PRODUCT INSERT

GENETAQ Green DNA Polymerase

Store at -20°C

Cat. No. PGP0217

Pack Size 500U 5U/µl

Ordering Information

Component	GENETAQ DNA, Polymerase 5 U/µL	10X GENETAQ Green Buffer*
PGP0217	500 U	2X1.25 mL

* includes 20 mM $MgCl_2$

Description

Puregene's GENETAQ Green DNA Polymerase is a combination of GENETAQ DNA Polymerase and 10X GENETAQ Green Buffer. GENETAQ DNA Polymerase is an enhanced Taq DNA Polymerase optimized for high throughput PCR applications. It ensures higher sensitivity, longer PCR products and higher yields compared to conventional Taq DNA polymerase. GENETAQ Green DNA Polymerase incorporates modified nucleotides, but is inhibited by dUTP.

The 10X GENETAQ Green Buffer includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The colored buffer does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, phosphorylation, ligation and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation.

Features

- Save time – go directly from PCR to gel electrophoresis.
- High yields of PCR products with minimal optimization.
- Higher sensitivity compared to conventional Taq DNA Polymerase.
- Amplification of long targets (up to 6 kb from genomic DNA, up to 20 kb from viral DNA).
- Robust amplification of difficult templates.

Applications

- Routine PCR amplification of DNA fragments up to 6 kb from from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

Concentration

- 5 U/µL

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 70°C.

Enzyme activity is assayed in the following mixture: 67 mM Tris-HCl (pH 8.8 at 25°C), 6.7 mM MgCl2, 1 mM 2-mercaptoethanol, 50 mM NaCl, 0.1 mg/mL BSA, 0.75 mM activated calf thymus DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [3H]-dTTP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol.

10X GENETAQ Green Buffer

In addition to the PCR buffer components, includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The 10X GENETAQ Green Buffer contains KCl and (NH4)2SO4 at a ratio optimized for robust performance in PCR and includes MgCl2 at a concentration of 20 mM.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and GENETAQ DNA Polymerase. Prepare enaugh master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

10X GENETAQ Green Buffer*	5 µL
dNTP Mix, 2 mM each	5 µL (0.2 mM of each)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
GENETAQ Green DNA Polymerase	1.25 U
Water, nuclease-free	to 50 µL
Total volume	50 µL

*10X GeneTaq Green Buffer contains 20 mm MgCl2, which is optimal for most applications. If additional optimization is required, 25 mm MgCl2 can be added to the master mix. The volume of water should be reduced accordingly.

PRODUCT INSERT

GENETAQ Green DNA Polymerase

Store at -20°C

Volumes of 25 mM MgCl2, required for specific final MgCl2 concentration:

Final concentration of MgCl2	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM MgCl2 to be added for 50 µL reaction	0 µL	1 µL	2 µL	4 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Place the reactions in a thermal cycler. Perform PCR using recommended thermal cycling conditions:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	
Annealing	Tm-5	30 s	25-40
Extension*	72	1 min	
Final Extension	72	5-15 min	1

** The recommended extension step is 1 min for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb.*

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION
During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, .
- Always perform “no template control” (NTC) reactions to check for contamination.

GENETAQ DNA Polymerase does not incorporate dUTP, therefore it is not possible to perform carryover contamination prevention with UDG. For this application we recommend using Taq DNA Polymerase Hot Start Taq DNA polymerase.

GUIDELINES FOR PRIMER DESIGN
follow general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.
- When introducing restriction enzyme sites into primers, refer to the table “Cleavage efficiency close to the termini of PCR fragments” located on www.onebio.com to determine the number of extra bases required for efficient cleavage.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

Estimation of primer melting temperature
For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:
Tm= 4 (G + C) + 2 (A + T),
where G, C, A, T represent the number of respective nucleotides in the primer. If the primer contains more than 25 nucleotides specialized computer programs is recommended to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE
Template DNA
Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.
All routine DNA purification methods are suitable for template preparation. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

MgCl2 concentration
GENETAQ Green DNA Polymerase is provided with an optimized 10X GENETAQ Green Buffer which includes MgCl2 at a concentration of 20 mM. A final MgCl2 concentration of 2 mM is generally ideal for PCR. The MgCl2 concentration can be further increased up to 4 mM by the addition of 25 mM MgCl2.
If the DNA samples contain EDTA or other metal chelators, the Mg2+ ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg2+).

PRODUCT INSERT

PUREGENE GENETIX BRAND

GENETAQ Green DNA Polymerase

Store at -20°C

dNTPs
The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) present in the reaction mixture.
To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, please refer to the table below:

Volume of PCR mixture	dNTP Mix, 2 mM each	dNTP Mix, 10 mM each	dNTP Mix, 25 mM each
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Primers
The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.
For degenerate primers and primers used for long PCR, we recommend higher primer concentrations in the range of 0.3-1 µM.

CYCLING PARAMETERS

Initial DNA denaturation
It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient. For GC-rich templates this step should be prolonged up to 10 min. If a longer initial denaturation step is required, or if the DNA is denatured at a higher temperature, GENETAQ DNA Polymerase should be added after the initial denaturation step to avoid a decrease in its activity.

Denaturation
A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol, 10% DMSO, 5% formamide or 1-1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.
In addition, 10% DMSO and 5% formamide inhibit DNA polymerases by 50%. Thus, the amount of the enzyme in the reaction should be increased if these additives are used.

Primer annealing
The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments. When additives which change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension
The optimal extension temperature for GENETAQ DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb. For amplification of templates >6 kb a reduction of the extension temperature to 68°C is recommended to avoid enzyme inactivation during prolonged extension times.

Number of cycles
The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.
If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension
After the last cycle, it is recommended to incubate the PCR mixture at 72°C for additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product will be cloned into TA, the final extension step may be prolonged to 30 min to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning, the final extension step can be omitted.

CERTIFICATE OF ANALYSIS
Endodeoxyribonuclease Assay
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 units of GENETAQ Green DNA Polymerase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Exodeoxyribonuclease Assay
No degradation of DNA was observed after incubation of 1 µg lambda DNA/HindIII fragments with 10 units of GENETAQ Green DNA Polymerase for 4 hours at 37°C.

Ribonuclease Assay
No contaminating RNase activity was detected after incubation of 10 units of GENETAQ Green DNA Polymerase with 1 µg of [3H]-RNA for 4 hours at 37°C.

Functional Assay
GENETAQ DNA Polymerase was tested for amplification of 956 bp single copy gene from human genomic DNA and for amplification of 20 kb lambda DNA fragment.