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Roche

Pwo SuperYield DNA Polymerase, dNTPack

With additional ready-to-use 10 mM PCR Grade Nucleotide Mix

Cat. No. 04 743 750 001 100 U

Version 10
Content version: March 2016

Store the kit at −15 to −25°C

1. What this Product Does

Number of PCR Reactions

The kit is designed for approximately 40 reactions.

Contents

Vial	Label	Contents
1	Pwo SuperYield DNA Polymerase	20 µl (5 U/µl) Enzyme Storage Buffer: 20 mM Tris-HCl, pH 7.5 (+20°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Nonidet P40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v)
2	Pwo SuperYield PCR Buffer	1 ml 10× conc. with 15 mM $MgSO_4$
3	GC-RICH Solution	1 ml 5× solution
4	PCR Grade Nucleotide Mix	1 × 200 µl 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 primer pair
- Water, PCR Grade*
- Thermal block cycler
- 0.2 ml thin-walled PCR tubes
- Sterile reaction tubes for preparing master mixes and dilutions

Application

Pwo SuperYield DNA Polymerase, dNTPack is ideal for high-fidelity amplification of DNA up to 3 kb. Pwo SuperYield DNA Polymerase, dNTPack combines the recombinant enzyme Pwo DNA Polymerase with an optimized buffer system. This buffer system enhances the enzymatic properties of the polymerase, resulting in higher yields of the amplification reaction with 18-fold increased fidelity compared to Taq DNA Polymerase.

- High Fidelity PCR
- Site-directed mutagenesis
- Cloning
- Gene expression

Enzyme Properties

Unit defintion and assay	One unit Pwo SuperYield 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: a) 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. b) Incubation procedure: 12.5 mg activated calf thymus DNA and 0.1 mCi [^{32}P]dCTP are incubated with 0.01 to 0.1 U Pwo SuperYield 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.
Volume activity	5 U/µl
Standard enzyme concentration	2.5 U per 50 µl reaction
Proofreading activity	Yes
Error rate	Pwo SuperYield DNA Polymerase has approximately 18-fold higher fidelity of DNA synthesis, compared to Taq DNA Polymerase
Optimal elongation temperature	+72°C
Optimal denaturation temperature	+92 to +95°C
Final Mg^{2+} concentration	1.5 mM
PCR product size	Up to 3 kb (for human genomic DNA, after optimization amplification of longer fragments is also possible)
PCR cloning	Pwo SuperYield DNA Polymerase generated PCR products can be used directly for blunt-end ligation without prior filling in the ends with Klenow Enzyme.
Carry-over prevention	dUTP is an inhibitor for B-type DNA Polymerases. Therefore, Pwo SuperYield DNA Polymerase is not compatible with carryover prevention using the dUTP/Uracil DNA-Glycosylase procedure.
Incorporation of modified nucleotides	Pwo SuperYield 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 generating 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. Please refer to DIG Product Selection Guide or DIG Manuals for detailed protocols.

2. How To Use this Product

2.1 Before You Begin

General

The optimal reaction conditions (incubation temperatures and times, concentration of template DNA and primer) depend on the template/primer system and must be determined individually.

Sample Material

- Use any template DNA (*e.g.,* 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 the MagNA Pure LC Instrument or the MagNA Pure Compact Instrument together with a dedicated nucleic acid isolation kit or
  - the High Pure PCR Template Preparation Kit*.
- Use 50 to 200 ng complex genomic DNA or 10 pg to 100 ng plasmid DNA/cDNA.

In initial experiments to determine the optimum amount of cDNA template, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel. Adding too much cDNA may inhibit the PCR.

Enzyme

Optimal enzyme concentrations range from 0.5 to 5 U per assay. The standard concentration is 2.5 U.

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 last.

Primers

Use PCR primers at a final concentration of 0.1 to 0.4 µM. The recommended starting concentration is 0.3 µM each.

⚠ Always use equimolar primer concentrations.

The design of the PCR primers determines amplicon length, melting temperature, amplification efficiency, and yield. Several programs for primer design are freely available to the public on the web (*e.g.,* Primer3).

