EUROGENTEC HEADQUARTERS:
LIEGE SCIENCE PARK • 4102 Seraing • Belgium • Tel.: +32 4 372 74 00
Fax: +32 4 372 75 00 • www.eurogentec.com • info@eurogentec.com

EUROGENTEC NORTH AMERICA, INC.
11111 Flintkote Avenue • San Diego CA 92121-1222USA
Tel.:+1 858 793 2661 • Fax:+1 858 793 2666 • info.usa@eurogentec.com
www.eurogentec.com

DAp GoldStar® DNA polymerase

ME-0068-01 • ME-0068-05 • ME-0068-SA

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

Description

DAp GoldStar® DNA polymerase is a high-performance proprietary complex of enzymes specifically designed for difficult/problematic applications requiring high processivity with high fidelity. DAp GoldStar® possesses 3'→5' exonuclease activity. DAp GoldStar® DNA polymerase is recommended for longer Genomic DNA fragments of between 2-20 Kb, or up to 30 Kb Lambda DNA fragments. With Lambda DNA as template, the best performance is achieved in the 2-20 Kb range. The HI-Spec additive buffer and DMSO improve specificity of the reaction, especially on GC rich or difficult templates.

Package content

Reagent	Volume	Description
DAp GoldStar®	62.5 µl 125 µl 6.25 µl	250 units DNA pol. (4 U/µl) ME-0068-01 500 units DNA pol. (40 U/µl) ME-0068-05 25 units DNA pol. (40 U/µl) ME-0068-SA (Sample)
10x reaction buffer	2x 1.5 ml	OptiBuffer™ without $MgCl_2$
5x HI-Spec additive	2x 1.2 ml	HI-Spec additive is a specificity enhancer. If necessary, re-dissolve HI-Spec by heating to 70 °C and vortexing
$MgCl_2$	2x 1.2 ml	Separate 50 mM $MgCl_2$
DMSO	1 ml	Separate 100 % DMSO

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.

Storage conditions

DAp GoldStar® DNA Polymerase can be stored at -20 °C, in a constant temperature freezer for 12 months. DAp GoldStar® will remain stable if stored as specified.

Storage buffer

20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 50 % glycerol and stabilizers.

Analysis conditions

25 mM TAPS, pH 9.3 (at 25 °C); 50 mM KCl; 2 mM $MgCl_2$; 1 mM ß-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

Endonuclease and exonuclease activities were not detectable after 4 hours of incubation of 1 µg of pBR322 plasmid DNA and 0.5 µg *Hind*III-digested lambda DNA at 72 °C in the presence of 20 units of DAp GoldStar®.

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 50 µl volume

10x OptiBuffer (provided)	5 µl
$MgCl_2$, 50 mM Solution (provided)	2 µl
dNTP final concentration	250 µM each dNTP
20 mM dNTP Mix (related product)	2.5 µl
Template and Primers	as required
DAp GoldStar® DNA polymerase (4 U/ µl)	0.5-2 µl
DMSO* (recommended)	2.5 µl
Water (ddH_2O)	up to 50 µl

*The use of DMSO is strongly recommended. If using Hi-spec Additive, add 10-20 µl to get 1.0-2.0x final concentration. Adjust the volume of water accordingly. It is not recommended to use both DMSO and Hi-Spec Additive together. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

Always keep on ice

$MgCl_2$ concentration: *this polymerase is a magnesium-dependent enzyme. The supplied 25 mM $MgCl_2$ solution should be used to adjust magnesium ion concentration. We recommend a magnesium concentration higher than 1.5 mM Mg^2+ for DNA fragments > 5 kb. Excess Mg^{2+} 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.*

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Cycling conditions

Denaturation	94-96 °C
Annealing	see below
Elongation suggested	40-60 sec/ 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. This data is intended for use as a guide only; conditions will vary from reaction to reaction and many need optimisation.

Related products

Reagent	Package size	Reference
dNTP Mix 20 mM total	1 X 20 µmol 5 X 20 µmol 10 X 20 µmol	NU-0010-10 NU-0010-50 NU-0010-100
dNTP Set 5 mM each NTP	4 X 5 µmol 4 X 25 µmol	NU-0020-10 NU-0020-50

Features and applications

> **Long-Region Applications:** The optimal mix of enzymes in DAp GoldStar® enables primer extension over long sequences by reducing premature strand termination and template degradation. Using long primers at elevated Mg^{2+} concentration, >30 kb lambda templates or 20 kb genomic DNA can be generated.

> **Difficult Templates:** DAp GoldStar® provides high performance and specificity, even with 'dirty' DNA or difficult templates with an unfavorable nucleotide composition. In contrast to standard 3'→5' exonucleases, DAp GoldStar® can be used in combination with degenerate or non-perfect matching primers.

> **A' Overhang:** DAp GoldStar® is recommended for direct gene cloning without the need to verify the sequence prior to expression. DAp GoldStar® leaves an A' overhang such that the primer extension product is suitable for effective integration into TA cloning vectors, even from difficult templates.

> **High Fidelity:** DAp GoldStar® is a mix of polymerases that possesses a 5'→3' DNA polymerase activity and 3'→5' exonuclease activity which reduces misincorporations during primer extension. This combination of properties provides a >17 fold higher fidelity than *Taq*. In contrast with other proof-reading enzymes, DAp GoldStar® does not degrade primers.

> **High Specificity:** DAp GoldStar® is supplied with a vial of DMSO. The use of 5% DMSO in the reaction mix increases the sensitivity and yield of the DAp GoldStar® polymerase and helps to prevent the formation of false background bands and smearing, especially on difficult templates. The Optimal amount required should be determined for each individual experiment (2-8 %).

Troubleshooting guide

Observation	Recommended solution(s)
No or low yield of extended product	Enzyme concentration too low – increase the amount enzyme in 0.5 U increments.
	Magnesium concentration too low – increase concentration in 0.25 mM increments with a starting concentration of 1.75 mM.
	Primer concentration not optimised. Titrate primer concentration (0.3-1 µM); ensuring that both primers have the same concentration.
	Primer annealing temperature too low. Increase annealing temperature. Primer annealing should be at least 5 °C below the calculated Tm of primers.
Multiple bands	Prepare master mixes on ice or perform a hot-start step.
	For problems with low specificity. Try Hi-Spec Additive to improve specificity.
	Template concentration too high. Prepare serial dilutions of template.
Smearing or artefacts	Too many cycles. Reduce the cycle number by 3-5 to remove non-specific bands.
	Enzyme concentration too high – decrease the amount of enzyme in 0.5 U increments.
	Extension time too long. Reduce extension time in 0.5-1 minute increments.

For further information please contact our Customer Help Desk:

For Europe:
E-mail: info@eurogentec.com
Tel: +32 4 372 76 65

For USA:
E-mail: info.usa@eurogentec.com
Tel: +1 858 793 26 61

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