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

Pwo Master

Version: 08
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Cat. No. 03 789 403 001 250 U
10 x 250 µl, 0.1 U/µl
100 reactions in a final volume of 50 µl

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Pwo Master, Master Mix, 2x conc.	▪ Each vial contains 25 U Pwo SuperYield DNA Polymerase, optimized reaction buffer with 4 mM $MgCl_2$, and PCR-grade dNTPs (dATP, dCTP, dGTP, dTTP, each 0.4 mM). ▪ Each vial is for 10 reactions.	10 vials, 250 µl each
2	Pwo Master, Water, PCR Grade	To adjust the final reaction volume.	4 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Pwo Master, Master Mix, 2x conc.	Store at +2 to +8°C.
2	Water, PCR Grade	

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
- Mineral oil (optional)

1.4. Application

Pwo Master is used for high fidelity amplification of fragments up to 3 kb and eliminates individual adjustment of the reagent compositions. 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 induced by the polymerase during PCR are of little concern. However, if only a small amount of template DNA 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 polymerization is of particular importance for:

- Cloning of PCR products.
- Characterization of a population of cells in culture.
- 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.

Product Description

Pwo Master is a premixed 2x-concentrated master mix containing Pwo SuperYield DNA Polymerase, an optimized buffer system, and PCR-grade deoxynucleotides. After adding the template DNA, primers, and adjusting the volume to a final volume of 50 µl with PCR-grade water, the reaction mix can directly be applied to the cycler. This ready-to-use mix offers a convenient solution for the high fidelity amplification of fragments up to 3 kb and eliminates individual adjustment of the reagent compositions.

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 5 to 500 ng complex genomic DNA or 100 pg to 10 ng plasmid DNA/cDNA.

⚠ ***Store the template DNA in either sterile, 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+}***

Primers

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.
- To overcome slow primer degradation, nuclease resistant dNTPs can be used for primer synthesis.
- Additionally, longer primers with maximized GC content and focused complementarity at the 5′ end may also be advantageous.

Forward and reverse primer

- For best results, start with 0.4 µM final concentration of each primer.
- For optimization, vary the concentration between 0.1 and 0.6 µM.

Mg2+ Concentration

2 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: 5 to 500 ng genomic DNA, 100 pg to 10 ng plasmid DNA. A concentration of 2.5 U will usually produce satisfactory results.
- The Pwo Master is formulated so that the final reaction mix contains 2 mM $MgCl_2$ and 0.2 mM dNTPs. Using these conditions, the ability to amplify different fragments of human genomic DNA ranging from 1.1 to 3 kb was demonstrated. All fragments were obtained with high yield and specificity.

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 only contains the Pwo Master; Master Mix 1 contains all other reaction components. The preparation of a separate Master Mix avoids that the enzyme interacts with primers or template which could lead to a partial degradation of primer and template by the 3′→5′ exonuclease activity of the enzyme.

1. Set up Master Mix 1 in a sterile thin-walled PCR tube on ice:

Reagent	Volume [µl]	Final conc.
Forward primer	X	400 nM
Reverse primer	X	400 nM
Template DNA	X	5 – 500 ng(1)
Water, PCR Grade	Add up to 25	–
Final Volume	**25**	

2. Mix reagents and centrifuge briefly to collect the sample at the bottom of the tube.

3. Set up Master Mix 2 as shown in the table:

Reagent	Volume [µl]	Final conc.
Pwo Master	25	2.5 U/reaction

4. Pipette the Pwo Master into the Master Mix prepared in Step 1.

5. Mix completely and overlay with 30 µl mineral oil if required by the thermal cycler.

(1) Up to 500 ng genomic or 10 ng plasmid DNA.

PCR protocol

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

1. Place your samples in a thermal block cycler and use the thermal profiles below to perform PCR.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94 (92 – 95)	2 min	1
Denaturation Annealing Elongation	94 (92 – 95) 50 – 68(1) 72	30 sec 30 sec 30 sec – 4 min(2)	20 – 30
Final Elongation	72	5 min	1

2. After cycling, use samples immediately or store them frozen for later use.

For downstream applications, check the PCR product on an agarose gel for size and specificity using an appropriate size marker.

(1) Optimal annealing temperature depends on the melting temperature of the primers and the system used.
(2) Elongation time depends upon length of the product to be amplified, approximately 1 minute per kb.

2.3. Parameters

Error Rate

Pwo SuperYield DNA Polymerase has approximately 18-fold higher fidelity of DNA synthesis compared to Taq DNA Polymerase.
Fidelity determined with the lacI assay.

Incorporation of Modified Nucleotides

No

Maximum Fragment Size

Up to 3 kb.

PCR Cloning

Blunt-end cloning
The PCR products generated with the Pwo SuperYield DNA Polymerase (Vial 1) are blunt ended and can be used directly for blunt-end ligation without any pretreatment of the ends.

Proofreading Activity

Yes

Temperature Optimum

Elongation temperature: +72°C

Volume Activity

0.1 U/µl

Working Concentration

2.5 U per 50 µl reaction (standard).

3. Results

Pwo SuperYield DNA Polymerase (Vial 1) can amplify up to 3 kb fragments from human genomic DNA and plasmid DNA with excellent yield an specificity.