The 3´→5´ exonuclease activity of Pwo SuperYield DNA Polymerase acts also on single stranded DNA (*e.g.,* PCR primers) in the absence and presence of dNTP. This activity does not usually 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 SuperYield DNA Polymerase is 20-35 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.

dNTP Concentration

The nucleotide concentration should be at least 200 µM of each dNTP.

⚠ Add the dNTPs to the incubation mixture directly before use. This will prevent decomposition of deoxynucleoside triphosphates.

Negative Control

To detect DNA contamination, always include a negative control in each run. To prepare this control, replace template DNA with Water, PCR Grade.

GC-Rich Templates

For the amplification of difficult templates like GC-rich DNA use the 5× GC-RICH Resolution Solution supplied with the kit.

2.2 Standard PCR Procedure

Preparation of the PCR mix

For each 50 µl reaction, prepare the following mix:.

❶ • Thaw the solutions and, for maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.
• Mix solutions carefully by pipetting them up and down, then store on ice.

❷ Prepare a 100× conc. solution of each respective PCR primer.

If you are using, *e.g.,* a final concentration of 0.3 µM for each primer, the 100× conc. solution would contain a 30 µM concentration of the respective 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.
	Standard template	GC-rich Template	
Water, PCR Grade	37.5 µl	27.5 µl	
Pwo SuperYield PCR Buffer, 10× conc. with Mg^{2+}	5 µl	5 µl	1×
PCR Grade Nucleotide Mix	1 µl	1 µl	200 µM (of each dNTP)
Downstream primer	0.5 µl	0.5 µl	0.3 µM
Upstream primer	0.5 µl	0.5 µl	0.3 µM
GC-RICH Resolution Solution 5× conc. **(optional)**	-	10 µl	1×
Pwo SuperYield DNA Polymerase (5 U/µl)	0.5 µl	0.5 µl	2.5 U
Total volume	**45 µl**	**45 µl**	

To prepare the PCR mix for more than one reaction, multiply the amounts in the "Volume" column by z, where z = the number of reactions to be run + one additional reaction.

❹ • Mix solution carefully by pipetting it up and down. Do not vortex.
• Pipet 45 µl PCR mix into each PCR reaction vessel or well of a PCR microplate (depending on your thermal block cycler).

❺ Add 5 µl of template DNA (*e.g.,* genomic DNA 50 to 200 ng; plasmid DNA 10 pg to 100 ng).

❻ **Performing PCR**
Thermal cycling
Place samples in a thermal block cycler, and start cycling using the thermal profiles mentioned below.

The thermal profiles were developed for the Applied Biosystems GeneAmp PCR System 2400, 2700, 9600. Other thermal block cyclers may require different profiles.

	Cycles	Temp	Time
Initial Denaturation[a]	1	92 to 95°C	2 min
Denaturation[a] Annealing[c] Elongation[d]	30 to 40[b]	92 to 95°C 44 to 65°C 72°C	15 sec 30 sec 45 sec to 3 min
Final extension	1	72°C	up to 7 min
Cooling		4°C	Unlimited time

[a] Denaturing temperature depends on the nature of the template used. Higher denaturing temperatures lead to increased depurination of template DNA and resulting in lower yield.

[b] 30 cycles are enough to produce an adequate amount of PCR product, if there is sufficient target (preferably $>10^4$ copies) in the sample. For low concentrations of target DNA, increase the number of cycles up to 40 cycles.

[c] Exact annealing temperature depends on the melting temperature of the primers.

[d] Elongation time depends on the length of target to be amplified. Recommended time is 1 min per 1 kb of the PCR fragment. PCR product yield can be increased by using a cycle elongation feature. Usually, the first 15 cycles are performed with a fixed elongation time, then to each of the remaining cycles 5 s are added (*e.g.,* cycle 15: 45 sec; cycle 16: 50 sec; cycle 17: 55 sec, *etc.*)

❼ After cycling, the samples may be frozen for later use.

