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DF *Taq* DNA-Polymerase E (DNA-free *Taq*)

Deoxynucleosidetriphosphate DNA nucleotidyltransferase from *Thermus aquaticus*

Component	Cat#	M3008.0250	M3008.0500	M3008.1000	M3008.2500	Colour code of cap
DNA-free *Taq* DNA polymerase		250 units	2x 250 units	4x 250 units	10x 250 units	colourless
10X Buffer E complete		1mL	2x 1mL	3x 1mL	7x 1mL	orange
10X Buffer E incomplete		1mL	2x 1mL	3x 1mL	7x 1mL	yellow
25mM MgCl2		1mL	1x 1mL	1x 1mL	1x 1mL	green

Product description

The Genaxxon bioscience DF *Taq* DNA Polymerase E is a highly processive 5' - 3' DNA polymerase, lacking 3' - 5' exonuclease activity. The high processivity and fidelity of Genaxxon bioscience DF *Taq* DNA Polymerase E allows amplification of DNA fragments >7 kb. Genaxxon bioscience DF *Taq* DNA Polymerase E is delivered with 10X reaction buffer and separate MgCL2. The enzyme is delivered with our buffer component 'Buffer-E'. The buffer is optimised for high specificity amplification of DNA-templates. Our complete buffer contains 15mM MgCl2. The special purification procedure guarantees DF *Taq* DNA Polymerase E free of DNA impurities, especially free of DNA from the conservative region of the 16S ribosomal gene.

Product Specifications

Concentration:	5 units/µL
Substrate analogs:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	2-4 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 *Taq* DNA-Polymerase E 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 DF *Taq* DNA Polymerase in PCR Buffer E.
Endonuclease activity: Plasmid DNA is incubated with DF *Taq* DNA Polymerase in PCR Buffer E.
RNase activity: RNA is incubated with DF *Taq* DNA Polymerase in PCR Buffer E.
Protease activity: **DF** *Taq* DNA Polymerase is incubated in storage buffer.
Self-priming activity: PCR is performed under standard conditions, without primers, using DF *Taq* DNA Polymerase E and human genomic DNA.

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Application

Standard PCR
PCR with high specificity
screening for microbial impurities

Stability

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

DF *Taq* DNA Polymerase E, 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 DF *Taq* DNA Polymerase E can also be stored at +2°C to +8°C up to 3 months.

Product Use Limitations

DF *Taq* DNA Polymerase E 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 DF *Taq* DNA Polymerase E

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
DF *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 MgCl2* (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, deionised water	up to 50µL

* if Buffer S complete is used, normally no MgCl2 has to be added.

Table 2: Final MgCl2 concentration in a **50µL reaction**

Final MgCl2 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 MgCl2 per 50µL reaction (µL)	0	1	2	3	4	5	6

Final MgCl2 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 MgCl2 per 50µL reaction (µL)	3	4	5	6	7	8	9

Note: The optimal Mg2+ 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 cycler.
4. **Add template DNA** 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.

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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.

Table 3: 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 lower 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.

7. **For simplified hot start**, proceed as described in step 8. Otherwise, place the PCR tube in the thermal cycler and start program.
8. **Simplified hot start:** Start PCR program. Once the thermal cycler has reached 94°C, place PCR tubes in the thermal cycler. In many cases, this simplified hot start improves specificity of PCR. For highly specific and convenient hot start PCR please use the Genaxxon bioscience HotStart DNA Polymerase (M3006), SuperHot Taq DNA Polymerase (M3307) or the HotStart Master mix (M3007).

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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