Ultra High Fidelity DNA Polymerase Master Mix

(1000 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22119-1000

www.zellx.de

PCR-Cloning | Primers extension | Long or difficult amplification | High-Throughput PCR

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Introduction

ZellX® Ultra High Fidelity DNA Polymerase Master Mix is an optimized ready-to-use master mix that contains all PCR reaction reagents including dNTPs, PCR buffer, $MgCl_2$ and Ultra High Fidelity DNA polymerase (except primers and DNA template) needed for running PCR reactions. ZELLX® Ultra High Fidelity DNA Polymerase is a second generation and the best High-fidelity DNA Polymerase in the market that exhibits magnificent performance for all PCR applications. It amplifies long templates with a robust accuracy and speed. The Fidelity of ZELLX® Ultra High Fidelity DNA Polymerase is more than **50-fold** higher comparing to the normal Taq DNA Polymerase.

It possesses the 5'→ 3´ DNA polymerase activity, combined with a robust 3´→ 5´ exonuclease activity to generate long PCR products with blunt ends for further cloning application. It is also suitable for amplification of long amplicons such as 10-20 kb of genomic DNA. With an amazing fidelity rate it is a great option to be used in cloning/subcloning DNA for protein expression, SNP analysis and next generation sequencing applications.

The ZellX® Ultra High Fidelity DNA Polymerase Master Mix formulation saves time and eliminates or decreases the risk of error and contamination due to a reduced number of pipetting steps.

Materials supplied in the Kit

Component	*Quantity*
Ultra High Fidelity DNA Polymerase Master Mix (2X)	10 mL (8 x 1.25 mL)
DMSO (100%)	1000 µL
$MgCl_2$ (25 mM)	200 µL

Storage instruction

All reagents should be stored at -20°C upon receipt. Avoid repeated freezing and thawing.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips (RNase & DNase free)

Nuclease-free tubes / strips / plates corresponding to the PCR device

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed. PCR machine / Thermocycler must be turned on and programmed in advance to avoid delays after setting up the reactions.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay Procedure

For 20 µL reaction

- Thaw Master Mix on ice and mix them well. Collect liquid at the bottom of the tube with a quick spin.
- Set up the following reaction mixture.
- **Important Note:** Ultra High Fidelity DNA polymerase Master Mix must be added as last component to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.

Component	*Quantity*
Ultra High Fidelity DNA Polymerase Master Mix	10 µL
Forward Primer	0.5 µM
Reverse Primer	0.5 µM
DNA template	20-50 ng (Plasmid DNA) 100-250 ng (Genomic DNA)
DMSO*	3%
Nuclease-Free Water	Up to 20 µL

***** Addition of DMSO is recommended for GC-rich amplicons. If DMSO is added in the PCR reaction, Tm must be decreased about 3° C**.**

Mix reagents thoroughly, and transfer to the thermocycler.

- Run the appropriate PCR cycling protocol on your PCR instrument.

Step	Number of Cycles	Temperature	Duration
Initial activation	1	98°C	3 min
Amplification	25-35	98°C	5-10 sec
		TM	10-35 sec
		72°C	15-30 sec/kb*
Cooling	1	72°C	5-10 min
		4°C	∞

* Extension time depends on amplicon length and complexity:

• For low complexity DNA (eg. Plasmid, lambda, or BAC DNA) use 15 s per Kb.

• For high complexity DNA (eg. gDNA) use 30s per kb. Do not exceed 1 min. per kb for amplicons that are <3 kb.

- The appropriate PCR cycling protocol must be optimized by the end user
- high primer concentrations result in nonspecific amplification and should be avoided