

# Hotstart *Taq* DNA polymerase

Hotstart*Taq* is a *Taq* DNA polymerase with aptamer technology inhibiting *Taq* activity

Component	Cat#	M3006.0200	M3006.1000	Colour code of cap
Aptamer inhibited hotstart <i>Taq</i> DNA polymerase		1x 200 units	1x 1000 units	colourless
10X Buffer E complete		1x 1mL	4x 1mL	orange
10X Buffer E incomplete		1x 1mL	4x 1mL	yellow
25mM MgCl <sub>2</sub>		1x 1mL	1x 1mL	green

## Product description

The Genaxxon bioscience HotStart *Taq* DNA polymerase with a specific aptamer offers all the benefits of the „normal“ Genaxxon bioscience *Taq* DNA Polymerase E plus a aptamer based, built-in hot start. The aptamer blocks polymerase activity below temperatures around 50 °C. Thus, during setup and the first PCR cycle, the enzyme is not active and misprimed primers are not extended. As a result, specificity and yield are increased compared to standard *Taq* DNA polymerase. Additionally, difficult targets with high GC-content can be amplified. Once temperature reaches optimal activating conditions, the antibody is inactivated during the first denaturation step, resulting in an active *Taq* DNA polymerase.

HotStart *Taq* DNA polymerase requires no prolonged heating or denaturing step as do other hot-start methods, making Genaxxon bioscience HotStart *Taq* DNA polymerase more convenient and easier to use.

Sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial saving of time and reagents.

## Product Specifications

Concentration:	5 units/μL
Substrate analogs:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	1-2 kb/min. at 72 °C
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No

## Unit definition

One unit of HotStart *Taq* DNA polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTP's into acid-insoluble fraction in 30 minutes at 72 °C under standard assay conditions.

## Quality Control

<b>Amplification efficiency:</b>	Amplification efficiency is tested in parallel amplification reactions and additionally against competitors' products.
<b>PCR reproducibility:</b>	PCR reproducibility is tested in parallel amplification reaction.
<b>Exonuclease activity:</b>	Linearized DNA is incubated with HotStart <i>Taq</i> DNA polymerase in PCR Buffer E.
<b>Endonuclease activity:</b>	Plasmid DNA is incubated with HotStart <i>Taq</i> DNA polymerase in PCR Buffer E.
<b>RNase activity:</b>	RNA is incubated with HotStart <i>Taq</i> DNA polymerase in PCR Buffer E.
<b>Protease activity:</b>	HotStart <i>Taq</i> DNA polymerase is incubated in storage buffer.
<b>Self-priming activity:</b>	PCR is performed under standard conditions, without primers, using HotStart <i>Taq</i> DNA polymerase and human genomic DNA..

## Application

HotStart PCR  
PCR with high specificity (Real time PCR / quantitative PCR)  
Multiplex PCR  
Detection of low target copy number

## Supplied buffers/solutions

- 10X PCR buffer with MgCl<sub>2</sub>
- 10X PCR buffer without MgCl<sub>2</sub>
- Magnesium stock solution with 25mM MgCl<sub>2</sub>

## Stability

Genaxxon bioscience HotStart *Taq* DNA polymerase is shipped on “wet ice” but retain full activity at RT (+15°C to +25°C) for at least 2 weeks.

HotStart *Taq* DNA polymerase, including buffers and reagents, should be stored immediately upon receipt at -20°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience HotStart *Taq* DNA Polymerase can also be stored at +2°C to +8°C up to 3 months.

## Product Use Limitations

HotStart *Taq* DNA polymerase is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

## Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request ([info@genaxxon.com](mailto:info@genaxxon.com)).

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience ([info@genaxxon.com](mailto:info@genaxxon.com))

## PCR Protocol Part

### Protocol using HotStart Taq DNA Polymerase

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

#### Important notes before getting started

Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis. Use disposable tips containing hydrophobic filters to minimize cross-contamination.

