x-VITA™ Proofreading DNA Polymerase

TAQP-PF1-001

Description

The x-VITA proofreading DNA polymerase, derived from the hyperthermophilic archaeon *Pyrococcus furiosus*, boasts superior thermostability and proofreading abilities compared to other thermostable polymerases. With a molecular weight of 90 kDa, it efficiently amplifies DNA targets up to 2 kb in length from simple templates, with an elongation rate of 1 kb/min at temperatures between 70 to 75°C. Notably, this DNA polymerase possesses 3' to 5' exonuclease proofreading activity, enabling it to correct nucleotide-misincorporation errors during DNA synthesis. Consequently, the generated PCR fragments typically exhibit fewer errors compared to those produced by other Taq polymerases. Furthermore, using x-VITA proofreading DNA polymerase in PCR reactions yields blunt-ended PCR products, making them well-suited for cloning into blunt-ended vectors. Due to its high-fidelity DNA synthesis capabilities, it is particularly advantageous for techniques requiring precise DNA replication.

Components

Proofreading Taq DNA Polymerase	100 µl
10× PCR Buffer (Mg^{2+} Plus)	1,25 ml
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 PCR Buffer I with Mg^{2+}: 500mM Tris-HCl (pH 8.8), 160mM $(NH_4)_2SO_4$, 25mM $MgCl_2$, 1% Triton X-100.

Applications

- ✓ High-fidelity PCR and primer-extension reactions
- ✓ High fidelity PCR for cloning into blunt ended vectors
- ✓ Site-directed mutagenesis

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 Proofreading Taq DNA Polymerase, primers, Mg^{2+}, 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

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× 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
Taq DNA Polymerase (5U/µl)	0.5-1.0 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 55-68°C 72°C	30 seconds 30 seconds 1 - 3 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

- ✓ The optimal reaction conditions (incubation time and temperature, concentration of DNA Polymerase, template DNA, Mg2+) depend on the template-primer pair and must be determined individually. It is especially important to titrate the Mg2+ concentration and the amount of enzyme required per assay. The standard concentration of Mg2+ is 2mM and

amount of DNA Polymerase is 1.25U per 50 µl of reaction mixture.

- ✓ DNA Polymerase remains 95% active after 2 hours incubation at 95°C.
- ✓ The error rate of DNA Polymerase in PCR is 2.6×10-6 errors per nt per cycle; the accuracy (an inverse of error rate), an average number of correct nucleotides incorporated before making an error is 3.8×10-5 (determined according to the modified method described in)
- ✓ DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labelled nucleotides) as substrates for the DNA synthesis.
- ✓ The enzyme has no detectable reverse transcriptase activity.
- ✓ Do not use dUTP in PCR.
- ✓ The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.