FT-1L7480

Long High Fidelity PCR Enzyme Mix

High Fidelity DNA Polymerase is a blend of DNA polymerases specially designed for the highly accurate and efficient amplification of fragments up to 30 kb including GC-rich or other difficult templates.

Product Description

Name :	**LongHigh Fidelity PCR Enzyme Mix**
Catalog Number :	1L7480, 100U, 2,5U/µl 1L7481, 500U, 2,5U/µl
Unit Definition :	One unit catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 minutes at 72°C.
Storage Buffer :	20 mM Tris/HCl (pH 8.0 @ 25°C), 100 mM KCL, 1 mM DTT, 0.1 mM EDTA, 0.1% (v/v) Nonidet P-40, 1 mM PMSF, 0.1% (v/v) Tween 20, 50% (v/v) Glycerol.
10X Reaction Buffer :	500 mM Tris/HCl, 140 mM (NH4)2SO4, 17.5 mM MgCl2 (pH 9.1 at 25°C).

Storage: Store at -20°C for 24 months.

Technical and Scientific Information

Endonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 15 units of pfu/High Fidelity DNA Polymerase with 0.5 µg of pUC-19-plasmid-DNA in 10 µl of 1x Reaction Buffer containing 1.5 mM $MgCl_2$ for 2 hours at 72°C.

Exonuclease Assay

No detectable degradation (smearing) of fragments was observed after incubation of 5 units of pfu/High Fidelity DNA Polymerase with 0.5 µg of pUC-19-plasmid-DNA (digested with Hpa II) in 10 µl of 1x Reaction Buffer containing 1.5 mM $MgCl_2$ for 2 hours at 72°C.

Ribonuclease Assay

No detectable degradation of 28S/18S bands was observed after incubation of 5 units of pfu/High Fidelity DNA Polymerase with 1 µg of total RNA (from rat liver) in 10 µl of 1x Reaction Buffer containing 1.5 mM $MgCl_2$ for 2 hours at 72°C.

Functional Assay

pfu DNA Polymerase and High Fidelity DNA Polymerase were tested for amplification of a 20 kb fragment of human genomic DNA.

Basic Protocol

Component	Volume (in µL)
10x pfu/HF Reaction Buffer	5
10 mM dNTP-Mastermix	1
Forward-Primer (10 pmol/µl)	2
Reverse-Primer (10 pmol/µl)	2
Template DNA	variable
5x Band Doctor	0 – 20
pfu/HF Polymerase (2.5 U/µl)	0.5
2x distilled, sterile water	Add to a final volume of 50
Total volume	**50**

Cycling Program

Step	Temperature (in °C)	Time	Cycles
Initial activation	95	2 min	1
Denaturation	95	20 sec	10 – 40
Annealing	AT*	40 sec	10 – 40
Extension	72	See chapter E (Application)	10 – 40
Final Extension	72	5 min	1

AT* : Annealing Temperature; Choose the lower Tm of both primers;

AT = Tm – 5°C ; Tm = 2°C x (A+T) + 4°C x (G+C)

Application

A. Template

Template DNA	DNA Amount	Number of Cycle
Animal genomic DNA	50 – 200 ng	25 – 35
	10 – 50 ng	30 – 40
Bacterial genomic DNA	10 – 50 ng	20 – 25
	1 – 5 ng	30 – 35
Plasmid and Lambda DNA	1 – 5 ng	20 – 30

B. DNA Polymerase

For amplification of longer fragments of an animal genomic DNA, the amount of enzyme should be increased to 2 or 2.5 U.

C. 5x Band Doctor

The use of Band doctor is not necessary in general, but helps in the amplification of DNA with high GC content and complex structures. The included 5x Band doctor can be added to the reaction mixture at the final concentration of 0.5x – 2x.

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Reaction mixture (conc. of band doctor)

Component	Volume (in µl)				
	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
10x pfu/HF Reaction Buffer	5	5	5	5	5
10 mM dNTP-Mastermix	1	1	1	1	1
Forward-Primer (10 pmol/µl)	2	2	2	2	2
Reverse-Primer (10 pmol/µl)	2	2	2	2	2
Template DNA	x	x	x	x	x
5x Band Doctor	0	5	10	15	20
pfu/HF Polymerase (2.5 U/µl)	0.5	0.5	0.5	0.5	0.5
2x distilled, sterile water	up to 50	up to 50	up to 50	up to 50	up to 50

D. 5x Primer Design

Primers can be designed manually or using a "primer design software". It is recommended that T_m of the designed primers is above 64°C, and AT above 58°C.

E. Extension Time

The extension time should be 2 min/kb for pfu Polymerase. For High Fidelity Polymerase the extension time should be 30 sec/kb for short fragments and 1 min/kb for fragments longer than 5 kb.

For the amplification of fragments longer than 5 kb, the temperature ought to be assigned at 68°C.

Related products

- UptiTherm DNA Polymerase, 5U/µl, UPS53921

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06
Order on-line or Contact your local distributor

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