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# Eurogentec

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## GoldStar® DNA polymerase

ME-0064-01 • ME-0064-05 • ME-0064-1ML

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®<br>Clear cap vial         | 20 µl   | 100   | 5 U/µl        | DNA pol. ME-0064-01  |
|                                     | 100 µl  | 500   | 5 U/µl        | DNA pol. ME-0064-05  |
|                                     | 1 ml  | 1000  | 5 U/µl        | DNA pol. ME-0064-1ML   |
| Reaction buffer<br>Blue cap vial    | 1 x 1.5 ml (ME-0064-01)<br>2 x 1.5 ml (ME-0064-05)<br>30 ml (ME-0064-1ML) |       | 10 x          | 750 mM Tris-HCl pH 8.8<br>(at 25°C),<br>200 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , Stabilizer<br>Without MgCl <sub>2</sub> |
| MgCl <sub>2</sub><br>Clear cap vial | 1.5 ml (30 ml, ME-0064-1ML)   |       | 25mM          | 25 mM MgCl <sub>2</sub>  |

### 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 MgCl<sub>2</sub>; 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 pipetting.

### For a 100 µl volume (keep on ice)

|                           |                              |
|---------------------------|------------------------------|
| 10x buffer                | 10 µl                        |
| H <sub>2</sub> 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 <sub>2</sub> (25 mM) | 6 µl (=1.5 mM, see below)    |
| GoldStar® DNA polymerase  | 0.8-1 unit to 1.25-2.5 units |

*MgCl<sub>2</sub> concentration: this polymerase is a magnesium-dependent enzyme. The supplied 25 mM MgCl<sub>2</sub> solution should be used to adjust magnesium ion concentration. We recommend a magnesium concentration higher than 1.5 mM Mg<sup>2+</sup> for DNA fragments > 5 kb. Excess Mg<sup>2+</sup> 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:

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