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

Taq DNA Polymerase, GMP Grade
from *Thermus aquaticus* BM, recombinant (*E. coli*)

Version: 08
Content Version: November 2020

Deoxynucleoside-triphosphate: DNA deoxnucleotidyltransferase

Cat. No. 03 734 927 001

1,000 U
5 U/µl
2,000 reactions of 20 µl final volume each containing 0.5 U Taq DNA Polymerase

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Taq DNA Polymerase, GMP Grade	▪ 5 U/µl ▪ Enzyme storage buffer: 20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1 M KCl, 0.5% Nonidet P-40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v), pH 8.0 (+4°C). ▪ Clear, colorless solution ▪ No animal-derived additives.	1 vial, 200 µl
2	Taq DNA Polymerase, GMP Grade, PCR Buffer with $MgCl_2$, 10x conc.	100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C)	2 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Taq DNA Polymerase, GMP Grade	Store at −15 to −25°C.
2	PCR Buffer with $MgCl_2$, 10x conc.	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For PCR

- PCR primers
- Template DNA
- PCR Nucleotide Mix, PCR Grade*
- Water, PCR Grade*
- High Pure PCR Product Purification Kit*

1.4. Application

Taq DNA Polymerase, GMP Grade is used in a variety of techniques:

- Amplification of DNA fragments by PCR or RNA fragments by RT-PCR.
- The polymerase accepts modified deoxyribonucleoside triphosphates as substrates for highly efficient DNA labeling using radionucleotides, digoxigenin, fluorescein, or biotin.
- Primer extension
- Cycle sequencing

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as genomic, plasmid, or cDNA suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent 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.

A good starting concentration is 250 ng genomic DNA or 1 ng plasmid DNA.

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

Mg2+ Concentration

1.5 to 5 mM (as $MgCl_2$) (optimal)
1.5 mM (as $MgCl_2$) when used with 200 µM of each dNTP (standard)

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg^{2+} vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg^{2+} concentration and the amount of enzyme used per assay to ensure 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/50 µl will usually produce satisfactory results.
- Optimal Mg^{2+} concentration can vary between 1.5 mM and 5 mM. In most cases, a Mg^{2+} concentration of 1.5 mM will produce satisfactory results if you use 200 µM of each dNTP.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. If you increase the dNTP concentration, you must also increase the Mg^{2+} concentration.

Good Manufacturing Practice

Taq DNA Polymerase, GMP Grade is produced 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:

- 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 <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.

Prevention of Carryover Contamination

Yes

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

Preparation of PCR master mixes

Prepare two PCR master mixes. Master Mix 2 contains enzyme and reaction buffer; Master Mix 1 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 the samples. For example, to prepare Master Mix 2 for 10 reactions, make 275 µl of the mix. The extra volume allows for losses during pipetting.

Preparation of master mix 1

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

2. Prepare a 10x-concentrated solution of each respective primer.
   If you are using, for example, the final concentration of 0.5 µM for each primer, the 10x-concentrated solution would contain a 5 µM concentration of the respective primer.

3. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to a final volume of 25	–
PCR primer mix, 10x conc.	5	0.1 – 0.6 µM
PCR Grade Nucleotide Mix* (10 mM of each dNTP)	1	200 µM of each dNTP
Template DNA	variable	0.01 – 500 ng
Final Volume	**25**	

4. Mix and centrifuge briefly.

Preparation of master mix 2

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

2. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	19.75	–
Taq DNA Polymerase buffer, 10x conc.	5	1x (1.5 mM Mg^{2+})
Taq DNA Polymerase (5 U/µl)	0.25	1.25 U
Final Volume	**25**	

3. Mix and centrifuge briefly.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

1. 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 the combined reaction mix on ice.***

2. Place your samples in a thermal block cycler and perform PCR.
   Thermal Profile A has a fixed extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 95[1]	2 min	1
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min	25 – 30
Final Elongation	72	7 min	1
Cooling	4	indefinitely	

Thermal Profile B has a gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 95[1]	2 min	1
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min	10
Denaturation Annealing Elongation	92 – 95[1] 55 – 65[2] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min + 5 sec cycle elongation for each successive cycle[3]	15 – 20
Final Elongation	72	7 min	1
Cooling	4	indefinitely	

3. After cycling, use samples immediately or store them frozen for later use.

For best results, check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker. In addition, purify the PCR product with the High Pure PCR Product Purification Kit, for example, before performing nested PCR.*

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

[2] 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 approximately +72°C; for PCR products >1 kb, elongation temperature should be approximately +68°C.

