GENAXXON bioscience
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ReproHot DNA Polymerase

Deoxynucleosidetriphosphate DNA nucleotidyltransferase
from *Thermus aquaticus*

Component	Cat#	M3012.0250	M3012.1000	Colour code of cap
ReproHot Hotstart DNA Polymerase with proof reading activity with monoclonal antibody mixture		1x 250 units	1x 1000 units	colourless
10X Buffer complete		1x 1mL	2x 1mL	purple

Product description

The Genaxxon bioscience ReproHot DNA Polymerase offers all the benefits of the Genaxxon Hotstart polymerase plus proof-reading activity with built-in hot start. ReproHot DNA Polymerase mix contains a monoclonal antibody, which blocks polymerase activity prior to the onset of thermal cycling. 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 or to Proof-Reading polymerases. 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 DNA polymerase. ReproHot DNA Polymerase requires no prolonged heating or denaturing step as do other hot-start methods, making Genaxxon bioscience ReproHot 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 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:	No
3'-5' exonuclease activity:	Yes
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No

Unit definition

One unit of ReproHot 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

Amplification efficiency:	Amplification efficiency is tested in parallel amplification reactions and additionally against competitors' products.
PCR reproducibility:	PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity:	Linearized DNA is incubated with ReproHot DNA-Polymerase in ReproHot PCR buffer.
Endonuclease activity:	Plasmid DNA is incubated with ReproHot DNA-Polymerase in ReproHot PCR buffer.
RNase activity:	RNA is incubated with ReproHot DNA-Polymerase in ReproHot PCR buffer.
Protease activity:	ReproHot DNA Polymerase is incubated in storage buffer.
Self-priming activity:	PCR is performed under standard conditions, without primers, using ReproHot DNA Polymerase and human genomic DNA.

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Application

High-Fidelity HotStart PCR
PCR with high specificity and accuracy (real time PCR / quantitative PCR)
Detection of low target copy number

Supplied buffers/solutions

- 10X PCR buffer with Mg2+

Stability

Genaxxon bioscience ReproHot DNA-Polymerase is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 4 weeks.

ReproHot 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 ReproHot DNA Polymerase can also be stored at +2°C to +8°C up to 3 months.

Product Use Limitations

ReproHot 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

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

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)

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PCR Protocol Part

Protocol using ReproHot 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 MgCl2** (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 MgCl2 concentration should be determined empirically but in most cases a concentration of 2.5mM, as provided in 10X E-buffer complete, will produce satisfactory results. Keep the master mix on ice.
   Note: The Mg2+ 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 Mg2+ concentration according to Table 2.

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

Components	Quantities
Template DNA	< 10ng plasmid DNA < 500ng genomic DNA
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP 1µL of 10mM ready-to-use dNTP mix.
10X amplification buffer	5µL
primer 1:	0.1 - 0.5µM (5 - 25 pmol absolute)
primer 2:	0.1 - 0.5µM (5 - 25 pmol absolute)
nuclease-free water	up to 50µL
ReproHot DNA Polymerase	0.25 - 0.5µL (1.25 - 2.5 units)

* normally no MgCl2 has to be added

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 cycler.
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.

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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	3 min.	94°C	
3-step cycling			
Denaturation:	0.5 - 1 min.	94°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C below Tm 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 4°C overnight, or -20°C for long term storage.

Table 3: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
Tm:	Design primer pairs with similar Tm values.
	Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence:	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 4: 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	10000bp - 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	

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