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

2.3 Related Procedures

Digestion with Restriction Enzyme

To facilitate downstream applications such as the direct cloning of amplified DNA, it is convenient to perform restriction enzyme digest directly in the PCR mix, without prior purification of the amplified fragment. The table below shows the activity of 22 selected restriction enzymes available from Roche Diagnostics in the Pwo SuperYield DNA Polymerase PCR Mix. Almost 50% of the restriction enzymes tested were fully active in the PCR mix and proved to be suitable for direct use in the investigated downstream application. In cases where star activity is observed and/or the activity of the enzyme in the PCR mix is low, purify the amplification product prior to the restriction enzyme digest using High Pure PCR Product Purification Kit*.

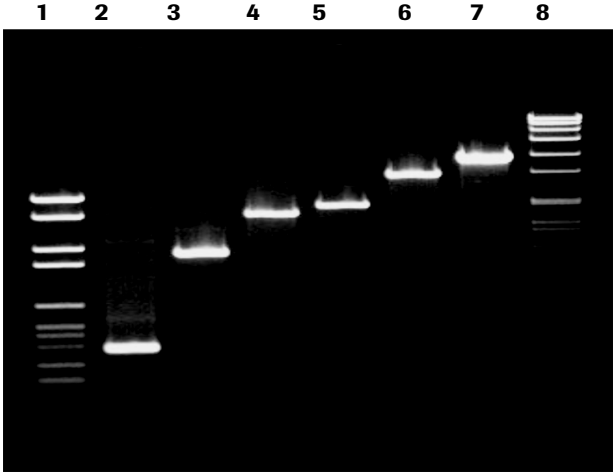
Table 1: Activity of restriction enzymes in Pwo SuperYield DNA Polymerase PCR Mix

Restriction enzyme	Recommended SuRE/Cut buffer	Relative activity (%) in PCR mix	Relative activity (%) in PCR mix with GC-RICH Solution
Apa I	A	10	>100
Bam HI	B	100	>100
Bgl II	M	85	100
Cla I	H	>100	>100
Dpn I [1)]	H	100	100
Eco RI	A	100	10 [2)]
Eco RV	B	25	25
Hind III	B	25	25
Kpn I	L	100	50
Nco I	H	100	100
Nde I	H	40	100
Nhe I	M	>100	>100
Not I	H	25	15
Nru I	B	10	25
Pst I	H	35	15
Sac I	A	100	20
Sal II	H	10	<10
Sma I	A	>100	100
Sph I	M	30	5-10
Xba I	H	25	100
Xho I	H	15	15
Xma CI	L	0	<5

[1)] Enzyme requires methylated DNA. pBR322 DNA was used as template.
[2)] Increased star activity was detected.

3. Typical Results

Pwo SuperYield DNA Polymerase is a very robust product which allows the amplification of various templates without extensive optimization. Mg^{2+} concentration is optimized for a variety of templates, wide range of applications and fragment lengths (see Fig. 1).



Lane 1: Molecular Weight Marker VI*
Lane 2: 0.3 kb tPA fragment from 200 ng human genomic DNA*
Lane 3: 1.1 kb Collagen fragment from 200 ng human genomic DNA*
Lane 4: 1.7 kb tPA fragment from 200 ng human genomic DNA*
Lane 5: 1.9 kb fragment from 200 ng potato DNA
Lane 6: 2.9 kb p53 fragment from 200 ng human genomic DNA*
Lane 7: 3.6 kb fragment from 5 ng pUCIQ 17 plasmid
Lane 8: Molecular Weight Marker VII*

4. Troubleshooting

	Cause	Recommendation
Little or no PCR product	Pipetting errors	Repeat PCR. Check all concentrations and storage conditions of reagents.
	Unbalanced reaction	Check final concentrations of your components.
	Primer problems due to • not optimal design • concentration • too high annealing temperature	 • If you use an established primer pair, check performance on an established PCR system (control template). • Design alternative primers. • Check and otimize primer concentration (0.2 to 0.5 µM). • Reduce annealing temperature.
	DNA template problems	• check quality/concentration of template DNA by analyzing an aliquot on an agarose gel using a serial dilution of template DNA • perform a control reaction with a different established primer pair/PCR system • repeat the purification of template DNA. • store template at +2 to +8°C. For long-term storage store at -15 to -25°C, avoid repeated freeze/thaw cycles. • use primers that amplify smaller genomic sequences.
	Cycle conditions are not optimal	• Decrease annealing temperature. • Check elongation time (1 min/1 kb PCR fragment). • Denaturation time should not be lower than 30 sec at +95°C. • Increase cycle number.
	Multiple contributing factors	• Test reaction with positive control template and primers of known performance. • Use freshly made solutions of master mix, template and primers.
	Annealing temperature too low	Increase annealing temperature.
Multiple bands or background smear	Primer design or concentration not optimal	• Redesign primers. • Titrate primer concentration.
	Too many cycles	Reduce cycles in steps of 3 cycles.

