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

PCR Master

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Cat. No. 11 636 103 001 1 kit
100 reactions in a final volume of 100 µl

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	PCR Master, 2x conc.	25 U Taq DNA Polymerase in 20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$, 0.01% Brij 35 (v/v), 0.4 mM each dATP, dCTP, dGTP, dTTP, pH 8.3 (+20°C).	10 vials, 500 µl each
2	Water, PCR Grade	To adjust the final reaction volume.	5 vials, 1 ml each

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	PCR Master	Store at −15 to −25°C. For short-term storage up to 6 months, 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
- Autoclaved 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 standard and hot start PCR

- PCR primers
- Template DNA
- Mineral oil (optional)

1.4. Application

The PCR Master offers a convenient solution for PCR amplifications not requiring individual adjustment of the reagent compositions. It contains everything required to perform a standard PCR except template DNA and primers, and eliminates time-consuming thawing and pipetting steps while minimizing contamination risk.

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 10^5 to 10^6 copies.

Primers

0.1 to 1 µM each (final concentration)

ℹ *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.*

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of template DNA and primers vary from system to system and must be determined for each individual experimental system.

ℹ *For optimal specificity of the amplification reaction, the annealing and elongation temperatures must be adjusted to primer length and sequence.*

Amplification of single-copy genes

Consider the following relationships as a guideline for amplification of single-copy genes.

Amount of DNA	Number of target molecules
1 µg human genomic DNA	3×10^5
10 ng yeast DNA	3×10^5
1 ng *E. coli* DNA	3×10^5

2.2. Protocols

Preparation of standard PCR mix

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.
   – Mix solutions carefully by pipetting them up and down, then store on ice.

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2. To an autoclaved 1.5 ml reaction tube on ice, add the components in the order listed for each 50 or 100 µl reaction.

Reagent	Volume [µl]	Final conc.
PCR Master, 2x conc.	25 or 50	1x
Forward primer 1	1	0.1 – 1 µM
Reverse primer 2	1	0.1 – 1 µM
Template DNA	variable	10^5 – 10^6 copies
Water, PCR Grade	variable	–
Total Volume	**50 or 100**	

3 Mix solution and centrifuge briefly to collect the sample at the bottom of the tube.

Downscaling to 25 µl total volume is possible but depends on the template and primer used.

4 Overlay with 100 µl of mineral oil to reduce evaporation of the mix during the amplification.

Standard PCR protocol

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

1 Prepare tubes or microplates for PCR according to the instrument supplier.

2 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	2 min	1
Denaturation Annealing Elongation	94 45 – 68[1] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min[2]	25 – 30
Final Elongation	72	7 min	1

3 Store samples at +2 to +8°C or −15 to −25°C.

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

[2] Elongation time depends on the length of fragment to be amplified. Use 45 second for fragments up to 1 kb. Use 1 minute for fragments up to 1.5 kb and 2 minutes for fragments up to 3 kb.

For optimal specificity and amplification rate, the temperature and cycling times must be optimized for each new target or primer pair.

Preparation of hot start PCR mix

1 Thaw the reagents and store on ice.
– Briefly vortex and centrifuge all reagents before setting up the reactions.
– Mix solutions carefully by pipetting them up and down, then store on ice.

2 To an autoclaved 1.5 ml reaction tube on ice, add the components in the order listed for each 25 or 50 µl reaction.

Reagent	Volume [µl]	Final conc.
Forward primer 1	1	0.1 – 1 µM
Reverse primer 2	1	0.1 – 1 µM
Template DNA	variable	10^5 – 10^6 copies
Water, PCR Grade	Add up to 25 or 50	–
Total Volume	**25 or 50**	

3 Mix solution and centrifuge briefly to collect the sample at the bottom of the tube.

4 Denature the template DNA by heating to +94°C for 2 minutes.

5 Prewarm the PCR Master to at least +50°C.

6 Add 25 µl or 50 µl of the prewarmed PCR Master to the denatured DNA/primer mixture.

Hot start PCR protocol

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

1. Prepare tubes or microplates for PCR according to the instrument supplier.
   – Overlay with 100 µl of mineral oil to reduce evaporation of the mix during the amplification if required by the thermal cycler.

2. 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	2 min	1
Denaturation Annealing Elongation	94 45 – 68[1] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min[2]	25 – 30
Final Elongation	72	7 min	1

3. Store samples at +2 to +8°C or −15 to −25°C.

[1] Exact annealing temperature depends on the melting temperature of the primers.
[2] Elongation time depends on the length of fragment to be amplified. Use 45 second for fragments up to 1 kb. Use 1 minute for fragments up to 1.5 kb and 2 minutes for fragments up to 3 kb.

⚠ ***For optimal specificity and amplification rate, the temperature and cycling times must be optimized for each new target or primer pair.***

2.3. Parameters

Working Concentration

2.5 U Taq DNA Polymerase per 100 µl reaction.

3. Additional Information on this Product

3.1. Test Principle

PCR is an *in vitro* method for enzymatically synthesizing defined sequences of DNA.
- The reaction uses two oligonucleotide primers that hybridize to opposite strands and flank the target DNA sequence which is to be amplified.
- A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by Taq DNA Polymerase results in exponential accumulation of a specific DNA fragment whose termini are defined by the 5′ ends of the primers.
- Because the primer extension products synthesized in a given cycle can serve as a template in the next cycle, the number of target DNA copies approximately doubles every cycle, therefore, theoretically 20 cycles of PCR yield about a million copies of the target DNA.
- The elongation of the primers is catalyzed by Taq DNA Polymerase, a heat stable DNA polymerase, that originally was isolated from the thermophilic eubacterium *Thermus aquaticus BM*.

3.2. Quality Control

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

4. Supplementary Information

4.1. 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.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

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

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

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

4.7. 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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