

Data sheet

Horse-Power™ Green-Taq DNA polymerase

Cat. No: P0023-GT

Introduction

Horse-Power Taq DNA polymerase is a thermostable recombinant enzyme produced in a *E.coli* strain, that carries the cloned pol gene from *Thermus aquaticus*. The enzyme has 5'→3' polymerase activity and a weak 5'→3' exonuclease activity but no 3'→5' exonuclease activity (proofreading).

The 10X Green Buffer contains an agarose loading buffer including two tracking dyes (blue and yellow dye) for visual tracking of DNA migration and a dense compound to facilitate the drop-down of the samples into the well agarose gels. The blue dye (migrates with 3 to 5 kb DNA fragments in 1% agarose gel) and the yellow dye (migrates faster than 10 bp DNA fragments in 1% agarose gel).

Features

- Molecular Weight: 94 kDa.
- Thermostable (half life at 94°C is 40 minute).
- Adds extra nucleotides (preferentially adenine) without template at 3' ends leaving 3' overhangs PCR fragments. This fact allows the popular TA-cloning or GC cloning.
- Save time – go directly from PCR to gel electrophoresis.

Applications

- Routine amplifications.
- Colony screening.
- Amplifications up to 6 kb using plasmid, viral or genomic DNA as template.
- PCR fragments amplification for TA or GC cloning

Package Content

	P0023-GT
Taq DNA Polymerase (5U/μl)	100 μl
10x Green Buffer*	2 X 1.25 ml

* Includes 20 mM MgCl₂

Storage: Store at -20°C.

Assay conditions

25mM Tris-HCl pH9.0 at 25°C, 50mM KCl, 2mM MgCl₂, 0.1mg/mL gelatine, 200 μM de dATP, dGTP, dTTP, 100μM[α32-P]dCTP (0.05μCi/nmol) and 12.5 μg activated salmon sperm DNA.

Unit definition: One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

Concentration: 5 U/μL

Quality Certifications

- Functionally tested in PCR.
- Undetected bacterial DNA (by PCR).
- Undetectable nucleases activity (endo-, exo, and ribo-).

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Basic Protocol

- Thaw on ice and mix all reagents well.
- Keep all reagents and reactions on ice.
- When setting up multiple reactions, prepare a master mix of water, buffer, dNTPs and polymerase. Prepare enough master mix for one more than the actual number reactions.
- Pipet the master mix into thin-walled 0.2 ml PCR tubes.
- Add template and primers separately if they are not used in all reactions.

Recommended PCR assay (20 µl assay)

(For total reaction volumes other than 20 µl, scale the reagents proportionally)

Components	Volume	Final con.
1. 10X Green buffer*	2 µL	1X
2. dNTPs 8 mM mixture	2 µL	0.8 mM
3. Primer Forward	variable	0.2–1 µM
4. Primer Reverse	variable	0.2–1 µM
5. Template DNA	0.2-10 µL	1.75-2.5 ng/µL
6. Horse-Power-Taq DNA polymerase (5 U/µL)	0.2 µL	0.05 U/µL
7. Nuclease free water	to 20 µL	-

*10X Green Buffer contains 20 mM MgCl₂, which is optimal for most applications. If additional optimization is required, 25 mM MgCl₂ (Cat. No BR0087) can be added to the master mix. The optimal Mg²⁺ concentration should be determined empirically.

Cycling instructions:

Step	Temperature	Time	Cycle
Initial activation	94°C	5min	1
Denaturation	94°C	35 sec	25-30
Annealing	55°C*	0.5 - 1 min.	
Extension	72°C**	1'/kb	
Final extension	72°C	10 min	1
Storage in the cycler	4°C	∞	1

*Recommended annealing temperature is 5°C below T_m of primers, or use gradient PCR to optimize the annealing temperature.

**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.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.