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

Pwo SuperYield DNA Polymerase, dNTPack

Version: 11
Content Version: November 2020

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

Cat. No. 04 743 750 001
100 U
5 U/µl
40 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	Content
1	Pwo SuperYield DNA Polymerase, dNTPack, Pwo SuperYield DNA Polymerase	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 P-40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v)(v/v).	1 vial, 20 µl
2	Pwo SuperYield DNA Polymerase, dNTPack, PCR Buffer, 10x conc. with $MgSO_4$	15 mM $MgSO_4$	1 vial, 1 ml
3	Pwo SuperYield DNA Polymerase, dNTPack, GC-RICH Solution, 5x conc.	For amplification of difficult templates.	1 vial, 1 ml
4	Pwo SuperYield DNA Polymerase, dNTPack, dATP, dCTP, dGTP, dTTP	Ready-to-use 10 mM dNTP solution.	1 vial, 200 µl

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 SuperYield DNA Polymerase	Store at −15 to −25°C.
2	PCR Buffer, 10x conc. with $MgSO_4$	
3	GC-RICH Solution, 5x conc.	
4	dATP, dCTP, dGTP, dTTP	

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

Pwo SuperYield DNA Polymerase, dNTPack is ideal for high-fidelity amplification of DNA up to 3 kb. It 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. Fidelity of *in vitro* DNA polymerization is one of the most important subjects in PCR. For many applications of PCR, 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.
The high fidelity of this enzyme makes it particularly suitable 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.

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 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, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel. Adding too much cDNA may inhibit the PCR.

⚠ ***Store the template DNA in either double-distilled water, 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+}.***

Control Reactions

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*.

Primers

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

⚠ ***Always use equimolar primer concentrations.***

Primer design

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 Internet.

- The 3′→5′ exonuclease activity of Pwo SuperYield 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 SuperYield 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 maximized GC content may also be advantageous. The 3′ end of the primer should be as homologous to the binding site as possible.

Mg2+ Concentration

1.5 mM final concentration.

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, and primer depend on the template/primer system and must be determined for each assay.
As a starting point, use the following guidelines:

- Optimal enzyme concentration: 0.5 to 5 U per assay. A concentration of 2.5 U will usually produce satisfactory results.

dNTP concentrations

Use a nucleotide concentration of at least 200 µM of each dNTP.

- Add dNTPs, such as the PCR Nucleotide Mix* to the incubation mixture directly before use to prevent decomposition of deoxynucleoside triphosphates.

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.

GC-Rich templates

For the amplification of difficult templates, such as GC-rich DNA, use the GC-RICH Solution, 5x conc. supplied with the kit.

Restriction enzyme digestion

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 following table shows the activity of restriction enzymes in Pwo SuperYield DNA Polymerase PCR Mix.

50% of the restriction enzymes tested were fully active in the PCR mix and suitable for direct use in downstream applications. In cases where star activity is observed and/or the activity of the enzyme in the PCR mix is low, first purify the amplification product prior to the restriction enzyme digest using High Pure PCR Product Purification Kit.*

Restriction enzyme	Recommended SuRE/ Cut buffer	Relative activity in PCR mix [%]	Release activity in PCR mix with GC-RICH Solution [%]
Dpn I[(1)]	H	100	100
Not I	H	25	15
Sma I	A	>100	100
Xba I	H	25	100

(1) Enzyme required methylated DNA. pBR322 DNA was used as template.

Prevention of Carryover Contamination

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.

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 mix

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

1. 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.

2. Prepare 100x-concentrated solutions of each respective primer.
   If you are using, for example, a final concentration of 0.3 µM for each primer, the 100x-concentrated solution would contain a 30 µM concentration of the respective primer.

3. In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 50 µl reaction by adding the following components in the order listed.
   – To prepare the PCR mix for more than one reaction, multiply the amounts in the Volume column by z, where z equals the number of reactions to be run plus one additional reaction.

Reagent	Volume [µl]		Final conc.
	Standard template	GC-rich template	
Water, PCR Grade*	37.5	27.5	–
PCR Buffer, 10x conc. with $MgSO_4$	5	5	1x
PCR Nucleotide Mix (10 mM of each dNTP)	1	1	200 µM of each dNTP
Forward primer	0.5	0.5	0.3 µM
Reverse primer	0.5	0.5	0.3 µM
GC-RICH Solution, 5x conc. (optional)	–	10	1x
Pwo SuperYield DNA Polymerase (5 U/µl)	0.5	0.5	2.5 U
Final Volume	**45**	**45**	

4. Mix solution carefully by pipetting it up and down. Do not vortex.

5. Pipette 45 µl PCR mix into each PCR reaction vessel or well of a PCR microplate, depending on your block cycler PCR instrument.

6. Add 5 µl template DNA, for example, 50 to 200 ng genomic DNA or 10 pg to 100 ng plasmid DNA.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

1. Prepare the tubes or microplates for PCR according to the instructions supplied with your instrument.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 95[1]	2 min	1
Denaturation Annealing Elongation	92 – 95[1] 45 – 65[3] 72[4]	15 sec 30 sec 45 sec – 3 min	30 – 40[2]
Final Elongation	72	up to 7 min	1
Cooling	4	Unlimited	–

2. After cycling, use samples immediately or store them frozen for later use.
   – For downstream applications, check the PCR product on a 0.6 to 1% Agarose MP gel.

