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ReproFast DNA Polymerase

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (DNA-directed); EC 2.7.7.7

Component	Cat#	M3003.0100	M3003.0250	M3003.1250	Colour code of cap
ReproFast DNA Polymerase (proof-reading polymerase)		100 units	250 units	5x 250 units	**colourless**
5X RCR Buffer with MgSO4		1mL	1mL	5x 1mL	purple

Description

The Genaxxon bioscience ReproFast DNA Polymerase is a thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proof reading exonuclease activities. The enzyme provides extremely high fidelity and a high efficiency. ReproFast high-fidelity DNA polymerase provides high overall performance and more robust amplification of longer targets due to Genaxxons polymerase-enhancing factor. The ReproFast factor improves the yield of DNA amplicons compared to that from pure *Pfu* DNA polymerase, enhances overall PCR performance, including shorter extension times, higher yield and greater target length capability.

Amplification efficiency is the measurement of fold amplification per PCR cycle and is expressed as a fraction or percentage relative to perfect doubling (a PCR resulting in perfect doubling would exhibit 100% amplification efficiency). A small change in amplification efficiency is magnified over a multiple-cycle PCR, causing significant changes in product yield. For example, in a 25-cycle PCR, a 5% improvement in amplification efficiency can translate to a two-fold increase in product yield. Lower-efficiency reactions not only produce lower yields but take longer to reach saturation than high-efficiency reactions, increasing the chances of generating primer-dimers and undesired side products.

Usage: As *Taq* DNA polymerase. Sometimes cycle-times should be extended.

Application:
High fidelity amplification.
High fidelity amplification of fragments up to 6 kb.
Primer extension reactions
PCR cloning and blunt-end amplification product generation

Concentration: 5 units/µL

Unit definition: One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 min at 72°C under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)methyl-amino-propane-sulphonic acid, sodium salt) pH 9.3 (at 25°C), 50 mM KCl, 2 mM MgCl2, 1 mM ß-mercaptoethanol) and activated calf thymus DNA as substrate.

Storage: -20°C

Storage buffer: 20 mM Tris-HCl (pH7.9; 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% Glycerol.

10X Reaction buffer: (complete) 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl pH 8.8 (at 25°C), 100 mM KCl, 20 mM MgSO4, 1% Triton X100, 1% BSA (nuclease free).

The 10X reaction buffer is delivered free of charge.

Quality control: Activity, SDS-PAGE purity, absence of endonucleases/nickases

Note: **Recommended elongation time is 1 minute per 1kb of target!**

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Suggested PCR Protocol

Pipette the following into a PCR tube, mix and make up to a final volume of 50µL. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl2 solutions before use to remove any gradients that may result from repeated freeze/thaw steps.
If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and thermal cycler protocol.

Components	Quantities
Template DNA	1 ng - 10 ng plasmid DNA or 5 ng - 500 ng genomic DNA
Nucleotides	1µL (10 mM) each dNTP
10X amplification buffer	5µL
primer 1:	4-7µL of 3 µM solution (10 - 20 pmol absolute)
primer 2:	4-7µL of 3 µM solution (10 - 20 mpol absolute)
sterile, nuclease free water	up to 50µL
ReproFast-Polymerase	0.25 - 0.5µL (1.25 - 2.5 units)

The following cycling protocol has to be seen as suggestion. Cycle numbers and cycle times have to be adjusted.

Cycling Profile *	
94°C for 5 minutes	step 1
94°C for 30 sec.	1 - 30 cycle(s)
annealing temp. for 15 sec.	1 - 30 cycle(s)
72°C for 15-30 sec. **	1 - 30 cycle(s)
94°C for 30 sec.	
72°C for 5-10 minutes	last step
cool down to 4°C	

* cycling times are proposals and have to be adjusted to the special needs.
** the longer the template to be amplified, the longer the amplification time

After running the amplification reaction, 5µL of each sample should be analyzed in a 1% agarose gel containing 0.5µg/mL of ethidium bromide.

Note: For every template/primer pair the optimal reaction conditions have to be evaluated empirically, changing the primer/template ratio, the ionic strength (with MgSO4) and the cycle parameters (time and temperatures).

Using PCR-product for T/A-cloning

As ReproFast (M3003), ReproHot (M3012), *Pfu* (M3004) and *Pwo* (M3002) are proof-reading DNA polymerases, they may generate end products that are not applicable for T/A-cloning. To use PCR-products produced by either of the above mentioned enzymes the following additional step has to be performed.

Addition of "A" to a blunt-end DNA fragment

1 - 2 µL of DNA fragment (PCR product isolated, purified and resuspended in water or TE buffer)
1 µL of 10X *Taq* buffer
1 µL of 25 mM $MgCl_2$
x µL of ATP solution (to a final conc. of 0.2 mM)
1 µL of *Taq* Polymerase (5 units/µL)

add nuclease free water to a final volume of 10µL
Incubate at 70°C for 10 to 30 minutes

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Trouble shooting:

No PCR Product

Check if annealing temperature has been calculated correctly.
The optimal annealing temperature of primers lies about 5°C below the TM of the oligo.

TM calculation: For oligos up to 15 bases: TM = 4x(G+C) + 2x(A+T)
For larger oligos: TM = 81.5+16.6([log10[J*])+0.4(%G+C)-(600/n)-0.63(%FA)

A = Adenosine, C = Cytosine, G = Guanine, T = Thymidine, FA = Formamide, J* = concentration of monovalent cations,
n = number of bases; TM = calculated melting temperature,

Remark: If primers with mismatches are used the *ReproFast* Polymerase **must** be added immediately before the start of the PCR®. Do **not** let the reaction mix stand at room temperature since primers will be degraded by the ReproFast DNA Polymerase.

Bands smear over the Gel

Increase annealing temperature
Add up to 5% DMSO to the Reaction mixture
Reduce the amount of template DNA
Check if the primers bind more than once on the template DNA. Perform control reactions with only one of the primers.

Low Yields

Increase annealing temperature

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