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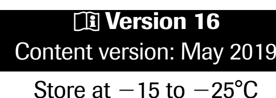
Roche

Pwo DNA Polymerase

From *Pyrococcus woesei*
Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7



Cat. No. 11 644 947 001 100 units
Cat. No. 11 644 955 001 500 units (2 × 250 units)

Store at −15 to −25°C

1. What this Product Does

Product Overview

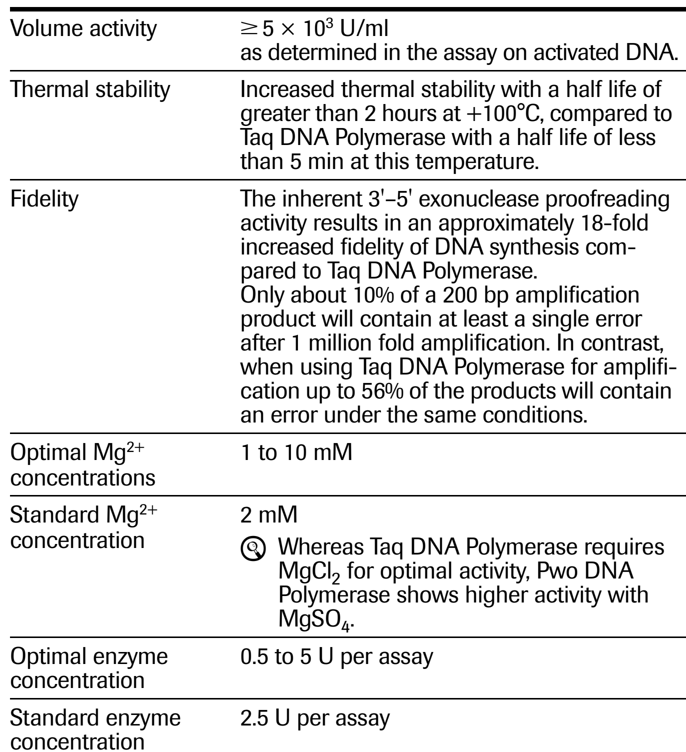
Vial	Content
Pwo DNA Polymerase	5 U/µl Enzyme storage and dilution buffer: 20 mM Tris-HCl, pH 7.5 (+20°C), 100 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.5% Nonidet P40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v).
PCR buffer, 10× conc. with $MgSO_4$	100 mM Tris-HCl, pH 8.85 (+20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$
PCR buffer, 10× conc. without $MgSO_4$	100 mM Tris-HCl, pH 8.85 (+20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$
$MgSO_4$ stock solution	25 mM $MgSO_4$

Product Description

Pwo DNA Polymerase was originally isolated from the hyperthermophilic archaebacterium *Pyrococcus woesei*.

The enzyme has a molecular weight of about 90 kDa. It is a highly processive 5'–3' DNA Polymerase and possesses an 3'–5' exonuclease activity also known as proofreading activity. The enzyme has no detectable 5'–3' exonuclease activity.

Enzyme Properties



Property	Value
Volume activity	≥ 5 × 10^3 U/ml as determined in the assay on activated DNA.
Thermal stability	Increased thermal stability with a half life of greater than 2 hours at +100°C, compared to Taq DNA Polymerase with a half life of less than 5 min at this temperature.
Fidelity	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.
Optimal Mg^{2+} concentrations	1 to 10 mM
Standard Mg^{2+} concentration	2 mM Whereas Taq DNA Polymerase requires $MgCl_2$ for optimal activity, Pwo DNA Polymerase shows higher activity with $MgSO_4$.
Optimal enzyme concentration	0.5 to 5 U per assay
Standard enzyme concentration	2.5 U per assay

Applications

Fidelity of *in vitro* DNA polymerization is one of the most important subjects in PCR. For many applications of PCR, where a homogenous DNA population is analyzed (*i.e.,* 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 for instance important for:

- cloning of PCR products
- direct sequencing of PCR products
- study of allelic polymorphism in individual RNA transcripts (1, 2)
- characterisation of the allelic stage of single cells (3) or single DNA molecules (4, 5)
- characterisation of rare mutations in tissue (6)
- characterisation 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.

