Hy-Fy High Fidelity Mix (x2)

#EZ-2021 1ml, 100rxn of 20µl

Contents: 2X High Fidelity Mix 1ml

Nuclease-free water 1ml

Store at -20°C

Shelf life: 2 years

Description

Hy-Fy High Fidelity Mix (x2) is a premixed, ready-to-use solution containing High Fidelity DNA Polymerase, dNTPs, MgSO4 and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only primers and template DNA are added. High Fidelity Mix contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher sensitivity by adding intensifier and optimizer. High Fidelity DNA polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has been shown to exhibit superior thermostability and proofreading properties compared to other thermostable polymerase. Unlike Taq DNA polymerase, highly thermostable High Fidelity DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that High Fidelity DNA polymerase-generated PCR fragments will have fewer errors than Taq-generated PCR inserts. Use of High Fidelity DNA polymerase in your PCR reactions results in blunt-ended PCR products, which are ideal for cloning into blunt-ended vectors. High Fidelity DNA polymerase is superior for techniques that require high-fidelity DNA synthesis.

Life Technologies (India) Pvt Ltd.
306, Agarwal City Mall, opposite M2K Pitampura, Delhi-110034 (India)
Tel # +91-11-4220-8000; 4220-8111; 4220-8222 Fax# +91-11-4220-8444, Mobile# +91-98105-21400
Email - customerservice@lifetechindia.com | customerservice@atzlabs.com

Applications

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

Feature

- Convenient –High Fidelity DNA Polymerase in a ready-to-use Mix.
- High yields of PCR products with minimal optimization.
- Fast -saves time due to reduced number of pipetting steps.
- Reproducible -lower contamination and pipetting error risk.

Composition of the 2x High Fidelity Ready Mix

High Fidelity DNA polymerase is supplied in 2X High Fidelity buffer, dNTPs, 3 mM MgSO4 and bromophenol blue. High Fidelity mix buffer is a proprietary formulation optimized for robust performance in PCR.

Basic PCR Protocol

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. **Add in a thin walled PCR tube on ice:**

   For a total 20µl reaction volume

Component of sample	Volume	Final concentration
High Fidelity Ready Mix (2X)	10 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM

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Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 20µl	—

Recommendations with Template DNA in a 20µl reaction volume

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.2 ng-3 ng
Phage DNA	0.1 ng-4 ng
E.coli genomic DNA	4 ng-40 ng

2. **Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.**
3. **Overlay the sample with mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid**.
4. **Perform PCR using the following thermal cycling conditions:**

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

Note: The elongation velocity is 1KB/min. For complex templates the elongation time should be longer.

5. **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.**
6. **Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.**

Notes on cycling conditions

- Hy-Fy High Fidelity Mix (x2) for High fidelity PCR applications.
- The error rate of High Fidelity DNA Polymerase in PCR is 2.6x10-6 errors per nt per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is 3.8x105 (determined according to the modified method described in)
- High Fidelity DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The enzyme has no detectable reverse transcriptase activity.
- 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.

Guidelines for preventing contamination of PCR reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

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Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests, functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25µl High Fidelity Mix (2X) with 1µg pBR322 DNA in 50µl for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25µl High Fidelity Mix (2X) with 1µg digested DNA in 50µl for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of High Fidelity Mix (2X) with 1µg E.coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C and 70°C.

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