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

Taq DNA Polymerase, 5 U/µl, dNTPack

from *Thermus aquaticus* BM, recombinant (E. *coli*)

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Taq DNA Polymerase with ready-to-use PCR Grade Nucleotide Mix.

Cat. No. 04 728 866 001	100 U 5 U/µl 80 reactions in a final volume of 50 µl
Cat. No. 04 728 874 001	500 U 2 x 250 U 400 reactions in a final volume of 50 µl
Cat. No. 04 728 882 001	1,000 U 4 x 250 U 800 reactions in a final volume of 50 µl
Cat. No. 04 728 904 001	2,500 U 10 x 250 U 2,000 reactions in a final volume of 50 µl
Cat. No. 04 728 858 001	5,000 U 20 x 250 U 4,000 reactions in a final volume of 50 µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Taq DNA Polymerase	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).	04 728 866 001	1 vial, 20 µl
			04 728 874 001	2 vials, 50 µl each
			04 728 882 001	4 vials, 50 µl each
			04 728 904 001	10 vials, 50 µl each
			04 728 858 001	20 vials, 50 µl each
2	PCR reaction buffer, 10x conc.	Buffer composition: 100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C).	04 728 866 001	1 vial, 1 ml
			04 728 874 001	3 vials, 1 ml each
			04 728 882 001	6 vials, 1 ml each
			04 728 904 001	15 vials, 1 ml each
			04 728 858 001	30 vials, 1 ml each
3	PCR Grade Nucleotide Mix	Ready-to-use 10 mM dNTP solution.	04 728 866 001	1 vial, 200 µl
			04 728 874 001	2 vials, 200 µl each
			04 728 882 001	4 vials, 200 µl each
			04 728 904 001	10 vials, 200 µl each
			04 728 858 001	20 vials, 200 µl 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	Store at −15 to −25°C.
2	PCR reaction buffer, 10x conc.	
3	PCR Grade Nucleotide Mix	

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 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For PCR

- PCR primers
- Template DNA
- Water, PCR Grade*

1.4. Application

Taq DNA Polymerase is used in a variety of techniques:

- The enzyme 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
- Sequencing/cycle sequencing

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as genomic or plasmid DNA 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 250 ng human genomic DNA or 0.1 to 15 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.

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 each 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 Grade Nucleotide Mix	1	200 µM of each dNTP
Forward primer 1	5	0.1 – 0.6 µM
Reverse primer 2	5	0.1 – 0.6 µM
Template DNA	variable	0.1 – 250 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	–
PCR reaction buffer, 10x	5	1x (1.5 mM $MgCl_2$)
Taq DNA Polymerase (5 U/µl)	0.25	1.25 U/reaction
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 use either of the thermal profiles below to perform PCR.

   Thermal Profile A has a fixed extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 55 – 65 72 or 68	15 – 30 sec 30 – 60 sec 45 sec – 3 min	25 – 30
Final Elongation	72 or 68	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	94	2 min	1
Denaturation Annealing Elongation	94 55 – 65 72 or 68	15 – 30 sec 30 – 60 sec 45 sec – 3 min	10
Denaturation Annealing Elongation	94 55 – 65 72 or 68	15 – 30 sec 30 sec 45 sec – 3 min + 5 sec cycle elongation for each successive cycle[1]	15 – 20
Final Elongation	72 or 68	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] 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.

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

– 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.

2.3. Parameters

Incorporation of Modified Nucleotides

Enzyme accepts modified nucleotides such as radiolabeled nucleotides, DIG-dUTP, and biotin-dUTP.

Maximum Fragment Size

Amplifies up to 3 kb products.
i *PCR is possible up to 10 kb, but yield diminishes as DNA fragment length increases.*

PCR Cloning

TA cloning
Enzyme adds a single, overhanging adenine (A).

pH Optimum

Approximately 9 (+20°C).

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

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.

Incubation procedure

1. M13mp9ss, M13 primer (17mer), and 1 µCi (α^{32P}) 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 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA within 60 minutes at +65°C under the described assay conditions.

Volume Activity

5 U/µl

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.	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 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.
	Primer design not optimal.	Design alternative primers.
	Primer concentration not optimal.	Both primers must have the same concentration.
		Titrate primer concentration (0.1 to 0.6 µM).
	Primer quality or storage problems.	If you use an established primer pair, check performance in an established PCR system, for example, 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 described in the protocol.
		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 templates, such as GC-rich templates.	Perform PCR with the GC-RICH PCR System*.
	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 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. 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		
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
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

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.

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