3′-mismatched primer correction assay

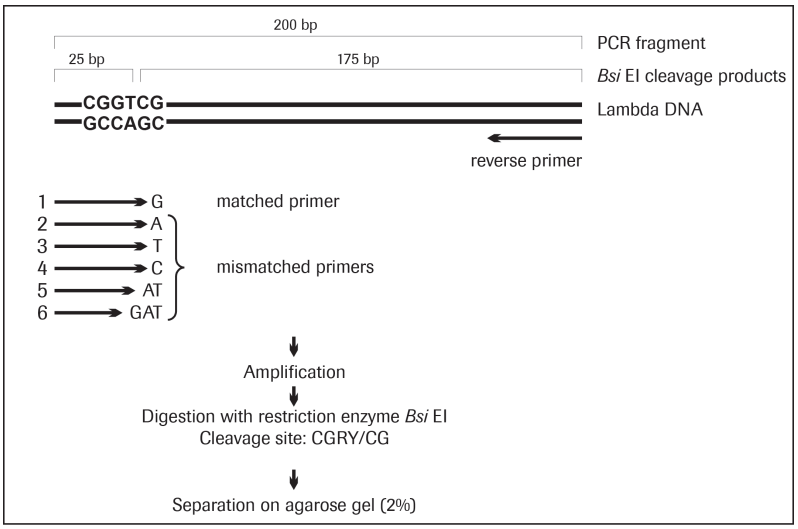

Fig. 1: Flowchart for 3′ mismatched and matched primers: Bsi EI recognizes CGRYCG.

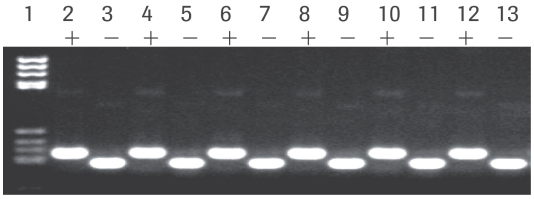

Fig. 2: PCR products of a 200 bp target from lambda DNA using perfectly matched and partially mismatched primers and Pwo DNA polymerase.
Lane 1: DNA Molecular Weight Marker V
Lanes 2, 3: Primer I (G:C match)
Lanes 4, 5: Primer II (A:C mismatch)
Lanes 6, 7: Primer III (T:C mismatch)
Lanes 8, 9: Primer IV (C:C mismatch)
Lanes 10, 11: Primer V (AT:CG mismatch)
Lanes 12, 13: Primer VI (GAT:AGC 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 Bsi EI (175 + 25 bp fragment)

Amplification of different targets from human genomic DNA

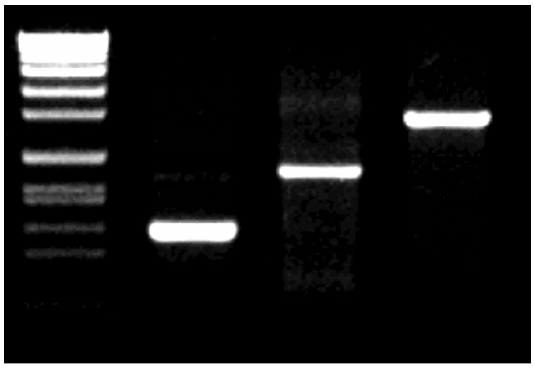

Fig. 3: The ability of the Pwo Master to amplify different targets without individual adjustments of the reagent compositions is demonstrated. The results show that fragments up to 3 kb can be obtained with high yield and specificity.
Lane 1: 1.1 kb collagen fragment
Lane 2: 1.7 kb tPA fragment
Lane 3: 2.8 kb p53 fragment

4. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Pipetting errors	Repeat PCR.
		Check all concentrations and storage conditions of reagents.
	Difficult template, such as GC-rich templates.	Add DMSO (titrate up to 8%). *Adding DMSO may negatively influence the accuracy of the reaction.*
		Use the GC-RICH PCR System
	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 problems due to too high annealing temperature.	Reduce annealing temperature.
		Determine the optimal annealing temperature by touchdown PCR.
	Primer specificity not optimal.	Perform nested PCR with nested primers.
	Primer quality or storage not optimal.	If using an established primer pair, check performance on an established PCR system (control template).
		Make sure that the primers are not degraded.
		Always store primers at −15 to −25°C.
	Formation of primer-dimers.	Reduce concentration of primers.
		Use FastStart Taq DNA Polymerase.
	DNA template problems.	Check quality and concentration of template DNA by analyzing an aliquot on an agarose gel to check for possible degradation.
		Perform a control reaction on template with an established primer pair or PCR system.
		Check or repeat purification of template DNA.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
		Make sure that the final elongation step was carried out.
Multiple bands or background smear present.	Annealing temperature too low.	Increase annealing temperature according to the primer length.
	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 and similar annealing temperatures.
		Perform nested PCR with nested primers.
	Difficult template, such as GC-rich templates.	Use the GC-RICH PCR System
	DNA template problems.	Use serial titration/dilution of template to avoid the influence of potential PCR inhibitors.
PCR products in negative control experiments.	Carryover contamination present.	Exchange all reagents, especially water.
		Use aerosol-resistant pipette tips.
		Set up PCR reactions in an area separate from that used for PCR product analysis.

5. Additional Information on this Product

5.1. Test Principle

Pwo DNA Polymerase was originally isolated from the hyperthermophilic archaebacterium *Pyrococcus woesei*. The core component of the Pwo Master is the proofreading polymerase Pwo DNA Polymerase. 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. The inherent 3′→5′ exonuclease proofreading activity of Pwo DNA Polymerase results in an approximately 18-fold increased fidelity of DNA synthesis compared to Taq DNA Polymerase.

How this product works

- The Pwo SuperYield enzyme is the same enzyme as the Pwo, but with an optimized storage buffer for yield. The master contains the Pwo SuperYield DNA Polymerase and an optimized reaction buffer.
- 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.

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. Trademarks

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

6.4. License Disclaimer

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

6.5. Regulatory Disclaimer

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

6.6. Safety Data Sheet

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

6.7. 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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