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AptaTaq Fast DNA Polymerase

Kit for fast end-point PCR

Cat. No. 06 879 110 001 100 U
Cat. No. 06 879 128 001 1,000 U

Version 04
Content version: June 2018

Store kit at +2 to +8°C

1. What this Product Does

Number of Reactions

- 50 reactions of 20 µl (100 U pack size)
- 500 reactions of 20 µl (1,000 U pack size)

Contents

Vial/Cap	Contents **A) 06 879 110 001** **B) 06 879 128 001**
1, colorless AptaTaq Fast DNA Polymerase	A) 40 µl B) 10 × 40 µl • AptaTaq Fast DNA Polymerase, 2.5 U/µl
2, colorless AptaTaq Fast DNA Polymerase Buffer, 5× concentrated	A) 400 µl B) 5 × 400 µl • 5× concentrated PCR buffer (without dNTPs and $MgCl_2$)
3, blue $MgCl_2$, 25 mM	A) 1 ml B) 5 × 1 ml
4, colorless Water, PCR Grade	A) 1 ml B) 5 × 1 ml

Storage and Stability

Stable at +2 to +8°C until the expiration date printed on the label. Kits are shipped on cool packs.

Applications

- AptaTaq Fast DNA Polymerase and its optimized reaction buffer are specifically designed for fast PCR with DNA or cDNA templates.
- AptaTaq Fast DNA Polymerase is optimized for products up to 500 bp length.
- Targets with a GC-content of up to 66% can be amplified without addition of GC-rich resolution solution.
- Its inbuilt hot-start feature allows reaction setup at ambient temperature.
- With this robust reagent and a one-for-all protocol, the need for PCR protocol optimization is minimized.

2. How to Use this Product

2.1 Before You Begin

General Considerations

For best results, start with a final concentration of 0.5 µM for upstream and downstream primer. For optimization, test the concentration range between 0.2 and 0.6 µM.

Sample Material

For amplification use:

- 0.5 to 200 ng high complexity DNA (*e.g.*, human genomic DNA),
- 0.5 to 200 ng cDNA,
- 0.25 pg to 0.5 ng plasmid DNA.

2.2 Procedure

Step	Action
❶	Thaw primer and nucleic acid template solutions; mix by vortexing.
❷	Prepare PCR primer solutions (*e.g.*, in a concentration of 10 µM for each primer).
❸	Vortex the AptaTaq Fast DNA Polymerase (vial 1).
❹	Vortex the AptaTaq Fast DNA Polymerase Buffer (vial 2).
❺	Spin down all vials in a microcentrifuge prior to opening to ensure recovery of the entire volume.
❻	To a sterile reaction tube add the components in the order listed below (for each 20 µl reaction):

Component	Volume	Final Concentration
Water, PCR Grade (vial 4)	8.8 µl	
AptaTaq Fast DNA Polymerase Buffer (vial 2)	4 µl	1× concentrated
PCR Nucleotide Mix^{PLUS} *, 10 mM	0.4 µl	0.2 mM
$MgCl_2$ (vial 3)	2 µl	2.5 mM
Forward primer, 10 µM	1 µl	0.5 µM
Reverse primer, 10 µM	1 µl	0.5 µM
AptaTaq Fast DNA Polymerase (vial 1)	0.8 µl	0.1 U/µl
Final volume	**18 µl**	

To prepare fast PCR reaction mixes for more than one reaction, multiply the amount in the column "Volume" by the number of reactions plus sufficient additional reactions.

Step	Action
❼	Mix by pipetting.
❽	In case of multiple reactions, dispense 18 µl of the reaction mix into individual PCR reaction tubes or wells of a multiwell plate.
❾	Add 2 µl nucleic acid template.
❿	Mix by pipetting.
⓫	Place the samples in a thermal block cycler and use the thermal profile below to perform the PCR:

Step	Cycles	Time	Temperature
Initial Denaturation	1	30 sec	95°C
Denaturation	25 to 35	1 sec	95°C
Annealing/Elongation		15 sec	60°C
Cooling	unlimited time		4°C

2.3 Optimization

If the recommended protocol does not fulfill assay requirements, try to optimize the reaction by increasing the annealing/elongation temperature to +63°C to achieve a higher specificity. In addition, use longer annealing/elongation times for longer PCR products.

3. Troubleshooting

	Possible Cause	Recommendation
No amplification/no product detectable	Error in the PCR program	Adjust the PCR program.
	Pipetting errors (*e.g.*, nucleic acid template not added)	Repeat experiment; check pipetting steps carefully.
	Amplicon too long	• Redesign primers to shorten the PCR product. • Prolong annealing/ elongation time.
	Inhibitory effects by impurities of the nucleic acid template	Repeat the isolation of the nucleic acid template.
	Suboptimal primer design	Redesign primers.
Amplification products in the negative (no template) control	Contamination with nucleic acid templates	• Replace solutions in which a contamination might occur (*e.g.,* water). • Clean lab environment (*e.g.*, bench). • Use UNG to prevent carryover contamination.

4. Additional Information on this Product

How this Product Works

AptaTaq Fast DNA Polymerase is optimized for fast end-point polymerase chain reaction assays. Enzyme and reaction buffer composition are optimized for fast activation and short PCR reaction times. AptaTaq Fast DNA Polymerase is optimized for high specificity by minimizing the formation of non-specific amplification products.

AptaTaq Fast DNA Polymerase is an optimized mixture of recombinant Taq DNA Polymerase and an oligonucleotide, known as aptamer, that reversibly binds to the enzyme (1). The aptamer blocks the active site of the DNA polymerase at low temperatures. As soon as the melting temperature greater than +55°C is reached, the oligonucleotide is released from the active site and the enzyme is activated immediately.

The advantage of this hot-start system is that it does not require an extra activation step and is due to this properties suitable to perform fast PCR reactions. AptaTaq Fast DNA Polymerase in combination with the PCR buffer included in this kit allows short reaction times, especially when a PCR cycler with fast ramp rates is used.

PCR products have an A added at the 3´-terminus and are suitable to perform TA cloning into vectors.

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination during PCR. This cross contamination prevention technique involves incorporating deoxyuridine triphosphate into amplification products, permitting pretreatment of subsequent PCR mixtures with UNG. When a dUTP containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template. Since target DNA templates contain thymidine rather than uridine, it is not affected by this procedure.

4.1 References

1 Dang, C., Jayasena, S. D. (1996) Oligonucleotide Inhibitors of Taq DNA Polymerase facilitate Detection of Low Copy Number Targets by PCR. *J.Mol.Biol.* **264**, 268-278.

4.2 Quality Control

Each lot of the AptaTaq Fast DNA Polymerase is function tested using PCR with human genomic DNA and primers specific for the human erythropoetin gene to yield a 195 bp product.

5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Conventions	Use
Numbered Instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the ordered list.
Asterisk *	Denotes a product availability from Roche Diagnostics.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Changes to Previous Version

Editorial changes.

Trademarks

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Regulatory Disclaimer

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

Disclaimer of License

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

5.2 Ordering Information

Product	Pack Size	Cat. No.
PCR Nucleotide Mix	200 µl 2,000 µl	11 581 295 001 11 814 362 001
PCR Nucleotide Mix^{PLUS}	2 × 100 µl	11 888 412 001
Didesoxynucleoside Triphosphate Set	4 × 100 µl (10 mM each)	03 732 738 001
Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001
Uracil-DNA Glycosylase	100 U	11 444 646 001
AptaTaq Fast PCR Master	100 reactions 1,000 reactions	06 879 080 001 06 879 101 001
Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001

Contact and Support

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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