

GCpro *Taq* DNA Polymerase



Technical Manual No. TM0216

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I. DESCRIPTION

GCpro *Taq* DNA Polymerase is a versatile and easy-to use enzyme, with powerful advantages for all PCR* applications. GCpro *Taq* DNA Polymerase is prepared from a recombinant clone expressed in *E. coli*, containing the DNA polymerase I gene from *Thermococcus litoralis*. The enzyme includes a highly processive 5'-3' DNA polymerase and shows no detectable 5'-3' exonuclease activity. Its inherent 3'-5' proofreading activity results in a greatly increased fidelity of DNA synthesis compared to *Taq* DNA Polymerase. The enzyme includes an optimized reaction buffer, which enables the amplification of GC-rich templates and problematic templates with secondary structures that interfere with the PCR process. GCpro *Taq* DNA Polymerase provides a very robust synthesis of longer GC-rich amplification products. GCpro *Taq* DNA Polymerase has all the properties of regular *Taq* DNA Polymerases for every standard PCR application.

II. APPLICATIONS

GCpro *Taq* DNA Polymerase can be used in most PCR applications including the following:

- High fidelity PCR amplification.
- High fidelity T/A cloning.
- Site-directed mutagenesis.

III. KEY FEATURES

Key features of GCpro *Taq* DNA Polymerase:

- Eight times more accurate than *Taq* DNA polymerase.
- Excellent yields with GC-rich and other complex templates.
- Cloning with TA end.
- High specific activity.
- High specificity due to combination of enzyme and unique PCR buffer.
- Highly thermostable, remaining 95% active after 2 hours incubation at 95°C.
- Incorporating modified nucleotides (e.g. biotin-, fluorescent-labeled nucleotides).



IV. RELATED PRODUCTS

GenScript also provides GCpro Taq DNA Polymerase Complete System to facilitate your experiment:

Components	E00009	E00010
GCpro Taq DNA Polymerase	250 U	250 U
2x Reaction Buffer	1.5 ml	1.5 ml
dNTPs		50 μ l (10 mM each)
Lyophilized Positive Control		50 rxns
Manual	TM0216	TM0216

V. SHIPPING AND STORAGE

This product is shipped on ice. Store the product at -20°C.

VI. GENERAL PCR PROTOCOL USING GCPRO TAQ POLYMERASE

This is a general PCR amplification protocol, optimization may be needed to get satisfactory results.

1. Set up 50 μ l PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently.

Reagent	Volume	Final Concentration
Water, PCR grade	20 μ l	
2X Buffer	25 μ l	1X
10 mM dNTP	1 μ l	200 nM each
20 μ M Forward primer	1 μ l	400 nM
20 μ M Reverse primer	1 μ l	400 nM
Template DNA	1 μ l	1-100 ng per reaction
5 U/ μ l GCpro Taq DNA Polymerase	1 μ l	0.1 U/ μ l
Total	50 μ l	

2. Suggested PCR profile:

- i. Initial Denaturation: 94-96°C for 2 min.
- ii. 25-30 cycles of Denaturation: 94-96°C for 30 sec.
- iii. Annealing: 50-60°C for 30 sec (primer T_m - 5°C).
- iv. Extension: 72°C for 30 sec to 5 min (1.5-2.5 kb/min).
- v. Final extension: 72°C for 7 min.



VII. TROUBLESHOOTING

The table below is guideline for troubleshooting.

Problem	Probable Cause	Solution
No PCR DNA	<p>Extension time is too short.</p> <p>One or more PCR components may be missing.</p> <p>PCR conditions are not optimized. The annealing temperature may be too high; more cycles may be needed; the denaturation time may be too short.</p> <p>The primers may not be designed optimally.</p> <p>Target template is highly GC-rich.</p>	<p>GCpro Taq DNA Polymerase require a minimum extension time of 1-2 min for 1 kb template.</p> <p>Run a positive control to make sure the PCR reaction is properly set-up.</p> <p>Optimize the PCR conditions by decreasing annealing temperature in 2-4°C increments, or increasing the number of cycles, or increasing the denaturation time in 10 sec increments. It is recommended to change only one parameter each time.</p> <p>The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.</p> <p>The target will be difficult to denature even with a longer denaturation step.</p>
Non-specific DNA products	<p>The primers may not be designed optimally.</p> <p>Annealing temperature is too low.</p>	<p>See above.</p> <p>Optimize the PCR conditions by increasing annealing temperature in 2-4°C increments, or decreasing the number of cycles.</p>
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

VIII. ORDER INFORMATION

GCpro Taq DNA Polymerase, Cat. No. E00009
 Telephone: 732-885-9188, 732-357-3839
 Fax: 732-210-0262, 732-885-5878
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For Research Use Only.

* The PCR process is covered by US. Patent numbers 4683195 and 4683202 issued to Cetus and owned by Hoffman-La Roche Inc. GenScript does not encourage or support the unauthorized use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

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