

HotGoldstar™

DNA Polymerase*

Reference : ME-0073-01 100 U
ME-0073-05 500 U

Concentration: 5 U/μl

Source :

Purified from an *E. coli* strain which carries a *Thermus aquaticus* DNA polymerase overproducing plasmid

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.

Storage & Dilution buffer :

20mM Tris HCl (pH8.0), 100mMKCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Nonidet P40, 50 % glycerol.

Reaction Buffer (10x) :

Vial 1 Green Cap (1.25 ml): 150 mM Tris-HCl pH 8.0 (at 25°C) 500 mM KCl ,0.1% (v/v) Tween 20. Without MgCl₂

Vial 2 (1.25 ml): 25 mM MgCl₂

Associated activities :

The enzyme has 5' → 3' polymerisation-dependent exonuclease replacement activity but lacks a 3' → 5' exonuclease activity.

The enzyme has the "extendase activity", allowing TA cloning.

Operating conditions :

It is recommended to add the components in the following order: 10xbuffer, H₂O, dNTPs (20 nmol/100 μl), DNA template (1 ng/100 μl), primers (0,1 nmol/100 μl) MgCl₂ (see below for MgCl₂ concentration) and last the polymerase (0.8-1unit/100 μl to 1.25-2.5 U/100μl).

Important :

Add 10 minutes pre PCR cycle at 95C to the end user normal PCR program to activate the enzyme. No amplification will be observed without this prior cycle.

Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advice to check primers design using a primer design software. We suggest a primer extension of 1 minute/kb at 72°C.

MgCl₂ concentration: this polymerase is a magnesium-dependent enzyme. The supplied 25mM MgCl₂ solution should be used to adjust magnesium ion concentration. We recommend a magnesium concentration of 2 mM, with a typical range between 1 to 4 mM.

Shipping & storage conditions :

Shipping at ambient temperature has no detrimental effect on the performance of this enzyme (if lower than 35°C). However, routine storage at -20°C is recommended.

Troubleshooting guide :

No amplification :

Check initial denaturation step of 10 minutes at 95°C.

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 1.5mM.

Quality control :

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



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* ***The Polymerase Chain Reaction (PCR*) process is covered by patents owned by Hoffmann La Roche, Inc. Use of the PCR process requires a license.***