equipment and Reagents Required

Additional equipment and reagents required to perform PCR reactions with Taq DNA Polymerase, GMP Grade, but not provided, include:

- standard laboratory equipment
  - nuclease-free, aerosol-resistant pipette tips
  - pipettes with disposable, positive-displacement tips
  - sterile reaction tubes for preparing PCR mixes and dilutions
  - standard benchtop microcentrifuge
- for the PCR reaction
  - thermal block cycler (*e.g.,* the Applied Biosystems GeneAmp PCR System 9600)
  - PCR primers
  - template DNA
  - PCR reaction vessels (thin-walled PCR tubes or plates are recommended)
  - PCR Nucleotide Mix, PCR Grade*
  - Water, PCR Grade*

** available from Roche Diagnostics*

Enzyme Characteristics

Origin	*Thermus aquaticus* BM, recombinant in *E.coli*
Appearance	clear, colorless solution
Purity	> 98% (SDS-PAGE)
Specific activity	≥ 130,000 U/mg
Ribonucleases	not detectable
Endonucleases	not detectable
Nicking activity	not detectable
Bioburden	≦ 50 cfu/ml
Animal-derived additives	none
Optimal enzyme concentration	0.5 to 2.5 U/50 µl reaction
Typical enzyme concentration	1.25 U/50 µl reaction
Optimal pH	≈ 9 (+20°C)
Optimal elongation temperature	≈ +72°C
Optimal Mg^{2+} concentration	1.5 mM (when using 200 µM dNTP each)
PCR product size	up to 3 kb
PCR cloning method	TA cloning
Thermostability	Enzyme retains over 80% activity after 30 cycles (1 min +95°C, 1 min +37°C, 3 min +72°C).
Incorporation of modified nulceotides	yes (*e.g.,* DIG-dUTP, Biotin-dUTP)
Prevention of carryover contamination	yes

2. How to Use this Product

2.1 Before You Begin

General Considerations

The optimal conditions (incubation times and temperatures, concentrations of enzyme, template DNA, Mg^{2+}) are system dependend 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 to 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's. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. Increase concentration of Mg^{2+} when increasing the concentration of dNTPs.

Sample Material

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids use:
  - either the MagNA Pure 96 Instrument, the MagNA Pure LC Instrument, or the MagNA Pure Compact Instrument together with a dedicated nucleic acid isolation kit (for automated isolation)
  - or a HIGH PURE nucleic acid isolation kit (for manual isolation)
- Use 10 to 500 ng complex genomic DNA or 0.1 to 10 ng plasmid DNA/cDNA. Recommended starting concentrations are 250 ng genomic DNA or 1 ng plasmid DNA.

⚠ Store the template DNA either in Water, PCR Grade or 5 to 10 mM Tris-HCl (pH 7 to 8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.

2.2 Procedure

Preparation of PCR Master Mixes

Prepare two PCR master mixes: the first one contains enzyme and reaction buffer, the second one contains all other reaction components. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template during the reaction setup.

If you are setting up multiple reactions, the volume of each master mix should be 110% of the volume needed for all your samples (*e.g.,* to prepare Master Mix 2 below for 10 reactions, make 275 µl of the mix). The extra volume allows for losses during pipetting.

Preparation of Master Mix 1

Step	Action
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 the PCR primers. 🔍 If you want to achieve for each primer, *e.g.,* a final concentration of 0.5 µM, the 10× conc. solution should contain 5 µM of each primer.
❸	For each 50 µl reaction, add the components in the order listed below to a sterile reaction tube on ice:

Reagent	Volume	Final Concentration
Water, PCR Grade	add up to 25 µl	
PCR primer mix (10×)	5 µl	0.1 to 0.6 µM
PCR Nucleotide Mix, PCR-grade (10 mM)	1 µl	0.2 mM
template DNA	variable	0.01 to 500 ng
Total Volume	**25 µl**	

Step	Action
❹	Mix gently and centrifuge briefly.

Preparation of Master Mix 2

Step	Action
❶	• Thaw the reagents and store on ice. • Briefly vortex and centrifuge all reagents before setting up the reactions.
❷	• To a sterile reaction tube on ice, add the components in the order listed below: (For each 50 µl reaction)

Reagent	Volume	Final Concentration
Water, PCR Grade	19.75 µl	
Taq DNA Polymerase buffer (10×)	5 µl	1× (1.5 mM Mg^{2+})
Taq DNA Polymerase (5 U/µl)	0.25 µl	1.25 U
Total Volume	**25 µl**	

Step	Action
❸	Mix gently and centrifuge briefly.