#### Things to do before starting

- If required, prepare a dNTP-mix containing 10mM of each dNTP (or ready-to-use product M3016 from Genaxxon bioscience). Store this mix in aliquots at -20°C.

#### Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl<sub>2</sub>** (if required) at RT or on ice. Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.
2. **Prepare a master mix according to Table 1.**  
 The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment. The optimal MgCl<sub>2</sub> concentration should be determined empirically but in most cases a concentration of 1.5mM, as provided in 10X S-buffer complete, will produce satisfactory results. Keep the master mix on ice.  
**Note:** The Mg<sup>2+</sup> concentration provided by the supplied PCR-buffer will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg<sup>2+</sup> concentration according to Table 2.

Table 1: PCR reaction components (50µL PCR reaction)

Components	Quantities
HotStart Taq DNA Polymerase	0.25 - 0.5µL (1.25 - 2.5 units)
10X PCR buffer	5µL
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP or 1µL of 10mM ready-to-use dNTP mix.
25mM MgCl <sub>2</sub> * (optional)	5µL (if no complete buffer is used)
primer 1:	0.5µL (0.25 - 2.5µL) 0.1µM (0.05 - 0.5µM (5 - 25 pmol absolute))
primer 2:	0.5µL (0.25 - 2.5µL) 0.1µM (0.05 - 0.5µM (5 - 25 pmol absolute))
Template DNA	<10ng plasmid DNA or <500ng genomic DNA
sterile, bidistilled water	up to 50µL

\* if Buffer complete is used, normally no MgCl<sub>2</sub> has to be added.

Table 2: Final MgCl<sub>2</sub> concentration in a 50µL reaction

Final MgCl <sub>2</sub> conc. in reaction (mM) complete buffer E	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Additional volume of 25mM MgCl <sub>2</sub> per 50µL reaction (µL)	0	1	2	3	4	5	6
Final MgCl <sub>2</sub> conc. in reaction (mM) incomplete buffer E	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl <sub>2</sub> per 50µL reaction (µL)	3	4	5	6	7	8	9

Note: The optimal Mg<sup>2+</sup> concentration should be determined empirically but in most cases a concentration of 2.5mM, as provided in the 10X complete buffer E will produce satisfactory results.

3. **Mix the master mix thoroughly** and dispense appropriate volumes into PCR tubes. Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cyclers.
4. **Add template DNA** (<1µg/reaction) to the individual tubes containing the master mix. For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.

5. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL - 100µL mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions. A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
7. Place PCR tubes in the thermal cycler and start program.

Table 3: PCR conditions (Thermal cycler)

Step	time	temperature	comments
Initial denaturation:	2-4 min.	94°C	for HotStart <i>Taq</i> with aptamer inhibition
3-step cycling			
Denaturation:	0.5 - 1 min.	94°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C* below lower T <sub>m</sub> of primers.
Extension:	0.5 - 1 min.	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	25 - 35		
Final extension	10 min.	72°C	

**Note:** After amplification, samples can be stored at +2°C to +8°C overnight, or at -20°C for long term storage.

Table 4: Recommendations for Standard PCR-Primers

<b>Length:</b>	18-30 nucleotides
<b>GC-Content:</b>	40-60%
<b>T<sub>m</sub>:</b>	Design primer pairs with similar T <sub>m</sub> values. Optimal annealing temperature may be above OR below the estimated T <sub>m</sub> . As a starting point, use an annealing temperature of 3°C to 5°C below T <sub>m</sub> of the primer with the lower T <sub>m</sub> -Value.
<b>Sequence:</b>	Avoid complementarities of two or more bases at the 3' ends of primer pairs. Avoid runs of 3 or more Gs or Cs at the 3' end. Avoid a 3'-end T. Avoid complementary sequences within primer and between primer pairs.

Table 5: Migration Chart of some Gel Tracking Dyes

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	1000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	