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Not for use in diagnostic procedures.

Pwo DNA Polymerase
from *Pyrococcus woesei*

Version: 17
Content Version: November 2020

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase

Cat. No. 11 644 947 001	100 U 5 U/µl 40 reactions in a final volume of 100 µl
Cat. No. 11 644 955 001	2 x 250 U 5 U/µl 200 reactions in a final volume of 100 µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Pwo DNA Polymerase	Enzyme storage and dilution buffer: 20 mM Tris-HCl, pH 7.5 (+20°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Nonidet P-40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v).	11 644 947 001	1 vial, 20 µl
			11 644 955 001	2 vials, 50 µl each
2	Pwo DNA Polymerase, PCR buffer, 10x conc. with $MgSO_4$ for Pwo Pol.	Buffer composition: 100 mM Tris-HCl, pH 8.85 (+20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$.	11 644 947 001	1 vial, 1 ml
			11 644 955 001	2 vials, 1 ml each
3	Pwo DNA Polymerase, PCR buffer, 10x conc. without $MgSO_4$ for Pwo Pol.	Buffer composition: 100 mM Tris-HCl, pH 8.85 (+20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$.	11 644 947 001	1 vial, 1 ml
			11 644 955 001	1 vial, 1 ml
4	Pwo DNA Polymerase, $MgSO_4$ Stock Solution	25 mM $MgSO_4$	11 644 947 001	1 vial, 1 ml
			11 644 955 001	1 vial, 1 ml

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	Pwo DNA Polymerase	Store at −15 to −25°C.
2	PCR buffer, 10x conc. with $MgSO_4$ for Pwo Pol.	
3	PCR buffer, 10x conc. without $MgSO_4$ for Pwo Pol.	
4	$MgSO_4$ Stock Solution	

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
- PCR Nucleotide Mix, PCR Grade*
- Water, PCR Grade*
- Agarose MP*
- Triton X-100* (optional)

1.4. Application

Fidelity of *in vitro* DNA polymerization is one of the most important subjects in PCR. For many PCR applications, where a homogeneous DNA population is analyzed, that is, direct sequencing or restriction endonuclease digestion, the mutations that are introduced by the polymerase during PCR are of little concern. However, if only a small amount of template DNA or RNA is used as starting material and if after PCR, single DNA molecules are analyzed, PCR artifacts can be a significant problem. Fidelity of DNA polymerization is important for:

- Cloning of PCR products.
- Direct sequencing of PCR products.
- Study of allelic polymorphism in individual RNA transcripts.
- Characterization of the allelic stage of single cells or single DNA molecules.
- Characterization of rare mutations in tissue.
- Characterization of a population of cells in culture.
- PCR products generated by Pwo DNA Polymerase are blunt-ended and can therefore be used directly for blunt-end ligation without any pretreatment of the ends.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as 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 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 0.1 to 0.75 µg complex genomic DNA.

Primers

Primer design

The 3′→5′ exonuclease activity of Pwo DNA Polymerase also acts on single-stranded DNA, such as PCR primers, in the absence and presence of dNTP. This activity does not usually interfere with PCR performance, however, it should be taken into consideration for primer design.
- The first fifteen 5′ bases should be protected completely from degradation. A good primer length for use with the Pwo DNA Polymerase is 20 to 35 bases.
- To overcome slow primer degradation, nuclease resistant dNTPs, such as phosphorothionate nucleotides can be used for primer synthesis.
- Additionally, longer primers with high GC content may also be advantageous. The 3′ end of the primer should be as homologous to the binding site as possible.

Mg2+ Concentration

1 to 10 mM (optimal)
2 mM (standard)

Whereas Taq DNA Polymerase requires $MgCl_2$ for optimal activity, Pwo DNA Polymerase shows higher activity with $MgSO_4$.

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 5 U/50 µl. A concentration of 2.5 U/50 µl will usually produce satisfactory results.
- $MgSO_4$ is preferred to $MgCl_2$. The standard concentration is 2 mM. The Mg^{2+} concentration should be optimized if little or no PCR product is obtained. The effect of magnesium on PCR efficiency is particularly pronounced for PCR products >2 kb.

dNTP concentrations

Use a nucleotide concentration of at least 200 µM for each dNTP. Lower nucleotide concentrations might increase fidelity, but may also lead to degradation of primers and products by elevated 3′→5′ exonuclease activity.
- Add dNTPs, such as the PCR Nucleotide Mix* to the incubation mixture directly before use to prevent decomposition of deoxynucleoside triphosphates that can occur at the alkaline pH required for optimal enzyme activity. The recommended length of the template DNA is 3 kb.

In the absence of dNTPs, the 3′→5′ exonuclease activity associated with Pwo DNA Polymerase will begin to degrade template and primer DNA. Therefore, always add Pwo DNA Polymerase to the reaction mixture as the last component.

Detergents and other additives

Usually detergents will not improve PCR performance. In some cases, improvements can be achieved by using up to 100 µg/µl BSA and/or 0.1% Triton X-100*.