2.3 PCR

🔍 The thermal profiles were developed for the Applied Biosystems GeneAmp PCR System 9600. Other thermal cyclers may require different profiles.

Step	Action
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 samples in a thermal cycler and use either of the thermal profiles below to perform PCR.

Thermal profile A: fixed elongation time

Program	Cycles	Time	Temp
Initial denaturation	1×	2 min	92 to 95°C [a]
Denaturation Annealing Elongation	25 to 30×	15 to 30 sec 30 to 60 sec 45 sec to 3 min	92 to 95°C [a] 55 to 65°C [b] 72°C
Final elongation	1×	7 min	72°C

Thermal profile B: gradually increasing extension time

🔍 This procedure ensures a higher yield of amplification products.

Program	Cycles	Time	Temp
Initial denaturation	1×	2 min	92 to 95°C [a]
Denaturation Annealing Elongation	10×	15 to 30 sec 30 to 60 sec 45 sec to 3 min	92 to 95°C [a] 55 to 65°C [b] 72°C
Denaturation Annealing Elongation	15 to 20×	15 to 30 sec 30 to 60 sec 45 sec to 3 min + 5 sec cycle elongation for each succ. cycle [c]	92 to 95°C [a] 55 to 65°C [b] 72°C
Final elongation	1×	7 min	72°C
Cooling		indefinitely	4°C

[a] The denaturation temperature can vary between +92°C and +95°C. The standard denaturation temperature is +94°C.

[b] Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system. 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.

[c] For example, cycle no. 11 is 5 sec longer than cycle 10, cycle no. 12 is 10 sec longer than cycle 10, cycle no. 13 is 15 sec longer than cycle 10, *etc.*

Step	Action
❸	After cycling, if the samples are not used immediately, store them frozen for later use.
	For best result, 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).

3. Troubleshooting

Problem	Possible Cause	Recommendation
Little or no PCR product	Pipetting errors	Check all concentrations and storage conditions of reagents.
	Difficult template (*e.g.,* GC-rich)	• Use the GC-RICH PCR System*. • Add DMSO (at 8%) and titrate enzyme concentration down to 0.5 U/reaction.
	DNA template problems	• Analyze an aliquot of the template on an agarose gel to check for possible degradation. • Perform a control reaction on template using an established primer pair. • Check or repeat purification of template.
	Enzyme concentration too low	Increase enzyme concentration (in 0.5 U steps) to 2 U/50 µl reaction.
	$MgCl_2$ concentration too low	Increase the $MgCl_2$ concentration in steps of 0.25 mM.
	Cycle conditions not optimal	• Decrease annealing temperature. • Increase cycle number. • Make sure the final elongation step is carried out.
Primer problems	Primer design	Design alternative primers
	Primer concentration	• Make sure both primers have the same concentration. • Titrate primer concentration.
	Annealing temperature too high	• Reduce annealing temperature. • Determine the optimal annealing temperature by touch-down PCR.
	Primer specificity	• Perform nested PCR.
	Quality or storage	• If you use an established primer pair, check performance on a control template. • Make sure the primers are not degraded. • Always store primers at −15 to −25°C.
	Formation of primer-dimers	• Use two reaction mixes according to the protocol above. • Check primer design. • Use FastStart Taq DNA Polymerase*.
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature according to the primer length.
	Primer design or concentration	• Check primer design. • Titrate primer concentration (0.1 to 0.6 µM) • Make sure both primers have the same concentration. • Perform nested PCR.
	Difficult template (*e.g.,* GC-rich)	• Use the GC-RICH PCR System*. • Add DMSO (at 8%) and titrate enzyme concentration down to 0.5 U/reaction.
	DNA template	Use serial dilutions of template.
PCR products in negative control experiments	Carryover contamination	• Exchange all reagents, especially water. • Use aerosol resistant tips. • Set up PCR reactions in an area separated from that used for PCR product analysis. • Use dUTP (600 µM) instead of dTTP in combination with thermolabile UNG* (1 U/50 µl reaction) and increase Mg^{2+} concentration to a maximum of 4 mM.
Specific problems in RT-PCR application	No product, additional bands, background smear	• The volume of unpurified cDNA template should not exceed 1/10th of the PCR reaction. • Increase $MgCl_2$ by titration in steps of 0.25 mM. • Follow the troubleshooting hints above.

