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Not for use in diagnostic procedures.

Taq DNA Polymerase, dNTPack

5 U/µl Taq DNA Polymerase with ready-to-use PCR Grade Nucleotide Mix

Cat. No. 04 728 866 001	100 U
Cat. No. 04 728 874 001	2 × 250 U
Cat. No. 04 728 882 001	4 × 250 U
Cat. No. 04 728 904 001	10 × 250 U
Cat. No. 04 728 858 001	20 × 250 U

Version 06
Content version: April 2018

Store the kit at −15 to −25°C

1. What this Product Does

Number of Reactions

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

- 80 reactions (Cat. No. 04 728 866 001)
- 400 reactions (Cat. No. 04 728 874 001)
- 800 reactions (Cat. No. 04 728 882 001)
- 2,000 reactions (Cat. No. 04 728 904 001)
- 4,000 reactions (Cat. No. 04 728 858 001)

Contents

Label	Contents
Taq DNA Polymerase (5 U/µl)	• 20 µl (100 U pack size) • 2 × 50 µl (2 x 250 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 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 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: 100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C)
PCR Grade Nucleotide Mix	• 1 × 200 µl (100 U pack size) • 2 × 200 µl (500 U pack size) • 4 × 200 µl (4 x 250 U pack size) • 10 × 200 µl (10 x 250 U pack size) • 20 × 200 µl (20 x 250 U pack size) Ready-to-use 10 mM dNTP solution

Storage and Stability

The undiluted solutions are 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
- 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

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 (4, 5)
- Sequencing / cycle sequencing (4, 6)

Enzyme Properties

Volume Activity	5 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 pH	Around 9 (adjusted at +20°C)
Optimal Elongation Temperature	Around +72°C
Optimal Mg^{2+} Concentration	Varies between 1.5 and 5 mM (as $MgCl_2$)
Standard Mg^{2+} Concentration	1.5 mM (as $MgCl_2$) when used with 200 µM of each dNTP
Size of PCR Products	Enzyme optimally amplifies up to 3 kb products. (PCR is possible up to 10 kb, but yield diminishes as DNA fragment length increases.)
PCR Cloning	T/A-cloning (Enzyme adds a single, overhanging A.)
Incorporation of Modified Nucleotides	Enzyme accepts modified nucleotides like radiolabeled nucleotides, DIG-dUTP, biotin-dUTP.
Thermostability	Enzyme retains over 80% activity after 30 cycles (1 min +95°C, 1 min +37°C, 3 min +72°C).

2. How To Use this Product

2.1 Before You Begin

General considerations

The optimal conditions (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 (7). At the very least, you should 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 (2, 3) 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.
- Template concentration: Typical concentrations are 10 ng to 250 ng human genomic DNA and 0.1 ng to 15 ng plasmid DNA.
- The optimal buffer for the template DNA is either double-distilled water or 5 to 10 mM Tris (pH 7 to 8).

  ⚠ Do not dissolve the template in TE buffer because EDTA chelates Mg^{2+}.

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. For example, 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

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

❸ To a sterile reaction tube on ice, add the components in the order listed below: (For each 50 µl reaction)

Component	Volume	Final conc.
Water, PCR Grade	to make a final volume of 25 µl	
PCR Grade Nucleotide Mix	1 µl	200 µM (of each dNTP)
PCR primer mix, 10×	5 µl	0.1 to 0.6 µM
Template DNA	variable	100 to 250 ng
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 To a sterile reaction tube on ice, add the components in the order listed below: (For each 50 µl reaction)

Component	Volume	Final conc.
Water, PCR Grade	19.75 µl	
PCR reaction buffer, 10×	5 µl	1× (1.5 mM $MgCl_2$)
Taq DNA Polymerase (5 U/µl)	0.25 µl	1.25 U/reaction
Final volume	25 µl	

❸ Mix and centrifuge briefly.

