x-VITA™ MasterMix for Proofreading PCR (2X)

TAQM-PF1-001

Description

Premixed, ready-to-use solution that includes x-VITA proofreading DNA polymerase, dNTPs, Mg^{2+}, and Reaction Buffer at optimal concentrations for efficient DNA template amplification via PCR. To prepare the final PCR mixture, you only need to add primers and template DNA. This premixed formulation saves time and reduces contamination risks, as it requires fewer pipetting steps during PCR setup. Additionally, it enhances sensitivity, thereby contributing to improved overall performance.

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.

Composition

0.15U/μl Pfu DNA Polymerase, 2X Pfu Buffer, 0.4mM dNTPs, 4mM MgSO4, 0.02% bromophenol blue.

Applications

- ✓ High fidelity PCR
- ✓ Routine PCR with high reproducibility
- ✓ Site-directed mutagenesis
- ✓ Generation of PCR products for TA cloning

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

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

Reagent	Quantity	Final concentration
2X Proofreading Taq Mix	25 µl	1×
Forward Primer	variable	0,4 - 1 µM
Reverse Primer	variable	0,4 - 1 µM
Template DNA	variable	10pg – 1 µg
Water, nuclease-free	to 50 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 in the 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 - 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

- ✓ Recombinant Taq DNA Polymerase is suitable for most PCR applications.
- ✓ The half-life of Taq DNA Polymerase is >40 minutes at 95°C.
- ✓ The error rate of Taq DNA Polymerase in PCR is 2.2×10-5 errors per nt per cycle
- ✓ Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labelled nucleotides) as substrates for the DNA synthesis.
- ✓ 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.