[3] For example, cycle number 11 is 5 seconds longer than cycle 10. Cycle number 12 is 10 seconds longer than cycle 10. Cycle number 13 is 15 seconds longer than cycle 10, etc.

2.3. Parameters

Bioburden

≤50 cfu/ml

EC-Number

EC 2.7.7.7

Incorporation of Modified Nucleotides

DIG-dUTP, Biotin-dUTP, etc.

Maximum Fragment Size

Amplifies up to 3 kb products.

Molecular Weight

Approximately 95 kD.

PCR Cloning

TA cloning

pH Optimum

Approximately 9 (+20°C).

Purity

>98% (SDS-PAGE)

Specific Activity

≥130,000 U/mg

Temperature Optimum

Approximately +72°C (elongation).
Optimal elongation temperature

Temperature Stability

Enzyme retains over 80% activity after 30 cycles (1 minute +95°C, 1 minute +37°C, 3 minutes +72°C).

Unit Assay

Unit assay on activated DNA

Incubation buffer

67 mM Tris-HCl; pH 8.3 (+25°C), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.2% polydocanol, 0.2 mg/ml gelatin, 0.2 mM each dATP, dGTP, dTTP, and 0.1 mM dCTP, pH 8.3 (+25°C).

Incubation procedure

1. 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 minutes.

2. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Unit Definition

One unit of Taq DNA Polymerase is defined as the amount of enzyme that incorporates 20 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA within 60 minutes at +65°C under the described assay conditions.

Working Concentration

0.5 to 2.5 U per 50 µl reaction (optimal).
1.25 U per 50 µl reaction (standard).

3. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Pipetting errors	Check all concentrations and storage conditions of reagents.
	Difficult templates, such as GC-rich templates.	Perform PCR with the GC-RICH PCR System*.
		Add DMSO (final concentration, 8%), and reduce enzyme concentration, for example, 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. ▪ Test the template with an established primer pair or PCR system. ▪ Check or repeat template purification.
	Enzyme concentration too low.	Increase enzyme concentration in 0.5 U steps to 2 U Taq DNA Polymerase, GMP Grade per 50 µl reaction.
	$MgCl_2$ 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.
Problem with primers.	Primer design not optimal.	Design alternative primers.
	Primer concentration not optimal.	Both primers must have the same concentration.
		Titrate primer concentration.
	Annealing temperature too high.	Reduce annealing temperature.
		Determine the optimal annealing temperature using touchdown PCR.
	Primer specificity not optimal.	Perform nested PCR.
	Primer quality or storage problems.	If you use an established primer pair, check performance on a control template.
		Make sure that primers are not degraded.
		Always store primers at −15 to −25°C.
	Formation of primer-dimers.	Use two master mixes, as described in the protocol.
		Use FastStart Taq DNA Polymerase* instead of Taq DNA Polymerase, GMP Grade.
		Check primer design.
Multiple bands or background smear.	Annealing temperature too low.	Increase annealing temperature according to primer length.
	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 templates, such as GC-rich templates.	Perform PCR with the GC-RICH PCR System*.
		Add DMSO (final concentration, 8%), and reduce enzyme concentration, for example, down to 0.5 U per reaction.
	DNA template problems.	Use serial dilution of template.

PCR products in negative control experiments.	Carryover contamination present.	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 Uracil-DNA Glycosylase* (1 U/50 µl reaction). ▪ Increase Mg^{2+} concentration to a maximum of 4 mM to compensate for higher dNTP concentration.
Problems specific to RT-PCR.	No product, additional bands, background smear observed.	The volume of unpurified cDNA template (RT reaction) should not exceed 10% of the final volume of the PCR reaction.
		Follow all troubleshooting tips.
		Increase $MgCl_2$ in 0.25 mM steps.

4. Additional Information on this Product

4.1. Test Principle

Taq DNA Polymerase is a highly processive 5′→3′ DNA polymerase that lacks 3′→5′ exonuclease activity. It is 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 manufactured under strict GMP regulations.

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
FastStart Taq DNA Polymerase, 5 U/µl	100 U, 1 x 100 U, 50 reactions in a final volume of 50 µl	12 032 902 001
	500 U, 2 x 250 U, 250 reactions in a final volume of 50 µl	12 032 929 001
	1,000 U, 4 x 250 U, 500 reactions in a final volume of 50 µl	12 032 937 001
	2,500 U, 10 x 250 U, 1,250 reactions in a final volume of 50 µl	12 032 945 001
	5,000 U, 20 x 250 U, 2,500 reactions in a final volume of 50 µl	12 032 953 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001

5.4. Trademarks

FASTSTART and MAGNA PURE are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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.

1120.04445872001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany