

MasterTaq® Kit

Licensed for PCR

For research use only

1 Introduction

The MasterTaq Kit contains recombinant Taq DNA polymerase purified from *Escherichia coli* DH1, a 10x Taq Buffer with 15 mM magnesium, a separate 25 mM Magnesium Solution, and the special 5x TaqMaster® PCR enhancer to help with performance of sensitive and sophisticated PCR applications on difficult template DNA.

TaqMaster is a buffer additive that improves thermostability (enzyme half-life) and processivity of Taq DNA polymerase by stabilizing the enzyme during PCR. TaqMaster makes the enzyme less sensitive to exogenous PCR-inhibiting contaminants. TaqMaster is chemically stable, inert, and does not change the optimal primer annealing temperature or the melting behavior of template DNA.

2 Precautions and warnings

Appropriate safety apparel such as lab coat, gloves, and eye protection should be worn. For more information, please consult the appropriate material safety data sheets which are available for this kit online at www.eppendorf.com/msds

3 Materials

3.1 Materials supplied with the kits

Component	100 U	250 U	500 U	1000U
Taq DNA Polymerase (5 U/μl)	20 μl	50 μl	100 μl	200 μl
10x Taq Buffer with Mg ²⁺	1 x 1.8 ml	1 x 1.8 ml	2 x 1.8 ml	3 x 1.8 ml
25 mM Magnesium Solution	1 x 1.5 ml	1 x 1.5 ml	1 x 1.5 ml	2 x 1.5 ml
5x TaqMaster PCR Enhancer	1 x 1.7 ml	1 x 1.7 ml	3 x 1.7 ml	6 x 1.7 ml

3.2 Materials required but not supplied

- Template DNA
- Primers
- dNTPs*
- Molecular Biology Grade Water*

*Eppendorf products

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4 Enzyme concentration

The enzyme concentration is 5 U/ μ l*

*One unit is defined as the amount that incorporates 10 nmoles of dNTPs into acid insoluble form in 30 minutes at 74°C under the assay conditions, using reaction conditions: 25 mM TAPS (N-tris-(hydroxymethyl)-methyl-3-amino-propanesulfonic acid, sodium salt) pH 9.3 (at 25°C); 50 mM KCl; 2 mM Mg(OAc)₂; 1 mM b-mercaptoethanol; 200 μ mol each dATP, dGTP, dTTP; 100 mM dCTP (a mix of unlabeled and [α -³²P]-labeled); 12.5 μ g activated salmon sperm DNA in a final volume of 50 μ l.

5 Composition of buffers

Enzyme storage buffer: 20 mM Tris-HCl pH 8.0 (at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween® 20, 0.5% Igepal® CA-630.

10x Taq Buffer with Mg²⁺: 500mM KCl, 100mM Tris-HCl pH 8.3 (at 25°C), 15mM Mg(OAc)₂.

6 Storage and stability

Store the MasterTaq Kit at -20°C.

The 5x TaqMaster PCR Enhancer must be heated at 65°C and vortexed vigorously until completely dissolved before using or returning to -20°. Never thaw and re-freeze without heating to 65°C and thoroughly mixing.

If stored and handled as recommended, the kit is stable at least until the expiration date on the label.

7 Quality assurance

Non-specific endonuclease and exonuclease activities were not detectable after a 14 to 16 hour incubation of 1 μ g of supercoiled plasmid DNA and 1 μ g of *Hind* III digested lambda DNA at 37°C in the presence of 15-20 units of Taq DNA Polymerase.

8 Associated activities

The recombinant Eppendorf Taq DNA Polymerase in the MasterTaq Kit possesses a 5'-3' DNA dependent polymerase activity and the intrinsic 5'-3' exonuclease activity of the native Taq DNA polymerase from *Thermus aquaticus*. The extension rate of the DNA polymerase activity ranges from 30 to 60 bp per second depending on the complexity of template DNA used in the reaction. The improved performance of the Eppendorf Taq DNA Polymerase in the MasterTaq Kit when using difficult or contaminated templates relies upon the supporting action of the TaqMaster PCR Enhancer.

9 Protocol

Heat frozen TaqMaster at 65°C and vortex vigorously until completely dissolved. Add TaqMaster to a final concentration of 1x*. Mix the reaction thoroughly after adding TaqMaster.

Mix the following components on ice in a thin-walled PCR tube:

Component	Volume	Final concentration
Molecular Biology Grade Water	variable	
5x TaqMaster	5-10 μ l	0.5x - 1x
10x Taq Buffer with Mg ²⁺	5 μ l	1x (1.5 mM Mg ²⁺)
10 mM dNTP Mix	1 μ l	0.2 mM
Forward Primer	variable	0.1 - 0.2 μ M
Reverse Primer	variable	0.1 - 0.2 μ M
Template DNA	variable	1 - 150 ng
Taq DNA Polymerase (5 U/ μ l)	0.2 - 0.5 μ l	0.5 - 2.5 U
Total volume	50 μl	

*10 μ l is the standard amount of TaqMaster to add into a 50 μ l reaction, although for some primer/template systems this volume might not be the optimum. For those cases it is recommended to vary the volume of added TaqMaster between 5 - 10 μ l in 1 μ l increments. For long-range PCR (> 5 kb) the addition of up to 20 μ l TaqMaster PCR Enhancer can improve the product yield.

Mix well after addition of the 5x TaqMaster. Individual reaction conditions (temperatures and times, concentrations of template DNA, primers, Mg²⁺, and enzyme) need to be determined empirically.

10 References

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11 Ordering information

Product	Package size	International order number	North American order number
MasterTaq® Kit	100 units	0032 002.404	954 14 003-2
	250 units	0032 002.501	954 14 004-1
	500 units	0032 002.552	954 14 008-3
	1000 units	0032 002.650	954 14 009-1
Taq DNA Polymerase	100 units	0032 002.200	954 14 000-8
	250 units	0032 002.307	954 14 001-6
	500 units	0032 003.419	954 14 006-7
	1000 units	0032 007.724	954 14 007-5
	5000 units	0032 003.354	954 14 520-4
TripleMaster® PCR System Plus	100 units + 20 controls	0032 008.208	954 14 020-2
TripleMaster® PCR System	100 units	0032 008.216	954 14 022-9
	200 units	0032 008.224	954 14 024-5
	500 units	0032 008.232	954 14 026-1
Eppendorf® MasterMix	100 reactions*	0032 002.250	954 14 040-7
	1000 reactions*	0032 002.447	954 14 042-3
Eppendorf® Buffer Pack	300 reactions*	0032 008.240	954 14 050-4
	1000 reactions*	0032 008.259	954 14 052-1
HotMaster™ Taq DNA Polymerase	100 units	0032 002.676	954 14 015-6
	250 units	0032 002.684	954 14 016-4
Eppendorf® HotMasterMix	100 reactions*	0032 002.714	954 14 018-1
	1000 reactions*	0032 002.722	954 14 019-9
cMaster™ RT Kit	50 reactions [†]	0032 002.323	954 14 012-1
	200 reactions [†]	0032 002.331	954 14 013-0
cMaster™ RT _{plus} PCR System	20 reactions*	0032 002.358	954 14 010-5
	100 reactions*	0032 002.366	954 14 011-3
dNTP Mix (10mM)	200 µl	0032 003.001	954 14 300-7
	1000 µl	0032 003.109	954 14 301-5
dNTP Set (100mM)	4 x 100 µl	0032 003.206	954 14 302-3
	4 x 250 µl	0032 003.303	954 14 303-1
Molecular Biology Grade Water	10 x 50 ml	0032 006.302	955 15 503-3
	1 L	0032 006.159	955 15 500-9
Magnesium Solution (25mM)	1.5 ml	0032 002.749	954 14 102-1

[†] 1 reaction = 20 µl

* 1 reaction = 50 µl

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