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 without dNTPs which could lead to a partial degradation of primer and template through the 3′→5′ exonuclease activity of Pwo DNA Polymerase.
For the standard PCR setup, use a total reaction volume of 100 µl. For special amplifications, such as circular plasmids, GC-rich templates, or low amounts of template but high yield of product desired, set up a total reaction volume of 50 µl.

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

2. Prepare 2 Master Mixes of reagents in sterile microcentrifuge tubes on ice.

Master Mix 1 for one reaction of 100 µl (50 µl)

Reagent	Volume [µl]	Final conc.
PCR Grade Nucleotide Mix* (10 mM of each dNTP)	2 (1)	200 µM of each dNTP
Forward primer 1	X	300 nM
Reverse primer 2	X	300 nM
Template DNA, such as human genomic	X	0.1 – 0.75 µg
Water, PCR Grade*	add up to 50 (25)	–
Final Volume	**50 (25)**	

Master Mix 2 for one reaction of 100 µl (50 µl)

Reagent	Volume [µl]	Final conc.
PCR buffer, 10x conc. with 20 mM $MgSO_4$	10 (5)	1x
Pwo DNA Polymerase	0.5	2.5 U/reaction
Water, PCR Grade*	add up to 50 (25)	–
Final Volume	**50 (25)**	

– Mix each Master 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 Master Mix 1 and Master Mix 2 in a 0.2 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 the thermal profiles below to perform PCR.
   When changing from Taq to Pwo DNA Polymerase, occasionally differences are observed for the amplification of the same target sequence. Readjust the optimal annealing temperature because improperly annealed primers might be degraded by the 3′→5′ exonuclease activity of Pwo DNA Polymerase.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 45 – 65 72	15 sec 30 sec 45 sec – 2 min	10
Denaturation Annealing Elongation	94 45 – 65(1) 72	15 sec 30 sec 45 sec – 2 min(2) + 5 sec cycle elongation for each successive cycle(3)	15 – 20
Final Elongation	72	up to 7 min	–

3. Analyze samples on a 0.6 to 1% Agarose MP* gel.
   Lack of an amplification product might be due to non-optimal $MgSO_4$ concentration. Set up Master Mix 2 using PCR buffer, 10x conc. without $MgSO_4$ and add the amount of 25 mM $MgSO_4$ as indicated in the table to achieve a certain final concentration of Mg^{2+} in 100 µl total reaction volume.

25 mM $MgSO_4$ [µl]	6	8	10	12	14	16
Final Mg^{2+} conc. [mM]	1.5	2	2.5	3	3.5	4

4. Proceed as indicated in the general PCR protocol.

(1) Optimal annealing temperature depends on the melting temperature of the primers used.
(2) Use elongation times as shown in the table. Be sure to use cycle extension features.

Elongation time [sec]	45	60	120
PCR Fragment length (kb)	≤1	1.5	3

(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

EC-Number

EC 2.7.7.7

Error Rate

The inherent 3′→5′ exonuclease proofreading activity results in an approximately 18-fold increased fidelity of DNA synthesis compared to Taq DNA polymerase. Only about 10% of a 200 bp amplification product will contain at least a single error after 1 million fold amplification.

In contrast, when using Taq DNA polymerase for amplification, up to 56% of the products will contain an error under the same conditions.

Incorporation of Modified Nucleotides

Pwo DNA Polymerase accepts modified nucleotides, such as DIG-dUTP, Biotin-dUTP, and Fluorescein-dUTP.

- Use a concentration of 50 µM (50 µM modified dUTP, 150 µM dTTP) to generate probes for Southern analysis.
- For Biotin-dUTP, increase the magnesium concentration to 4 mM $MgSO_4$.
- For ELISA-based detection systems, a concentration of 10 µM modified dUTP is normally sufficient.

Molecular Weight

Approximately 90 kD.

PCR Cloning

Pwo DNA Polymerase-generated PCR products can be used directly for blunt-end ligation without prior filling in the ends with Klenow enzyme.

Temperature Stability

Increased thermal stability with a half life of >2 hours at +100°C, compared to Taq DNA Polymerase with a half life of <5 minutes at this temperature.

Unit Assay

Incubation buffer for assay on activated DNA

20 mM Tris-HCl, pH 8.8 (+20°C), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.2 mM of each dATP, dCTP, dGTP, dTTP.

Incubation procedure

1. 12.5 mg activated calf thymus DNA and 0.1 µCi [α-^{32}P] dCTP are incubated with 0.01 to 0.1 U Pwo DNA Polymerase in 50 µl Incubation buffer with a paraffin oil overlay at +70°C for 30 minutes.

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

Unit Definition

One unit Pwo DNA Polymerase is defined as the amount of enzyme that catalyzes the incorporation of 10 nmol of total deoxynucleoside triphosphates into acid precipitable DNA within 30 minutes at +70°C under the described assay conditions.

