enzynomics

*n*Pfu-Forte

Cat.#	Size	Size
P410	100 units	2.5 units/μl
P425	250 units	2.5 units/μl

Store at -20℃

Supplied with: 10X *n*Pfu-Forte Buffer
dNTP Mixture
Sterile water

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

Product description

*n*Pfu-Forte is *n*Pfu (Cat.# P325, P350) supplemented with an enhancer, which maintains the activity of *n*Pfu DNA Polymerase in the presence of inhibitors produced during high-temperature PCR cycles. Thus, high-fidelity DNA polymerization is obtained with *n*Pfu-Forte DNA Polymerase, allowing amplification of DNA up to 10 kb long. *n*Pfu-Forte DNA Polymerase is more effective than *n*Pfu DNA Polymerase and has a high amplification rate (1 kb/min). Higher levels of PCR products are often obtained even with low-abundance template DNA.

Characteristics

- Molecular weight: 90 kDa
- Error rate: 2.8 X 10^{-7}
- Thermal stability: Half life of 4 hrs at 95℃
- Blunt end products

Applications

- High fidelity DNA amplification for cloning
- Amplification of long DNA fragments (>5-15 kb)
- DNA amplification to generate blunt end products
- Site-directed mutagenesis
- DNA sequencing

For Research Use Only. Not for use in diagnostic procedures.

ISO9001 ISO14001 ISO13485

Quality control

- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free
- RNase free
- Inhibitor-free

Unit definition

One unit is defined as the amount of enzyme required to incorporate 10 nmol of dNTP into acid insoluble materials in 30 min at 74℃ in a 50-μl reaction mixture (20 mM Tris-HCl/pH 8.8, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 12.5 μg of calf thymus DNA).

Storage buffer

20 mM Tris-HCl (pH 7.9), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% NP-40, 0.5% Tween-20, 50% glycerol.

10X *n*Pfu-Forte Buffer

Containing 20 mM Mg^{2+}

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Standard PCR conditions

- PCR mixture[a]

10X nPfu-Forte Buffer	2 μl
*n*Pfu-Forte DNA polymerase[b] (2.5 units/μl)	0.5 μl
dNTP mixture (2 mM each)	2 μl
Template DNA[c]	1 μl
Primer 1 (5 pmole/μl)	1 μl
Primer 2 (5 pmole/μl)	1 μl
Sterile water	up to 20 μl
[a]Assemble the reaction mixture on ice	
[b]Add the PCR polymerase at the final step	
[c]Plasmid DNA, 0.1 ng~30 ng; genomic DNA, 50 ng~500 ng	

- PCR cycle

Initial denaturation	95℃	2 min
Denaturation	95℃	30 sec
Annealing[a]	55℃~65℃	30~60 sec
Elongation	72℃	1 min/kb
Number of cycles	25~35 times	
Final elongation	72℃	5 min
When cycles are over, keep the reaction mixture at 4℃; may add 10 mM EDTA until use to prevent DNA degradation.		
[a]Recommended annealing temperatures is 5 to 10℃ below the lower Tm of the two primers used.		

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