(1) Denaturing temperature depends on the nature of the template used. Higher denaturing temperatures lead to increased depurination of template DNA, resulting in lower yield.
(2) 30 cycles are enough to produce an adequate amount of PCR product if there is sufficient target, preferably >1 × 10^4 copies in the sample. For low concentrations of target DNA, increase the number of cycles up to 40 cycles.
(3) Exact annealing temperature depends on the melting temperature of the primers.
(4) Elongation time depends on the length of target to be amplified. Use 1 minute 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 seconds are added. For example, cycle 15 is 45 seconds, cycle 16 is 50 seconds, cycle 17 is 55 seconds, etc.

2.3. Parameters

Error Rate

Pwo SuperYield DNA Polymerase has approximately 18-fold higher fidelity of DNA synthesis, compared to Taq DNA Polymerase.

Incorporation of Modified Nucleotides

Pwo SuperYield 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.

Maximum Fragment Size

Up to 3 kb.
For human genomic DNA, after optimization, amplification of longer fragments is also possible.

Molecular Weight

Approximately 90 kD.

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.

Proofreading Activity

Yes

Temperature Optimum

Elongation temperature: +72°C.
Denaturation temperature: +92 to +95°C.

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 SuperYield DNA Polymerase in 50 µl Incubation buffer with a paraffin oil overlay at +70°C for 30 minutes.

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

Unit Definition

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 minutes at +70°C under the described assay conditions.

Volume Activity

5 U/µl

Working Concentration

2.5 U per 50 µl reaction.

3. 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 and a wide range of applications and fragment lengths.

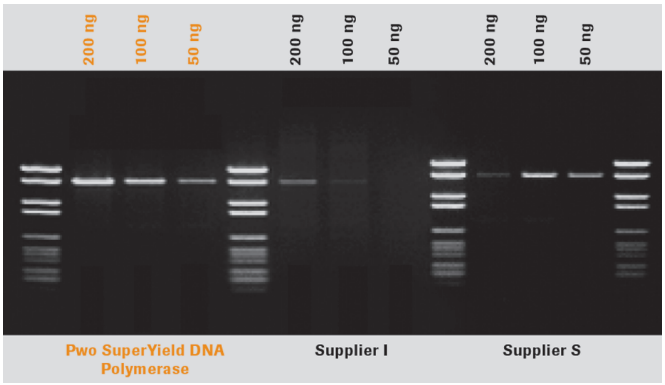

Fig. 1: Amplification of a 1.7 kb fragment of the tPA exon from human genomic DNA.
Result: Pwo SuperYield DNA Polymerase showed highest yield and consistency compared to enzymes from two other suppliers.

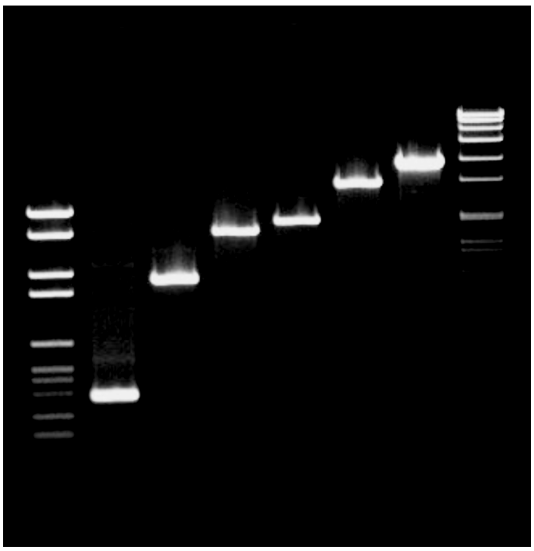

Fig. 2: Amplification of various templates with Pwo SuperYield DNA Polymerase.
Lane 1: Molecular Weight Marker VI
Lane 2: 0.3 kb tPA fragment from 200 ng human genomic DNA.
Lane 3: 1.1 kb collagen fragment from 200 ng human genomic DNA.
Lane 4: 1.7 kb tPA fragment from 200 ng human genomic DNA.
Lane 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 DNA.
Lane 8: Molecular Weight Marker VII

4. Troubleshooting

Observation	Possible 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 design not optimal.	If you use an established primer pair, check performance on an established PCR system (control template).
		Design alternative primers.
	Primer concentration not optimal.	Check and optimize primer concentration (0.2 to 0.5 µM).
	Primer problems due to too high annealing temperature.	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 purification of template DNA.
		Store template at +2 to +8°C or store at −15 to −25°C for long-term storage. ⚠ ***Avoid repeated freezing and thawing.***
		Use primers that amplify smaller genomic sequences.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Check elongation time (1 minute/1 kb PCR fragment).
		Denaturation time should not be <30 seconds at +95°C.
		Increase cycle number.
	Multiple contributing factors.	Test the reaction with positive control template and primers of known performance.
		Use freshly prepared solutions of master mix, template, and primers.
	Annealing temperature too low.	Increase annealing temperature.
Multiple bands or background smear present.	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

5.1. Test Principle

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 approximately 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.

How this product works

Pwo SuperYield DNA Polymerase combines the recombinant enzyme Pwo DNA Polymerase with an optimized buffer system.

- The buffer system enhances the enzymatic properties of the polymerase, resulting in higher yields of the amplification reaction without changing the fidelity of DNA synthesis.
- The enzyme delivers excellent results due to its enzyme design and optimized buffer system. Amplify fragments up to 3 kb; even longer amplicons are possible from simple templates.

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		
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
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001

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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