Volume Activity

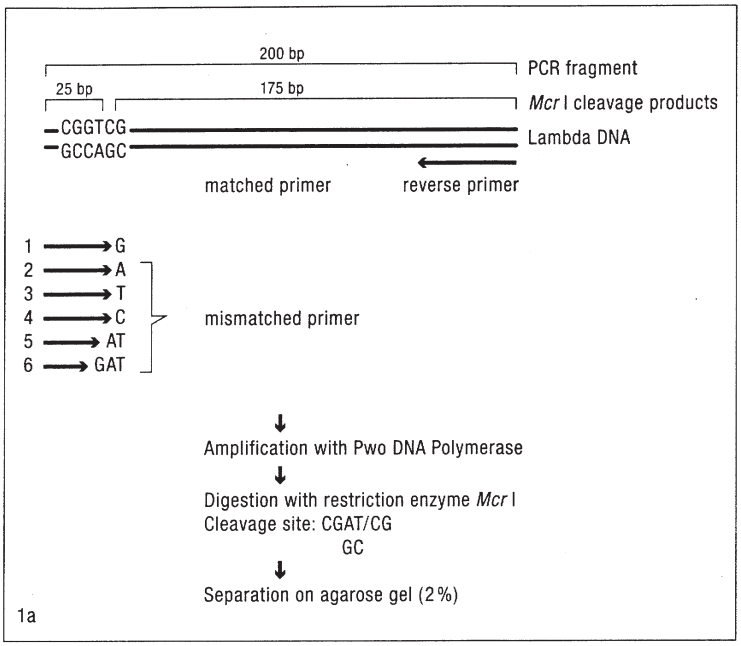
≥5 U/µl as determined in the assay on activated DNA.

Working Concentration

0.5 to 5 U per assay (optimal).
2.5 U per assay (standard).

3. Results

3′ mismatched primer correction assay



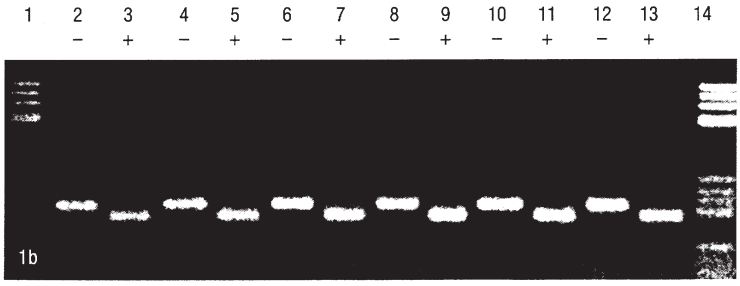


Fig. 1a: Flowchart for 3' mismatched and matched primers: Mcr I recognizes CGPuPyCG.

Fig. 1b: PCR products of a 200 bp target from lambda DNA using perfectly matched and partially mismatched primers and Pwo DNA Polymerase.
Lanes 1, 14: DNA Molecular Weight Marker V
Lanes 2, 3: Primer I (G:C match)
Lanes 4, 5: Primer II (G:A mismatch)
Lanes 6, 7: Primer III (G:T mismatch)
Lanes 8, 9: Primer IV (G:G mismatch)
Lanes 10, 11: Primer V (2 base pair mismatch)
Lanes 12, 13: Primer VI (3 base pair mismatch)
Lanes 2, 4, 6, 8, 10, 12 (-): without restriction enzyme digestion (200 bp fragment)
Lanes 3, 5, 7, 9, 11, 13, (+): restriction enzyme digestion with Mcr I (175 + 25 bp fragment)

4. Troubleshooting

Observation	Possible cause	Recommendation
Decreased fidelity in PCR.	Impure or damaged template DNA.	Recheck template quality.
	Non-optimal Mg^{2+} concentration.	Perform a titration of $MgSO_4$ to find the optimum concentration for fidelity.
	Use of organic solvents such as DMSO.	Avoid using organic solvents. *If addition of DMSO is necessary due to a GC-rich template, perform a titration to find the optimum concentration.*
	Residues remaining from DNA isolation, such as ethanol and phenol.	Avoid using organic solvents.
	Too many cycles.	Perform PCR reaction with a moderate number of cycles (<20), by increasing at the same time, the input template DNA amount to achieve sufficient sensitivity.
	Too high concentration of dNTPS.	See section, **General Considerations** for additional information.

5. Additional Information on this Product

5.1. Test Principle

Pwo DNA Polymerase is a highly processive 5′→3′ DNA polymerase and possesses a 3′→5′ exonuclease activity also known as proofreading activity. The enzyme has no detectable 5′→3′ exonuclease activity and has a molecular weight of approximately 90 kD.
Pwo DNA Polymerase was originally isolated from the hyperthermophilic archaebacterium *Pyrococcus woesei*.

5.2. Quality Control

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

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

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

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
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
Triton X-100	50 ml, 5 x 10 ml	11 332 481 001

6.4. Trademarks

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

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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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