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

• **Thermal Profile A:** fixed extension time

	Cycles	Time	Temp
Initial Denaturation	1	2 min	94°C
Denaturation Annealing Elongation	25 to 30	15 sec to 30 sec 30 sec to 60 sec 45 sec to 3 min	94°C 55 to 65°C 72 or 68°C
Final Elongation	1	7 min	72 or 68°C
Cooling	indefinitely		4°C

• **Thermal Profile B:** gradually increasing extension time (This procedure ensures a higher yield of amplification products.)

	Cycles	Time	Temp
Initial Denaturation	1	2 min	94°C
Denaturation Annealing Elongation	10	15 sec to 30 sec 30 sec to 60 sec 45 sec to 3 min	94°C 55 to 65°C 72 or 68°C
Denaturation Annealing Elongation	15 to 20	15 sec to 30 sec 30 sec 45 sec to 3 min + 5 sec cycle elongation for each succ. cycle[a]	94°C 55 to 65°C 72 or 68°C
Final Elongation	1	7 min	72 or 68°C
Cooling	indefinitely		4°C

[a] 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.*

🔍 The denaturation temperature can vary between +92°C 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 around +72°C; for PCR products larger than 1 kb, elongation temperature should be around +68°C.

❸ 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).

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.
	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 (*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

Taq DNA Polymerase (1, 2) is a highly processive 5'→ 3' DNA polymerase that lacks 3'→ 5' exonuclease activity (3). It is a single polypeptide chain with a molecular weight of approximately 95 kDa.

Taq DNA Polymerase was originally isolated from the thermophilic eubacterium *Thermus aquaticus BM*, a strain lacking Taq I restriction endonuclease. The enzyme preparation obtained from *E. coli* is free of nonspecific endo- or exonucleases according to the current quality control procedures.

Unit Definition

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

Unit Assay

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.

4.1 References

1 Chien, A., Edgar, D. B. & Trela, J. M. (1976) Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. *J. Bacteriol.* **127,** 1550-1557.

2 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.

3 Tindall, K. R. & Kunkel, T. A. (1988) Fidelity of DNA synthesis by the Thermus aquaticus DNA polymerase. *Biochemistry* **27,** 6008-6013.

4 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.

5 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.
6 Taq polymerase: increased enzyme versatility in DNA sequencing (1988) Applied Biosystems.
7 Erlich, H. A. (ed.) (1989) PCR Technology: Principles and Application for DNA Amplification, Stockton Press, New York.
8 Mesquita, P. (2003) Human MUC2 mucin gene is transcriptionally regulated by cdx homeodomain proteins in gastrointestinal carcinoma cell lines. *J. Biol. Chem.* **278:** 51549-51556.
9 Zhu, Y. (2002) Hemin induces neuroglobin expression in neural cells. *Blood* **100:** 2494-2498.

4.2 Quality Control

Each lot of Taq DNA Polymerase, dNTPack 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

Lambda DNA (1 µg) is incubated with Taq DNA Polymerase in 50 µl test buffer for 16 hours at +37°C. The amount of enzyme that shows no degradation of the lambda DNA is > 30 U.

Absence of Nicking Activity

Supercoiled pBR322 DNA (1 µg) is incubated with Taq DNA Polymerase in 50 µl test buffer for 4 hours at +37°C. The amount of enzyme that shows no relaxation of the supercoiled DNA is > 30 U.

Absence of Exonucleases

Different amounts of Taq DNA Polymerase are incubated in 100 µl test buffer containing [^{3}H]-labeled DNA for 4 hours at +65°C. The amount of enzyme that shows no exonuclease activity is >15 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 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.

5.2 Ordering Information

	Product	Pack Size	Cat. No.
DNA Purification	High Pure PCR Template Preparation 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) 125 nmol (125 µl) 5 x 125 nmol (5 x 125 µl)	11 093 088 910 11 558 706 910 11 570 013 910
	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 x 1 ml) 25 ml (1 x 25 ml) 100 ml (4 x 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001

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