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GoldStar® DNA polymerase

ME-0064-01 • ME-0064-05 • ME-0064-SA

Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.

Source

Purified from an *E. coli* strain containing 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.

Shipping conditions

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

Package contents

Reagent	Volume	Units	Concentration	Description
GoldStar® Clear cap vial	10 µl	50	5 U/µl	DNA pol. ME-0064-SA
	20 µl	100	5 U/µl	DNA pol. ME-0064-01
	100 µl	500	5 U/µl	DNA pol. ME-0064-05
Reaction buffer Blue cap vial	1 x 1.5 ml ME-0064-01 2 x 1.5 ml ME-0064-05		10 x	750 mM Tris-HCl pH 8.8 (at 25°C), 200 mM (NH ₄) ₂ SO ₄ , Stabilizer Without MgCl ₂
MgCl ₂ Clear cap vial	1.5 ml		25mM	25 mM MgCl ₂

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, 50 % glycerol, stabilizers.

Analysis conditions

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

Associated activities

The enzyme has 5' → 3' polymerisation-dependent exonuclease replacement activity but lacks 3' → 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 under the analysis conditions.

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**Reaction Conditions**

Thaw all required reagents completely and put them on ice.
 Mix all reagents well by inversion and spin them down prior to pipeting.

For a 100 µl volume (keep on ice)

10x buffer	10 µl
H ₂ O	as required
dNTP final concentration	200 µM each dNTP
> 20 mM dNTP Mix	4 µl (see related products)
DNA template	1 ng
Primers	0.1 nmol
MgCl ₂ (25 mM)	6 µl (=1.5 mM, see below)
GoldStar® DNA polymerase	0.8-1 unit to 1.25-2.5 units

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.

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 Hot start technique

Related products

Reagent	Package size	Reference
dNTP Mix 20 mM total	1 X 20 µmol	NU-0010-10
	5 X 20 µmol	NU-0010-50
	10 X 20 µmol	NU-0010-100
dNTP Set 5 mM each NTP	4 X 5 µmol	NU-0020-10
	4 X 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 Pol	500 Units	ME-0073-05
SilverStar DNA Polymerase	500 Units	ME-0074-05

For further information please contact our Customer Help Desk:**For Europe:**

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