

Version: 2 Revision date: 14/06/2023 Canvax Reagents, S.L.U. Luis de Mercado Street, 19 Boecillo Technological Park

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# 1. Identification

Product name Horse-Power<sup>TM</sup> Green-Tag DNA polymerase

(5 U/µl) 500U

Cat. No P0023-GT

# 2. Description

**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  $5' \rightarrow 3'$  polymerase activity and a weak  $5' \rightarrow 3'$  exonuclease activity but no  $3' \rightarrow 5'$  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).

## 3. Composition

Item	Quantity
Horse-Power Taq DNA polymerase (5U/µL)	100 µL
10X Green Buffer	2 x 1.25 mL

## 4. Features

# Molecular Weight: 94 kDa.

- > Thermostable (half-life at 94 °C is 40 minutes).
- 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.
- Incorporates modified nucleotides (biotinylated, fluorescently labelled, etc).

# Quality:

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

### 5. Storage specifications

Store at -20° C

#### 6. 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.





# DATA SHEET

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## 7. Further information

Product Use Limitation This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

## **Assay conditions**

25 mM Tris-HCl pH 9.0 at 25 °C, 50 mM KCl, 2 mg MgCl2, 0.1 mg/mL gelatine, 200  $\mu$ M dATP, dGTP, dTTP, 100  $\mu$ M [ $\alpha$ 32-P] dCTP (0.05  $\mu$ Ci/nmol) and 12.5  $\mu$ g 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.

# RECOMMENDED PCR ASSAY (20µL assay)

- ·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.

Components	Volume	Final con.
10X Green buffer	2 μL	1X
dNTPs 8mM mix	2 µL	0.8 mM
Primer Forward (15mM)	ΧμL	0.2 - 1 µm
Primer Reverse (15mM)	ΧμL	0.2 – 1 µm
Template DNA	0.2-10 µL	1.75-2.50 ng/μL
Horse-Power Taq DNA polymerase (5 U/µl)	0.2 μL	0.05 U/μL
Autoclaved distilled water	to 20 µL	-

<sup>\*10</sup>X 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	1X	1
Denaturation	94°C	0.8 mM	
Annealing	55°C*	0.2 <b>-</b> 1 μm	25-30
Extension	72°C**	0.2 – 1 µm	
Final extension	72°C	1.75-2.50 ng/μL	1
Storage in the cycler	4°C	0.05 U/µL	1

<sup>\*</sup>Recommended annealing temperature is 5°C below Tm of primers, or use gradient PCR to optimize the annealing temperature.

<sup>&</sup>quot;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.

