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

PCR Core Kit

Version: 09
Content Version: October 2020

For amplification of specific DNA fragments by PCR.

Cat. No. 11 578 553 001

1 kit
5 U/µl
100 reactions in a final volume of 50 µl

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Content
1	colorless	PCR Core Kit, Taq DNA polymerase	▪ Enzyme storage buffer: 20 mM Tris-HCl, 100 mM DTT, 0.1 mM EDTA, 0.5% Nonidet P-40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v), pH 8.0 (+4°C). ▪ 5 U/µl Taq DNA polymerase	1 vial, 250 U
2	red	PCR Core Kit, dNTP mix	dNTP stock solution, containing 10 mM dATP, dCTP, dGTP, dTTP each.	1 vial, 200 µl
3	green	PCR Core Kit, PCR reaction buffer, 10x conc.	Buffer composition: 100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3 (+20°C).	2 vials, 1 ml each
4	blue	PCR Core Kit, $MgCl_2$, 25 mM	For optimization of Mg^{2+} concentration.	1 vial, 1 ml
5	yellow	PCR Core Kit, PCR reaction buffer, 10x conc. without $MgCl_2$	Buffer composition: 100 mM Tris-HCl, 500 mM KCl, pH 8.3 (+20°C).	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	colorless	Taq DNA polymerase	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	red	dNTP mix	
3	green	PCR reaction buffer, 10x conc.	
4	blue	$MgCl_2$, 25 mM	
5	yellow	PCR reaction buffer, 10x conc. without $MgCl_2$	

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 (0.1 to 1 mM each)
- Template DNA
- Water, PCR Grade*
- Mineral oil (optional)
- Agarose MP*

1.4. Application

The PCR Core Kit is used for PCR amplification.

- Optimize conventional PCRs with maximum sensitivity and specificity.
- Separate vials of each reagent allows easy optimization of the Mg^{2+} concentration.

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 1 mg complex genomic DNA, 10 ng yeast DNA, or 1 ng *E. coli* DNA, corresponding to 3 × 10^5 target molecules.

Primers

0.1 to 1 mM of each primer.

Mg2+ Concentration

1.5 to 5 mM (as $MgCl_2$) (optimal)
1.5 mM (as $MgCl_2$) when used with 200 µM of each dNTP (standard)

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, primers, Mg^{2+} vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg^{2+} concentration and the amount of enzyme used per assay to ensure optimal efficiency of DNA synthesis.
As a starting point, use the following guidelines:
- Optimal enzyme concentration: 1 to 5 U/50 µl. A concentration of 2.5 U/50 µl will usually produce satisfactory results.
- Optimal Mg^{2+} concentration can vary between 1 mM and 5 mM. In most cases, a Mg^{2+} concentration of 1.5 mM will produce satisfactory results if you use 200 µM of each dNTP.

  For individual optimization of the Mg^{2+} concentration, a buffer without $MgCl_2$ and a $MgCl_2$ stock solution are supplied separately. The table shows the volumes of the $MgCl_2$ stock solution which give the designated $MgCl_2$ concentrations when added to a 100 µl PCR mixture. All other steps for preparing of the reaction mix are the same as described.

$MgCl_2$ [mM]	1	1.25	1.5	1.75	2	2.5	5
Volume [µl]	4	5	6	7	8	10	20

- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. If you increase the dNTP concentration, you must also increase the Mg^{2+} concentration.
- For optimal specificity of the amplification reaction, adjust the annealing and elongation temperature to primer length and sequence.

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 contains enzyme and reaction buffer; Master Mix 1 contains all other reaction components. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template during the reaction setup.

Preparation of master mix 1

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. To a sterile 1.5 ml reaction tube on ice, add the components in the order listed:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to a final volume of 25	–
dNTP mix (10 mM of each dNTP)	1	200 µM of each dNTP
Forward primer 1	variable	0.1 – 0.6 µM
Reverse primer 2	variable	0.1 – 0.6 µM
Template DNA	variable	0.1 – 0.25 µg/reaction
Final Volume	**25**	

3. Mix and centrifuge briefly.

Preparation of master mix 2

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	19.75	–
PCR reaction buffer, 10x conc.	5	1x
Taq DNA polymerase (5 U/µl)	0.25	1.25 U/reaction
Final Volume	**25**	

3. Mix and centrifuge briefly.

PCR protocol

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

1. For each reaction, combine 25 µl Master Mix 1 and 25 µl Master Mix 2 in a thin-walled PCR tube on ice.
   – Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
   If the thermal cycler does not have a top heater, overlay the reaction mixture with 30 µl mineral oil.

2. Place your samples in a thermal block cycler and perform PCR.
   Thermal Profile A has a fixed extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 50 – 65[1] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min	25 – 30
Final Elongation	72	7 min	1

Thermal Profile B has a gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 50 – 65[1] 72	15 – 30 sec 30 – 60 sec 45 sec – 3 min	10
Denaturation Annealing Elongation	94 50 – 65[1] 72	15 – 30 sec 30 sec 45 sec – 3 min + 5 sec cycle elongation for each successive cycle[2]	15 – 20
Final Elongation	72	7 min	1

3. Analyze the PCR samples on a 0.6 to 1% agarose MP gel*.

(1) Annealing temperature depends on the melting temperature of the primers used.
(2) For example, cycle number 11 is 5 seconds longer than cycle 10. Cycle number 12 is 10 seconds longer than cycle 10. Cycle number 13 is 15 seconds longer than cycle 10, etc.

2.3. Parameters

Working Concentration

Enzyme concentration

1 to 5 U per 50 µl reaction (optimal).
2.5 U per 50 µl reaction (standard).

3. Additional Information on this Product

3.1. 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.	
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.

4.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
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

4.4. Trademarks

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

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

1020.11574051001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany