Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Maxima® Hot Start Green PCR Master Mix (2X)

#K1062 500 rxns of 50 µl

Lot: __ **Expiry Date:** __

Store at -20°C

06040100029366031139

Ordering Information

Component	#K1061 100 rxns of 50 µl	#K1062 500 rxns of 50 µl
Maxima® Hot Start Green PCR Master Mix (2X)	2x1.25 ml	10x1.25 ml
Water, nuclease-free	2x1.25 ml	10x1.25 ml

ISO 9001 ISO 14001
www.fermentas.com

Description

Maxima® Hot Start Green PCR Master Mix (2X) is a ready-to-use solution containing Maxima® Hot Start *Taq* DNA Polymerase, optimized hot start PCR buffer, Mg^{2+}, and dNTPs. The master mix is supplemented with two tracking dyes and a density reagent that allows for direct loading of PCR products on gels. The dyes in the master mix do not interfere with PCR performance and are compatible with downstream applications such as fluorescent automatic DNA sequencing, ligation and restriction digestion. The master mix retains all features of Maxima® Hot Start *Taq* DNA Polymerase. It is capable of high yield amplification of targets up to 3 kb from genomic DNA. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless Maxima® Hot Start PCR Master Mix (2X) (#K0151).

Applications

- High throughput Hot Start PCR.
- RT-PCR.
- Highly specific amplification of complex genomic and cDNA templates.
- Amplification of low copy DNA targets.
- Generation of PCR products for TA cloning.

Maxima® Hot Start Green PCR Master Mix (2X) composition

Maxima® Hot Start *Taq* DNA polymerase is supplied in 2X hot start PCR buffer, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 4 mM Mg^{2+}. The master mix is also supplemented with a density reagent and two dyes for direct loading on agarose gels.

PROTOCOL

1. Gently vortex and briefly centrifuge Maxima® Hot Start Green PCR Master Mix (2X) after thawing.
2. Add the following components for each 50 µl reaction at room temperature:

Maxima® Hot Start Green PCR Master Mix (2X)	25 µl
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
Water, nuclease-free (#R0581)	to 50 µl
Total volume	50 µl

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation / enzyme activation	95	4 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension	72	1 min/kb	
Final Extension	72	5-15 min	1

6. Load 5-15 µl of PCR mixture directly on a gel.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform "no template control" (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the REviewer™ primer design software at www.fermentas.com/reviewer or follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3'-end.
- When introducing restriction enzyme sites into primers, refer to the table "Cleavage efficiency close to the termini of PCR fragments" located on www.fermentas.com to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides we recommend using specialized computer programs e.g., REviewer™ (www.fermentas.com/reviewer) to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µl reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods can be used to prepare the template e.g., Genomic DNA Purification Kit (#K0512) or GeneJET™ Plasmid Miniprep Kit (#K0502/3). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers we recommend higher primer concentrations in the range of 0.3-1 µM.

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CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. Maxima® Hot Start *Taq* DNA polymerase is inactive at room temperature during the reaction set up and is activated in 4 min during the initial denaturation / enzyme activation step.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for Maxima® Hot Start *Taq* DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72°.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product will be cloned into a TA vector (for instance, using InsTAclone™ PCR Cloning Kit (#K1213)), the final extension step may be prolonged to 30 min to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning using CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit www.fermentas.com.

QUALITY CONTROL

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25 µl of Maxima® Hot Start Green PCR Master Mix (2X) with 1 µg of pUC19 DNA in 50 µl of reaction mixture for 4 hours at 37°C.

Ribonuclease Assay

Less than 0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µl of Maxima® Hot Start Green PCR Master Mix (2X) with 1 µg of [^{3}H]-RNA in 50 µl of reaction mixture for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotides was observed after incubation with 1X master mix for 4 hours at 37°C.

Functional Assays

- PCR: amplification of a 354 bp DNA fragment from the single copy APOE gene from 50 pg human genomic DNA.
- RT-PCR: amplification of 496 bp DNA fragment from cDNA synthesized using RevertAid™ H Minus Reverse Transcriptase from 1 fg of RNA transcript.

Quality authorized by:  Jurgita Zilinskiene

Notice to purchaser