# x-VITA™ Taq Polymerase for long fragments

TAQP-PF1-001

## Description

x-VITA™ Long Taq Polymerase, a blend of two thermostable DNA polymerases, x-VITA Taq and x-VITA™ proofreading, a specialized formulation crafted for amplifying lengthy DNA fragments. This unique Long Taq DNA Polymerase formulation has demonstrated the capability to amplify extended DNA templates from the λ phage genome of up to 20 kb. It also serves as a superior option for amplifying complex templates, such as those rich in GC content.

It can effectively substitute ordinary Taq Polymerase in most applications. It boasts an elongation rate of 3 kb/min. The resulting products consist of a mixture of 3´-dA overhangs and blunt-ended products, facilitating their utility in TA cloning.

## Components

| | |
|---|---|
| Long Taq DNA Polymerase | 50 µl |
| 10× Long PCR Buffer ($Mg^{2+}$ Plus) | 1,25 ml |
| 10× Long PCR Buffer ($Mg^{2+}$ Free) | 1,25 ml |
| PCR Enhancer | 500 µl |
| 6× Loading Buffer | 1 ml |

- ✓ Storage Buffer: 20mM Tris-HCl (pH 8.0), 100mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (V/V) glycerol.
- ✓ 10X Long PCR Buffer I with $Mg^{2+}$: 500mM Tris-HCl (pH 8.8), 160mM $(NH_4)_2SO_4$, 25mM $MgCl_2$, 1% Triton X-100.
- ✓ 10X Long PCR Buffer without $Mg^{2+}$: 200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM $(NH_4)_2SO_4$, 16mM $MgSO_4$, 1% Triton X-100.

## Applications

- ✓ PCR amplification of complex template DNA
- ✓ PCR amplification of long DNA sequences
- ✓ DNA sequencing and PCR cloning

## Features

- ✓ High fidelity.
- ✓ Longer fragment: amplify long templates as long as 20 kb.
- ✓ Amplification of complex template (GC rich or repetitive sequence).
- ✓ Generates 3'-dA and blunt-end PCR products.

## Unit definition

One unit is defined as the amount of the enzyme required to catalyse the incorporation of 10 nM of dNTPs into an acid-insoluble form in 30 minutes at 70°C using herring sperm DNA as substrate.

## Storage

Store at -20 ºC.

## Product use limitation

This product is developed, designed, and sold exclusively for research purposes and use. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

## Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Long Taq DNA Polymerase, primers, Mg2+, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

1.1 Recommended PCR assay with Long PCR Buffer ($Mg^{2+}$ plus)

| Reagent | Quantity | Final concentration |
|---|---|---|
| Sterile deionized water | variable | - |
| 10× Long PCR Buffer ($Mg^{2+}$ plus) | 5 µl | 1× |
| dNTPs (10mM each) | 1 µl | 0.2mM each |
| Primer I | variable | 0.4-1µM |
| Primer II | variable | 0.4-1µM |
| Long Taq DNA Polymerase (5U/µl) | 0.25-0.5 µl | 1.25-2.5U/50 µl |
| Template DNA | variable | 10pg-1µg |
| Total* | | 50 µl |

* 4-16 µl PCR Enhancer can be added to the reaction system of 50 µl. By reducing the dissociation temperature of DNA template and promoting the effective amplification of DNA template, PCR Enhancer can increase the sensitivity and specificity of PCR reaction.

1.2 Recommended PCR assay with Long PCR Buffer ($Mg^{2+}$ free)

| Reagent | Quantity | Final concentration |
|---|---|---|
| Sterile deionized water | variable | - |
| 10× Long PCR Buffer ($Mg^{2+}$ free) | 5 µl | 1× |
| dNTPs (10mM each) | 1 µl | 0.2mM each |
| Primer I | variable | 0,4 - 1µM |
| Primer II | variable | 0,4 - 1µM |
| 25mM $Mg^{2+}$ | variable | 1,0 – 4,0mM |
| Long Taq DNA Polymerase (5U/µl) | 0,25 – 0,5 µl | 1,25 – 2,5U/50 µl |
| Template DNA | variable | 10pg - 1µg |
| Total | | 50 µl |

Table for selection volume of 25 mM $MgCl_2$ solution in a 50 µl reaction mix:

| Final $Mg^{2+}$ Conc. (mM) | 1,0 | 1,5 | 2,0 | 2,5 | 3,0 | 4,0 |
|---|---|---|---|---|---|---|
| $Mg^{2+}$ (25mM) | 2 µl | 3 µl | 4 µl | 5 µl | 6 µl | 8 µl |

Recommendation amounts of template DNA in a 50 µl reaction mix:

| | |
|---|---|
| Human genomic DNA | 0,1µg - 1µg |
| Plasmid DNA | 0,5ng - 5ng |
| Phage DNA | 0,1ng - 10ng |
| *E.coli* genomic DNA | 10ng - 100ng |

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

| | | |
|---|---|---|
| Initial Denaturation | 94°C | 3 minutes |
| 25-35 Cycles | 94°C<br>55-68°C<br>72°C | 30 seconds<br>30 seconds<br>1 - 10 minutes |
| Final Extension | 72°C | 10 minutes |

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
5. Analyse the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

***Notes on cycling conditions***

- ✓ Initial denaturation can be performed over an interval of 1-5 min at 94°C-95°C depending on the GC content of template.
- ✓ Denaturation for 30 sec to 2 min at 94-95°C is sufficient. If the amplified DNA has a very high GC content, denaturation time may be increased up to 3-4 min.
- ✓ Optimal annealing temperature is 5°C lower than the melting temperature of duplex primers. If nonspecific PCR products are obtained, optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
- ✓ The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR products. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.
- ✓ The final extension step can extend amplicons that will be cloned into T/A vectors.