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

PCR Optimization Kit

Kit for optimization of PCR

Cat. No. 11 636 138 001

Version 08

Content version: April 2016

Store at −15 to −20°C

1. What this Product Does

Kit Contents

The kit contains reagents for 100 one-step or 50 two-step optimization assays assuming a reaction volume of 100 µl.

- **Vial 1–16, PCR optimization buffers (10 × conc.)**

16 vials (buffer A–P), each containing 1 ml of 100 mM Tris-HCl, 500 mM KCl, 10–25 mM $MgCl_2$. Detailed concentrations of $MgCl_2$ and pH-value for each buffer are shown in the following table:

pH	$MgCl_2$ concentration [mM]			
	10	15	20	25
8.3	A	B	C	D
8.6	E	F	G	H
8.9	I	J	K	L
9.2	M	N	O	P

- **Vial 17 , DMSO (dimethylsulfoxide)**
  One vial containing 1 ml DMSO.
- **Vial 18, Gelatine stock solution**
  One vial containing gelatine, 1.0% (w/v) in water, 1 ml.
- **Vial 19, $(NH_4)_2SO_4$ stock solution**
  One vial containing 500 mM $(NH_4)_2SO_4$ in water, 1 ml.
- **Vial 20, Glycerol stock solution**
  One vial containing glycerol, 50% (v/v) in water, 1 ml.
- **Vial 21, $MgCl_2$ stock solution**
  One vial containing 25 mM $MgCl_2$ in water, 1 ml.

Storage and Stability

If stored at -15 to -25°C, all kit components are stable until the expiration date printed on the label.

Application

The PCR Optimization Kit can be used to optimize PCR conditions for a given template/primer combination. The PCR Optimization Kit is the convenient and reliable solution for optimizing PCR experiments. It contains a set of buffers with different pH and magnesium concentrations spanning the most-often-used reaction conditions. Four different additives allow additional optimization to increase yield and specificity. DMSO, for instance, is reported to reduce nonspecific priming (Figure 1), while gelatin and glycerol stabilize Taq DNA polymerase during PCR, which generally increases the yield. The ready-to-use solutions save time and minimize the risk of contamination.

A technical note describing the use of the PCR Optimization Kit for a Step-by-Step Protocol for Multiplex PCR with the FastStart High Fidelity PCR System* and the PCR Optimization Kit is available under http://lifescience.roche.com/PCR.

**available from Roche Diagnostics*

Additional Reagents Required

- Primer, 0.1–1 µM of each primer
- Template DNA
- Taq DNA polymerase, 5 U/µl *
- dNTP stock solution dATP, dCTP, dGTP, dTTP; 10 mM each, in water, pH 7.0 (*e. g.,* PCR Nucleotide Mix*)
- Water, PCR Grade*.

Depending on the type of thermocycler, the usage of mineral is optional. Mineral oil must be used in case a thermocycler without heating lid is used.

2. How to Use this Product

2.1 Optimization Strategy

Step 1

In the first step the concentration of $MgCl_2$ and the pH-values of the buffers are varied.

Set up amplification reaction using the buffer A-P. These buffers have pH-values of 8.3, 8.6, 8.9 and 9.2, each with $MgCl_2$ concentrations of 1.0, 1.5, 2.0 and 2.5 mM (final concentration in the PCR).

If the optimization in step 1 provides satisfactory results, there is no need to proceed to step 2.

- Before starting the experiment thaw all reagents and mix them thoroughly.
- Use a sterile microcentrifuge tube to prepare a master mix for 16 reactions as follows:

Component	Volume	Concentration
dNTP	34 µl	0.2 mM
Primer 1	variable	0.1–1 µM
Primer 2	variable	0.1–1 µM
Template DNA[1]	variable	10^5–10^6 copies
Taq DNA polymerase	3.4–17 µl	1–5 units
Water, PCR Grade	Fill up to a volume of 1530 µl	

1. Mix thoroughly, centrifuge briefly and add 90 µl each to 16 sterile microcentrifuge tubes.
2. Add 10 µl each of the buffers A–P to the reaction tubes.
3. Mix thoroughly, centrifuge briefly.
4. Optional: Overlay carefully with mineral oil.
5. Cycling conditions depend on the respective template, primers and the type of thermocycler used (5).

[1]Consider the following relationship as a guideline for amplification of single copy genes:

1 µg human genomic DNA = 3×10^5 target molecules
10 ng yeast DNA = 3×10^5 target molecules
1 ng E. coli DNA = 3×10^5 target molecules

Step 2

If one or more of the buffers used in step 1 yields an improved but not optimal amplification further optimization is required. In step 2 a selection of one or more buffers showing the best results in step 1 are tested in the presence of different additives.

1. Prepare a reaction mix in a sterile microcentrifuge tube. Place tube on ice during pipetting. Add one or a combination of the additives.

Reagent	Volume	Concentration
optimal buffer from step 1	10 µl	1×
dNTP	2 µl	0.2 mM
Primer 1	variable	0.1–1 µM
Primer 2	variable	0.1–1 µM
Template DNA	variable	10^5–10^6 copies
Taq DNA polymerase (5 U/µl)	0.2–1 µl	1–5 units
Additive, one of: DMSO (100%), glycerol (50%), gelatine (1%), $(NH_4)_2SO_4$, 500 mM, $MgCl_2$	 1–10 µl 10–30 µl 1–10 µl 1–6 µl 1 µl	 1–10% 5–15% 0.01–0.1% 5–30 mM + 0.25 mM
Water, PCR Grade	Fill up to a volume of 100 µl	
Final volume	**100 µl**	

2. Mix thoroughly and centrifuge briefly.
3. Overlay carefully with mineral oil.
4. Use cycling conditions as established for step 1.

3. Additional Information on this Product

3.1 How this Product Works

The amplification of DNA fragments by PCR (polymerase chain reaction) is a powerful method to amplify a specific segment of DNA out of a complex mixture of DNA. The optimal reaction conditions may vary significantly depending on the target DNA and the primers. By modification of the buffer composition for a specific amplification reaction an improvement of the yield and the specificity of the PCR can be achieved. As no general rule exists to predict the amplification efficiency of a certain template primer pair the buffer composition and the additives have to be determined empirically to get optimal results.

3.2 References

1 Pomp, D and Medrano, JF (1991). Organic solvents as facilitators of polymerase chain reaction. *BioTechniques* **10,** 58–59.

2 Filichkin, SA and Gelvin, SB (1992). Effect of dimethyl sulfoxide concentration on specificity of primer matching in PCR. *BioTechniques* **12**, 828–830.

3 Masoud, S. A., Johnson, L. B. & White, F. F. (1992) *PCR Methods and Applications* **2**, 89–90.

4 Rolfs, A. et al (1992). PCR: Clinical Diagnostics and Research. Springer Laboratory, 51–67.

5 Rychlik, W et al (1990). Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Res.* **18**, 6409–6412.

6 Knoll, S et al (2002).Rapid preparation of Fusarium DNA from cereals for diagnostic PCR using sonification and an extraction kit. *Plant Pathology* **51**, 728-734.

7 Waters, CM et al (2003). Role of the Enterococcus faecalis GelE Protease in Determination of Cellular Chain Length, Supernatant Pheromone Levels, and Degradation of Fibrin and Misfolded Surface Proteins. *J. Bacteriol.* **185,** 3613 – 3623.

8 Wong, AM et al (2003). Alleles of the proximal promoter of BAT1, a putative anti-inflammatory gene adjacent to the TNF cluster, reduce transcription on a disease-associated MHC haplotype. *Genes Cells* **8,** 403 – 412.

9 Schubert, M et al. (2005) Step-by-Step Protocol for Multiplex PCR with the FastStart High Fidelity PCR System and the PCR Optimization Kit. Roche Applied Science-Amplification Technical Note, Id. No. 04 788 958 001 or online at http://lifescience.roche.com

3.3 Quality Control

Each lot of the PCR Optimization Kit is function-tested in PCR using human genomic DNA.

According to our current Quality Control procedures no exonucleases, endonuclease and contaminating DNA are detectable in the kit components.

4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
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 package insert 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.

4.2 Ordering Information

Product	Pack size	Cat. No.
Taq DNA Polymerase, 5 U/µl	100 U 2 × 250U 4 × 250 U 10 × 250 U 20 × 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
PCR Nucleotide Mix	100 reactions 1,000 reactions	11 581 295 001 11 814 362 001
FastStart High Fidelity PCR System	500 U 2,500 U	03 553 400 001 03 553 361 001
Water, PCR Grade	25 ml 25 ml 100 ml	03 315 932 001 03 315 959 001 03 315 843 001

4.3 Changes to previous version

Editorial changes.

4.4 Regulatory Disclaimer

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

4.5 Trademarks

FASTSTART is a trademark of Roche.

All brands or product names are trademark of their respective holders.

4.6 Disclaimer of License

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

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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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