Storage and Stability

The undiluted enzyme solution is stable when stored at -15 to -25°C at least until the date stated on the label.

2. How to Use this Product

2.1 Before You Begin

General Considerations

The optimal reaction conditions (incubation times and temperatures, concentration of Pwo DNA Polymerase, template DNA, Mg^{2+}) depend on the template/primer pair and must be determined individually. It is especially important to titrate the Mg^{2+} concentration and the amount of enzyme required per assay.

Magnesium Concentrations

$MgSO_4$ 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 larger than 2 kb.

Detergens and Other Additives

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

dNTP Concentrations

The nucleotide concentration should be 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.

The dNTPs (*e.g.,* PCR Nucleotide Mix*) should be added to the incubation mixture directly before use.

This will prevent decomposition of deoxynucleoside triphosphates that can occur at the alkaline pH required for optimal enzyme activity. The recommended length of 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, it is important to always add Pwo DNA Polymerase to the reaction mixture as the last component.
- This can be achieved by using the hot start technique with AmpliWax. In this case, primer and template are separated from the polymerase by preparing appropriate upper and lower mixes. Both mixes are separated by a wax layer, that melts above +70°C and allows then thorough mixing of the reagents.

Primer Design

The 3’–5’ exonuclease activity of Pwo DNA Polymerase acts also on single stranded DNA (*e.g.,* PCR primers) in the absence and presence of dNTP. This activity does usually not interfere with PCR performance. But it should be taken into consideration for primer design. The first fifteen 5’ bases should be protected completely from degradation and therefore a good primer length for use with Pwo DNA Polymerase is 20 to 35 bases. To overcome slow degradation of primers, nuclease resistant dNTPs, *e.g.,* phosphorothionate nucleotides (8), can be used for primer synthesis. Additionally, longer primers with maximized GC content may be advantageous. The 3’ end of the primer should be as homologous to the binding site as possible.

Labeling with modified nucleotides

Pwo DNA Polymerase accepts modified nucleotides like DIG-dUTP*, Biotin-dUTP*, and Fluorescein-dUTP*.

The concentration of these nucleotides should be 50 μM (50 μM modified dUTP, 150 μM dTTP) for gene-rating probes for Southern analysis. For Biotin-dUTP the magnesium concentration should be increased to 4 mM $MgSO_4$. For ELISA based detection systems a concentration of 10 μM modified dUTP is normally sufficient.

Cloning

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

2.2 Protocol for DNA Amplification

Preparation of Reaction Mixes

The preparation of two separate master mixes helps to circumvent the need of hot start and in addition 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.

Alternatively use AmpliWax between mix 1 and mix 2. Only for 100 μl total PCR volume recommended, for 50 μl total PCR volume do not use AmpliWax.

Protocol

For standard PCR setup a total reaction volume of 100 μl.

For special amplifications, *e.g.,* circular plasmids, GC-rich templates, or low amount of template but high yield of product desired, set up a total reaction volume of 50μl.

Step	Action
1	Briefly centrifuge all reagents before starting.
2	• Prepare two mixes of reagents in sterile microcentrifuge tubes (on ice): • **Mix 1, for one reaction of 100 μl (50 μl):**

Reagents	Volume	Final conc.
dNTP mix (10 mM)	2 μl (1 μl)	200 μM
Downstream primer	× μl	300 nM
Upstream primer	× μl	300 nM
Template DNA *e.g.,* human genomic DNA	× μl	0.1 to 0.75 μg
Water, PCR grade	up to 50 μl (25 μl)	

Step	Action
3	• **Mix 2, for one reaction of 100 μl (50 μl):**

Reagents	Volume	Final conc.
10× PCR buffer with 20 mM $MgSO_4$	10 μl (5 μl)	1×
Pwo DNA Polymerase	0.5 μl	2.5 U
Water, PCR Grade	up to 50 μl (25 μl)	

Step	Action
4	• Combine Mix 1 and Mix 2 in a 0.2 ml thin-walled PCR tube (on ice). • Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the sample at the bottom of the tube. • Continue with thermal cycling immediately.

Thermal Cycling

An example for a cycle profile is given for the Perkin Elmer GenAmp 9600 Thermocycler. When using other Thermocyclers the cycle conditions have to be adjusted.

- When changing from Taq to Pwo DNA Polymerase in some cases problems have been observed for the amplification of the same target sequence.
  In this case we recommend to readjust the optimal annealing temperature, because improperly annealed primers might be degraded by the 3’-5’ exonuclease activity of Pwo DNA Polymerase.

Step	Temperature	Time	Cycle No.
Initial Denaturation	94°C	2 min	1 ×
Denaturation Annealing Elongation	94°C 45 to 65°C 72°C	15 sec 30 sec 45 sec to 2 min	10 ×
Denaturation Annealing Elongation	94°C 45 to 65°C[a)] 72°C	15 sec 30 sec 45 sec to 2 min[b)] + cycle elongation of 5 sec for each cycle[c)]	15 to 20 ×
Final Elongation	72°C	up to 7 min	

[a)] Annealing temperature depends on the melting temperature of the primer used.

[b)] We recommend elongation times as stated below (be aware that you should use cycle extension features.

[c)] For example, cycle no. 11 has in additionis extended by 5 sec, cycle no. 12 has in addition is extended by 10 sec, cycle no. 13 has in is extended addition by 15 sec, *etc.*

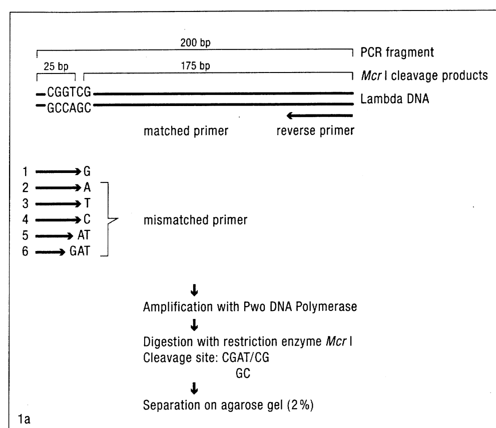
Elongation time	45 sec	1 min	2 min
PCR fragment length (kb)	up to 1	1.5	3 min

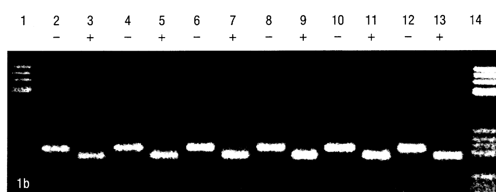
Analyze samples on a 0.6 to 1% Agarose MP* gel.

Lack of an amplification product might be due to non-optimal $MgSO_4$-concentration: In this case, set up Master Mix 2 using 10× PCR buffer without $MgSO_4$ and add the amount of 25 mM $MgSO_4$ as indicated in the table below 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

Proceed as indicated in the general PCR protocol.





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

1b: PCR products of a 200 bp target from lambda DNA using perfectly matched and partially mismatched primers and Pwo DNA Polymerase.

lane 1, 14:	DNA molecular weight marker V
lane 2, 3:	Primer I (G:C match)
lane 4, 5:	Primer II (G:A mismatch)
lane 6, 7:	Primer III (G:T mismatch)
lane 8, 9:	Primer IV (G:G mismatch)
lane 10, 11:	Primer V (2 base pair mismatch)
lane 12, 13:	Primer VI (3 base pair mismatch)

lane 2, 4, 6, 8, 10, 12 (-): without restriction enzyme digestion (200 bp fragment)
lane 3, 5, 7, 9, 11, 13, (+): restriction enzyme digestion with *Mcr* I (175 + 25 bp fragment)

3. Unit Assay

Unit Definition

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

Unit Assay on Activated DNA

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: 12.5 mg activated (1) calf thymus DNA and 0.1 mCi [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 min. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation followed by scintillation counting.

4. Quality Control

Each lot of Pwo DNA Polymerase is assayed for activity on activated DNA. Furthermore, a function test for PCR is performed using human genomic DNA (1.1 kb) and λ DNA (0.5 kb). Each lot of Pwo DNA Polymerase is assayed for contamination activities as stated below.

Absence of Endonucleases

1 µg λ DNA is incubated with Pwo DNA Polymerase and 200 µM dNTPs each at +37°C for 16 hours. Incubation with up to 30 U Pwo DNA Polymerase does not show degradation of λ DNA.

