x-VITA™ MasterMix for long fragment PCR (2X)

TAQM-LT1-001

Description

Premixed, ready-to-use solution that includes x-VITA Long Taq 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.

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

0.25U/μl Taq DNA Polymerase, 0.01U/μl Proofreading DNA Polymerase, 2× PCR Buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$, 0.02% bromophenol blue.

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

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

Reagent	Quantity	Final concentration
2X Long 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
PCR Enhancer	4 - 16 µl	–
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 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 – 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.