

GoldStar[®] DNA polymerase

Reference: ME-0064-1ML

Licensed for PCR

Products and procedures described in this protocol are intended for research purposes only.

Batch details

Units per vial : 1000 units

Concentration : 5 U/ μ l

Source

Purified from an *E. coli* incorporating a *Thermus aquaticus* DNA polymerase overexpressing Plasmid.

Description

GoldStar[®] DNA polymerase allows amplification of DNA fragments as long as 12 Kb, even in the presence of inhibiting impurities (e.g. cell lysate material).

Quality control

Each lot is tested for the absence of nicking and priming activities, exonucleases and non-specific endonucleases.

Package contents

Reagent	Volume	Description
Goldstar [®] Clear cap vial	1 ml	DNA pol. ME-0064-1ML
10x reaction buffer Blue cap vial	30 ml	750 mM Tris-HCl pH 8.8 (at 25 °C), 200 mM (NH ₄) ₂ SO ₄ , 0.1 % (v/v) Tween 20. Without MgCl ₂
MgCl ₂ Clear cap vial	30 ml	25 mM MgCl ₂

Shipping conditions

Shipping at ambient temperature has no detrimental effect on the performance of this enzyme (if lower than 35 °C).

Storage conditions

Storage at -20 °C is recommended.

Storage & Dilution buffer

20 mM Tris HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5 % Nonidet P40, 50 % glycerol.

Analysis conditions

25 mM TAPS, pH 9.3 (at 25 °C); 50 mM KCl; 2 mM MgCl₂; 1 mM β -mercaptoethanol; 250 μ M each dCTP, dGTP, dTTP; 250 μ M (3H) dATP (0.05 Ci/mmol); activated salmon sperm DNA (1.25 μ g/ μ l); total volume of 50 μ l.

Associated activities

The enzyme has 5' \rightarrow 3' polymerisation-dependent exonuclease replacement activity but lacks 3' \rightarrow 5' exonuclease activity. The enzyme has "extendase activity", allowing TA cloning.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

Reaction Conditions

For a 100 μ l Reaction

10x buffer	10 μ l
H ₂ O	as required
dNTP final concentration	200 μ M each dNTP
> 20 mM dNTP Mix	4 μ l (see related products)
DNA template	1 ng
Primers	0.1 nmol
MgCl ₂	6 μ l (=1.5 mM, see below)
polymerase	0.8-1 unit to 1.25-2.5 units

Always keep on ice

MgCl₂ concentration: this polymerase is a magnesium-dependent enzyme. The supplied 25 mM MgCl₂ solution should be used to adjust magnesium ion concentration. We recommend a magnesium concentration higher than 1.5 mM Mg²⁺ for DNA fragments > 5 kb. Excess Mg²⁺ stabilizes the DNA double strand and consequently prevents complete denaturation of DNA, which reduces the extension yield. It may also stabilize spurious primer/template annealing, thus decreasing specificity.

Cycling conditions

Denaturation	see below
Annealing	see below
Elongation suggested	1 minute/kb at 72 °C

Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advise that you check primer design using primer design software.

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Troubleshooting guide

Observation	Recommended solution(s)
Appearance of a smear	Reduce the quantity of polymerase. This should not exceed 1 U/50 µl, except for long fragments or not highly purified DNA
Appearance of contaminating bands	Reduce the magnesium concentration to 1.5 mM, or use Hotstart technique

Related products

Reagent	Package size	Reference
dNTP Mix 20 mM total	1X 20 µmol	NU-0010-10
	5X 20 µmol	NU-0010-50
	10X 20 µmol	NU-0010-100
dNTP Set 5 mM each NTP	4 X 5 µmol	NU-0020-10
	4X 25 µmol	NU-0020-50
GoldStar® reaction buffer	5 ml	ME-0000-01
	20 ml	ME-0000-02
	100 ml	ME-0000-03
HotGoldStar DNA Polymerase	500 Units	ME-0064-05
SilverStar DNA Polymerase	500 Units	ME-0074-05

For further information please contact our Customer Help Desk:

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