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

\[ Tm = 4(G + C) + 2(A + T) \]

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 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 Mg²⁺.

PROTOCOL

1. Gently vortex and briefly centrifuge Maxima Hot Start PCR Master Mix (2X) after thawing.

2. Add the following components for each 50 µl reaction at room temperature:

<table>
<thead>
<tr>
<th>Component</th>
<th>Maxima Hot Start PCR Master Mix (2X)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Template DNA</th>
<th>Water, nuclease-free</th>
<th>Total volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>#K1051</td>
<td>2x1.25 ml</td>
<td>0.1-1.0 µM</td>
<td>0.1-1.0 µM</td>
<td>10 pg - 1 µg</td>
<td>(#R0581)</td>
<td>50 µl</td>
</tr>
<tr>
<td>#K1052</td>
<td>10x1.25 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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:

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

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 amplions 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.
- 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 Thermo Scientific 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 conserved 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 Thermo Scientific GeneJET Plasmid Miniprep Kit (#K0602/3). 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.

(continued on reverse page)
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°C.

**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 Thermo Scientific 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 Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

**Troubleshooting**

For troubleshooting please visit www.thermoscientific.com/fermentas

---

**CERTIFICATE OF ANALYSIS**

**Endodeoxyribonuclease Assay**

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25 µl of Maxima Hot Start 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 PCR Master Mix (2X) with 1 µg of [3H]-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 Assay**

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

**Quality authorized by:** Jurgita Zilinskiene

---

**NOTICE TO PURCHASER: LIMITED LICENSE**

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,127,155, 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser’s own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City.

---

**PRODUCT USE LIMITATION**

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.

© 2011 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.