4. Additional Information on this Product

How this Product Works

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 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 purified and manufactured under strict GMP regulations.

Background Information

Roche Applied Science produces Taq DNA Polymerase, GMP Grade, under strict enforcement of Good Manufacturing Practice (GMP) guidelines. Good Manufacturing Practice is concerned with overall perspective of quality control. The general GMP rules are valid for regulated products. Manufacturers of raw materials are also encouraged to follow these guidelines. What we at Roche Applied Science established from these guidelines is as follows:

- Raw materials must meet high quality standards and regulatory demands.
- All manufacturing processes are clearly defined, systematically reviewed and shown to be capable of consistently manufacturing products of the required quality and complying with their specifications, and documented via a sophisticated computerized document management system.
- Materials having direct contact with the product are sterilized and used only once. Equipment in direct contact with the product are dedicated for a single product or parameter.
- Employment of a strict “one room, one product” policy in an established area specifically dedicated to the production of enzymes for molecular biology.
- The production areas are access controlled and class 100 000 with reference to the non-viable particle counts (> 0.5 µ/cft) and less than 200 cfu/m^3. The filling of bulk solutions takes place in a laminar flow box (class 100).
- Defined flow of personnel, material and equipment.
- Manufacturing processes and significant changes to the process are validated.

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 1 hour at +65°C under the assay conditions stated below.

- Incubation buffer for assay on activated DNA (9): 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 1 hour. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Quality Control

Taq DNA Polymerase, GMP Grade is tested for PCR performance in combination with supplied buffer, as well as contaminating activities, as described in the following.

Absence of endonucleases	1 µg λ DNA is incubated with Taq DNA Polymerase in 50 µl test buffer at +37°C for 16 hours. The amount of enzyme showing no degradation of λ DNA is 30 U.
Absence of nicking activity	1 µg supercoiled pBR322 DNA is incubated with Taq DNA Polymerase in 50 µl test buffer at +37°C for 16 hours. The amount of enzyme showing no relaxation of supercoiled DNA is 30 U.

Absence of exonucleases	1 µl of Taq DNA Polymerase is incubated in 29 µl test buffer containing an unlabeled DNA standard at +37°C for 1 hour. The amount of enzyme showing no exonuclease activity is 130 U.
Bioburden	Each lot of enzyme is tested for the total number of viable aerobic microorganisms. The number of colony forming units is determined using either the membrane filtration or the plate-count method.
PCR performance test 1	Each lot is tested in a functional test using a conventional block cycler. A 0.5 kb fragment is amplified from λ DNA (0.01 ng).
PCR performance test 2	Each lot of enzyme is tested in a LightCycler® assay. A fragment of the β-globin gene from human genomic DNA is amplified with a sensitivity of 30 pg.
PCR performance test 3	Each lot of enzyme is tested in a LightCycler® assay. A fragment of the cloned β-globin gene is amplified from a plasmid preparation with a sensitivity of 10^4 copies.

References

1 Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550-1557.
2 Lawyer, F.C. *et al.* (1989) J. Biol. Chem. 264, 6427-6437.
3 Tindall, K.R. and Kunkel, T.A. (1988) Biochemistry 27, 6008-6013.
4 Frey, B. and Suppmann, B. (1995) Biochemica 2, 8-9.
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.
7 Taq Polymerase: increased enzyme versatility in DNA sequencing (1988) Applied Biosystems.
8 Erlich, H.A. (ed.) (1989) PCR Technology: Principles and Application for DNA Amplification, Stockton Press, New York.

5. Supplementary Information

5.1 Conventions

Text Conventions

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

Symbol	Description
Numbered stages labeled ①, ②, *etc.*	Stages 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 Diagnostics

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.

Changes to previous version

- Editorial changes.
- Update of the quality control procedure used for determining the absence of exonucleases.

Abbreviations

In this document, the following abbreviations are used:

Abbreviation	Meaning
GMP	Good Manufacturing Practice
UNG	Uracil-DNA Glycosylase

5.2 Ordering Information

Product	Pack Size	Cat No.
PCR Nucleotide Mix	200 µl	11 581 295 001
	10 × 200 µl	11 814 362 001
Water, PCR Grade	25 ml (25 ×1 ml)	03 315 932 001
	25 ml (1 × 25 ml)	03 315 959 001
	100 ml (4 × 25 ml)	03 315 843 001

5.3 Trademarks

5.4 License Disclaimer

For patent license limitations for individual products please refer to: List of biochemical reagent products

5.5 Regulatory Disclaimer

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

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany