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Not for use in diagnostic procedures.

AptaTaq Fast PCR Master

Kit for fast end-point PCR

Cat. No. 06 879 080 001 100 reactions
Cat. No. 06 879 101 001 1,000 reactions

Version 03
Content version: June 2018

Store kit at +2 to +8°C

1. What this Product Does

Number of Reactions

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

Contents

Vial	Contents **A) 06 879 080 001** **B) 06 879 101 001**
1 AptaTaq Master, 5× concentrated	A) 400 µl B) 10 × 400 µl • AptaTaq Fast DNA Polymerase and the fast reaction buffer with dATP, dCTP, dGTP, dUTP, and $MgCl_2$.
2 Water, PCR Grade	A) 1 ml B) 10 × 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 PCR Master is a ready-to-use reagent mix that simplifies processing of Fast PCR assays.
- It is also optimized for high-throughput PCR using DNA or cDNA templates.
- AptaTaq Fast PCR Master 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.
- AptaTaq Fast PCR Master contains dUTP. PCR products can be digested using Uracil-DNA Glycosylase (UNG) to prevent false positives arising from carryover contamination.
- 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 of 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 PCR Master (vial 1).
❹	Spin down all vials in a microcentrifuge prior to opening to ensure recovery of the entire volume.
❺	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 2)	12 µl	
Forward primer, 10 µM	1 µl	0.5 µM
Reverse primer, 10 µM	1 µl	0.5 µM
AptaTaq Fast PCR Master, 5x concentrated (vial 1)	4 µl	1× concentrated
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
❻	Mix by pipetting.
❼	In case of multiple reactions, dispense 18 µl of the reaction mix into individual PCR reaction tubes or wells of a multiwell plate.
❽	Add 2 µl nucleic acid template.
❾	Mix by pipetting.
❿	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 PCR Master, 5x concentrated, contains all reagents, except primers and nucleic acid template, needed for fast end-point polymerase chain reaction assays. Enzyme and master mix composition are optimized for fast activation and short PCR reaction times. AptaTaq Fast PCR Master contains AptaTaq Fast DNA Polymerase for hot-start PCR with immediate activation to improve 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 PCR Master 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 later PCRs, it will be cleaved by a combination of 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 PCR Master 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.

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

APTATAQ is a trademark of Roche.

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Regulatory Disclaimer

For life science research only. Not for use in diagnostic 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.
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 DNA Polymerase	100 U 1,000 U	06 879 110 001 06 879 128 001
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.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany