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DAP GoldStar® DNA polymerase

“Difficult Application DNA polymerase”

Technical Data Sheet

References: ME-0068-01

ME-0068-05

Licensed for PCR

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

plasmid DNA and 0.5µg Hind III-digested lambda DNA at 72°C in the presence of 20 units of DAP GoldStar®.

Batch details

Units per vial: ME-0068-01 250 units
ME-0068-05 500 units
Concentration: 4 U/µl

Unit definition

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

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® DNA polymerase is recommended for longer Genomic DNA fragments of between 2-20Kb, or up to 30Kb Lambda DNA fragments. With Lambda DNA as template, the best performance is achieved in the 2-20Kb range. The HI-Spec additive buffer improves specificity of a reaction, especially on GC rich or difficult templates.

Reaction Conditions

For a 50µl reaction	
10x OptiBuffer (provided)	5µl
MgCl ₂ , 50mM Solution (provided)	2-8 µl
dNTP final concentration	250 –500 µM each dNTP
20mM dNTP Mix (related product)	2.5 –5 µl
Template and Primers	as required
Enzyme	0.5-2µl
HI-Spec additive if necessary (See features and applications)	as required
Water (ddH ₂ O)	up to 50µl

Denature: 94-96°C
Elongate: 68°C (40-60 seconds per 1Kb)

Package contents

Reagent	Volume	Description
DAP GoldStar®	62.5 µl 125 µl	DNA pol. ME-0068-01 DNA pol. ME-0068-05
10x reaction buffer	1.25 ml	OptiBuffer™ without MgCl ₂
5x HI-Spec additive	1.20 ml	HI-Spec additive is a specificity enhancer. If necessary, re-dissolve HI-Spec by heating to 70°C and vortexing
MgCl ₂	1.20 ml	Separate 50 mM MgCl ₂

Features and applications:

Long-Region Applications: Optimal composition of different enzymatic activities enables DAP GoldStar® to span the primer extension over long regions and demonstrate high processivity by reducing premature strand termination and template degradation. Using long primers at elevated Mg⁺⁺ concentrations, >30kb or 20kb products can be achieved from lambda templates or genomic DNA, respectively.

•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’ proof-reading polymerases, 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’ proof-reading activity which reduces misincorporations during primer extension. This combination of properties provides a >17 fold higher

Shipping conditions

At +4°C or -20°C.

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

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

Associated activities

Endonuclease and exonuclease activities were not detectable after 4 hours of incubation of 1µg of pBR322

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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 a unique specificity enhancer. 5x Hi-Spec additive helps to prevent the formation of false background bands and smearing, especially on difficult templates. Hi-Spec Additive should be used at 1.0-2.0x final concentration – the optimal amount required should be determined for each individual experiment. Hi-Spec Additive may also alter the ideal annealing temperature for primers – some optimisation may be required.

Troubleshooting guide

Observation	Recommended solution(s)
No or low yield of extended product	Enzyme Concentration too low – increase the amount enzyme in 0.5U increments.
	Magnesium Concentration too low – increase concentration in 0.25mM increments with a starting concentration of 1.75mM.
	Primer Concentration not optimised. Titrate primer concentration (0.3-1µM); 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 0.5U increments.
	Extension Time too Long. Reduce extension time in 0.5-1 minute increments.

Related products

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

For any further information required please contact our Customer Help Desk:

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