x-VITA™ Green-Taq Polymerase

TAQP-SG5-001

Description

xVITA™ Green-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 94ºC is 40 minute)
- ✓ Adds extra nucleotides (preferentially adenine) without template at 3´ends leaving 3´overhangs PCR fragments.
- ✓ 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

Components	
Taq DNA Polymerase (5U/µl)	100 µl
10x Green Buffer*	2x1.25 ml

*Includes 20 mM $MgCl_2$

Storage

Store at -20ºC.

Assay conditions

Store at 25mM Tris-HCl pH9.0 at 25°C, 50mM KCl, 2mM $MgCl_2$, 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.

Quality certifications

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

Protocol

1. Thaw on ice and mix all reagents well.
2. Keep all reagents and reactions on ice.
3. 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.
4. Pipet the master mix into thin walled 0.2 ml PCR tubes.
5. Add template and primers separately if they are not used in all reactions.

Recommended PCR assay (20ul assay)

Components	Volume	Final concentration
10X PCR buffer*	2 µL	1X
dNTPs 8 mM mixture	2 µL	0.8 mM
Primer Forward (15 mM)	variable	0.2 - 1 µm
Primer Reverse (15 mM)	variable	0.2 - 1 µm
Template DNA	0.2-10 µL	1.75-2.5 ng/µL
Taq DNA polymerase (5 U/µL)	0.2 µL	0.05 U/µL
PCR grade H_2O	to 20 µL	-

*10X Green Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. If additional optimization is required, 25 mM $MgCl_2$ can be added to the master mix. The optimal Mg^{2+}+ concentration should be determined empirically.

For total reaction volumes other than 20ul, scale the reagents proportionally.

Cycling instructions

Step	Temperature	Time	Cycle
Initial activation	94ºC	5 min	1
Denaturation	94ºC	35 sec	25 - 30
Annealing	55ºC*	30 sec -1 min	
Extension	72ºC**	1’ / kb	
Final extensión	72ºC	10 min	1
Storage in the cycler	4ºC	∞	1

*Recommended annealing temperature is 5°C below Tm 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.