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倍沃医学

High Fidelity DNA Polymerase

Store at -20 ℃

Description

High Fidelity DNA Polymerase (Hi Fi DNA Polymerase) is a highly thermostable DNA polymerase from the hyperthermophilic archaeum *Pyrococcus furiosus*. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5’→3’ direction. Hi Fi DNA Polymerase also exhibits 3’→5’ exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5’→3’ exonuclease activity and no detectable reverse transcriptase activity.

The error rate of Hi Fi DNA Polymerase in PCR is $2.6x10^{-6}$ errors per nt per cycle, as determined by a modified method described in.

Note

dUTP, dITP and primers containing these nucleotides should not be used in PCR with Hi Fi DNA Polymerase because the binding of this enzyme to DNA templates with uracil and hypoxanthine stalls DNA synthesis.

Applications

- High fidelity PCR.
- Generation of PCR products for cloning and expression.
- RT-PCR for cDNA cloning and expression.
- Generation of PCR product for blunt-end cloning.
- Site-directed mutagenesis.

Source

E.coli with a cloned pol gene from Pyrococcu

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 72°C.

Kit Contents

Component	HF1101-00/10	HF1101-01/11	HF1101-02/12
Hi Fi DNA Polymerase	50 Units (10 µL)	250 Units (50 µL)	500 Units (100 µL)
5 x Hi Fi PCR Buffer(with Mg^{2+})	0.4mL	2 x 1.5 mL	3 x 1.5 mL
10 mM dNTPs	-/400 µL	-/200 µL	-/400 µL
Nuclease-free water	2 mL	12 mL	18 mL

PROTOCOL

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and template DNA. Hi Fi DNA Polymerase should be the last component added. Prepare sufficient master mix for the number of reactions plus one extra to allow for pipeting error.

1. Gently vortex and briefly centrifuge all solutions after thawing.

2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

Hi Fi DNA Polymerase (5 U/µL)	0.25 µL
5 x Hi Fi PCR Buffer (with Mg^{2+})	10 µL
dNTP Mixture (2.5 mM each)	4 µL
Template DNA	50 pg-1 µg
Forward primer (20 µM)	1 µL
Reverse primer (20 µM)	1 µL
Nuclease-free water	Up to 50 µL

3. Gently vortex the samples and spin down.

4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25 μL of mineral oil.

5. Perform PCR using the following thermal cycling conditions:

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-35
Annealing	Tm-5	30 s	
Extension	72	0.5 kb/min	
Final extension	72	5-15 min	1

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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