5. Additional Information on this Product

How this Product Works

Pwo SuperYield DNA Polymerase combines the recombinant enzyme Pwo DNA Polymerase with an optimized buffer system. This buffer system enhances the enzymatic properties of the polymerase, resulting in higher yields of the amplification reaction without changing the fidelity of DNA synthesis. This enzyme delivers excellent results due to its enzyme design and optimized buffer system. It amplifies fragments up to 3 kb – even longer amplicons are possible from simple templates.

Pwo SuperYield DNA Polymerase exhibits 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.

Background Information

Pwo DNA Polymerase was originally isolated from the hyperthermophilic archaebacterium *Pyrococcus woesei*. Based on the original enzyme, Pwo SuperYield DNA Polymerase was specially developed to yield considerably high amounts of PCR product with consistent high fidelity. The recombinant enzyme has a molecular weight of about 90 kD. It 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 according to current quality control procedures. The inherent 3´→5´ exonuclease proofreading activity of Pwo SuperYield DNA Polymerase results in an approximately 18-fold increased fidelity of DNA synthesis compared to Taq DNA Polymerase.

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 important for:

- Cloning of PCR products
- Direct sequencing of PCR products
- Studying of allelic polymorphism in individual RNA transcripts (1, 2)
- Characterisation of the allelic stage of single cells (3) or single DNA molecules (4, 5)
- Characterization of rare mutations in tissue (6)
- Characterization of a population of cells in culture

Quality Control

Each lot of Pwo SuperYield DNA Polymerase, dNTPack 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 SuperYield DNA Polymerase is assayed for contaminating activities as stated below.

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

References

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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 Frey, B. & Suppmann, B. (1995) *Biochemica* **2**. 8-9.
8 Bar-Nahum,G. *et al.* (2005) *Cell* **120**, 183-199.
9 Jäger, S. *et al.* (2005) *J.Am.Chem.Soc.* **127,** 15071-15082.

6. Supplementary Information

6.1 Changes to Previous Version

- Editorial changes

6.2 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.

6.3 Ordering Information

	Product	Pack Size	Cat. No.
PCR	PCR Nucleotide Mix PCR Grade, Na-Salt (10 mM each)	200 µl 2,000 µl	11 581 295 001 11 814 362 001
	Pwo Master	1 kit	03 789 403 001
RT-PCR	Transcriptor Reverse Transcriptase	250 U 500 U 2,000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor High Fidelity cDNA Synthesis Kit	50 reactions 100 reactions 200 reactions	05 081 955 001 05 091 284 001 05 081 963 001
	Transcriptor First Strand cDNA Synthesis Kit	50 reactions 100 reactions 200 reactions	04 379 012 001 04 896 866 001 04 897 030 001
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 µl (25 nmol) 125 µl (125 nmol)	11 573 152 910 11 573 179 910
	Digoxigenin-11-dUTP (alkali-stable)	25 µl (25 nmol) 125 µl (125 nmol) 5 × 125 µl (5 × 125 nmol)	11 093 088 910 11 558 706 910 11 570 013 910
	Biotin-16-dUTP	50 µl (50 nmol)	11 093 070 910
	Fluorescein-12-dUTP	25 µl (25 nmol)	11 373 242 910
	Water, PCR Grade	25 ml (25 × 1 ml) 25 ml (1 × 25 ml) 100 ml (4 × 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001

6.4 Regulatory Disclaimer

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

6.5 Trademarks

6.6 Disclaimer of License

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

Contact and Support

To ask questions, solve problems, suggest enhancements and 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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