Absence of Nicking Activity

1 µg supercoiled pBR322 DNA is incubated with Pwo DNA Polymerase with 200 µM dNTPs each at +37°C for 16 hours. Incubation with up to 30 U Pwo DNA Polymerase does not show relaxation of supercoiled DNA.

5. Troubleshooting

For general troubleshooting hints regarding PCR, please visit our homepage under diagnostics.roche.com

There are a lot of parameters which could influence PCR fidelity negatively:

- organic solvents (like DMSO, ethanol, phenol; the latter two may be residues from DNA isolation),
- too long denaturating phases,
- too many cycles,
- too high concentration of dNTPs,
- impure or damaged template DNA,
- too high pH,
- non-optimal Mg^{2+} concentration.

In case you experience a decreased fidelity in PCR, a first measure should be to check the quality of template DNA and to perform a titration of $MgSO_4$ to find the optimum concentration for fidelity. Usage of DMSO or other organic solvents should be avoided. If addition of DMSO is necessary due to a GC-rich template perform a titration to find the optimum concentration. Perform the PCR reaction with a moderate number of cycles ($<$20) by increasing at the same time the input template DNA amount to achieve sufficient sensitivity.

References

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3 Li, H. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4580–4584.
4 Jeffreys, A. J. *et al.* (1990) *Cell* **60,** 473–485.
5 Ruano, G. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6296–6300.
6 Cha, R. S. *et al.* (1992) *PCR Methods Applic.* **2,** 14–20.
7 Cha, R. S. & Thilly, W. G. (1993) *PCR Methods Applic.* **3,** 18–29.
8 Skerra, A. (1992). *Nucl. Acids Res.* **20**, 3551-3554.
9 Bar-Nahum, G. *et al.* (2005) *Cell,* 183-193.

6. Supplementary Information

6.1 Conventions

Text Conventions

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

Text Conventions	Use
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.

Changes to Previous Versions

- Editorial changes.

Ordering Information

Product	Pack Size	Cat. No.
Taq DNA Polymerase, 5 U/µl	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
PCR Core Kit[PLUS]	1 kit	11 585 541 001
PCR Core Kit	1 kit	11 578 553 001
PCR Master	1 kit	11 636 103 001
Expand High Fidelity PCR System	100 U 2 × 250 U 10 × 250 U	11 732 641 001 11 732 650 001 11 759 078 001
Expand High Fidelity[PLUS] PCR System	2 × 250 U 10 × 250 U	03 300 226 001 03 300 234 001
Expand 20 kb[PLUS] PCR System	200 U	11 811 002 001
Expand Long Range dNTPack	175 U 700 U 3,500 U	04 829 034 001 04 829 042 001 04 829 069 001
High Fidelity PCR Master	1 kit	12 140 314 001
FastStart Taq DNA Polymerase, 5 U/µl	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
FastStart High Fidelity PCR System	2 × 250 U 10 × 250 U	03 553 400 001 03 553 361 001
Pwo SuperYield DNA Polymerase	100 U 2 × 250 U	04 340 868 001 04 340 850 001
Pwo Master	1 kit	03 789 403 001
Pwo DNA Polymerase	100 U 2 × 250 U	11 644 947 001 11 644 955 001
GC-RICH PCR System	100 U	12 140 306 001
Special Nucleotides Mixes		
Deoxynucleoside Triphosphate Set, PCR Grade, sodium salt	4 × 25 µmol 4 × 125 µmol	11 969 064 001 03 622 614 001
Deoxynucleoside Triphosphate Set, lithium salt	4 × 10 µmol 40 × 10 µmol	11 277 049 001 11 922 505 001
PCR Nucleotide Mix	200 µl 2,000 µl	11 581 295 001 11 814 362 001
PCR Nucleotide Mix[PLUS]	2 × 100 µl	11 888 412 001
Additional Reagents		
DIG-11-dUTP, alkali-stable	25 nmol (25 µl) 125 nmol (125 µl) 5 × 125 nmol (5 × 125 µl)	11 093 088 910 11 558 706 910 11 570 013 910
DIG-11-dUTP, alkali-labile	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
High Pure PCR Product Purification Kit	50 spin filter 250 spin filter	11 732 668 001 11 732 676 001

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