enzynomics

Life Technologies™
Your Molecular & Cell Technology Partner

*n*Taq-*multi* HOT

Cat.#	Size	Conc.
P725HC	250 units	5 units/μl
P750HC	500 units	5 units/μl

Store at -20℃

Supplied with: 10X *n*Taq-*multi* HOT Buffer
dNTP Mixture(2 mM each)
GC Melt I
GC Melt II
Sterile water

India Contact:
Life Technologies (India) Pvt. Ltd.
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Product description

A gene encoding the *Thermus aquaticus* (Taq) DNA polymerase was cloned and expressed in *E. coli*, and the enzyme was purified to homogeneity. *n*Taq-HOT DNA Polymerase is a hot start PCR polymerase which remains inactive at temperatures lower than 75℃ as a result of the chemical modifications. Therefore, a heat activation step is required to activate *n*Taq-HOT DNA Polymerase. Activation is accomplished at denaturation steps of PCR cycles and, thus, *n*Taq-HOT DNA Polymerase is active only after initiation PCR cycles. Nonspecific elongation of incorrectly annealed primers before initiation of PCR reaction is one major cause of nonspecific DNA amplification. For this reason, amplification of nonspecific bands can be effectively prevented by using *n*Taq-HOT DNA Polymerase, thus increasing the specificity and efficiency of target DNA amplification.

Characteristics

- Molecular weight: 94 kDa
- Error rate: 2.4 X 10^{-5}
- Thermal stability : half life of 40 min at 95℃
- A-tail formation at 3' ends of amplified duplex DNA.
- No activity at temperatures lower than 75℃. Heating up to 95℃ results in activation of enzyme.

Applications

- High specific amplification of DNA fragments shorter than 3 kb.
- Amplification of cDNA and genomic DNA.
- Amplification of template DNA with secondary or higherordered structure that is resistant to PCR amplification
- Well-suited for an automated PCR machines, for which PCR reaction mixtures are prepared at room temperature
- Primer extension
- Multiplex PCR

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, 1mM DTT, 0.5% NP-40, 0.5% Tween-20, 50% glycerol.

10X nTaq-HOT Buffer

Containing 15 mM Mg^{2+}

GC Melt I and II

- This product is useful for amplification of DNA with GC-rich sequences or to avoid amplification of non-specific bands (Use of GC Melt may reduce PCR efficiency).
- In general, use 1X strength in PCR reaction by diluting the 10X solution, but the amount should be adjusted for optimal results.

Cautions

When *n*Taq-*multi* HOT DNA Polymerase is used, initial denaturation of 10 min is required to ensure effective activation of the enzyme.

Standard PCR conditions

- PCR mixture

Component	Volume
10X *n*Taq-*multi* HOT Buffer	2 μl
*n*Taq-*multi* HOT[a] (5 units/μl)	0.2 μl
dNTP Mixture (2 mM each)	2 μl
Template DNA[b] (0.1~500 ng/μl)	1 μl
Primer 1 (5 pmole/μl)	1 μl
Primer 2 (5 pmole/μl)	1 μl
Sterile water	up to 20 μl

[a]Add the PCR polymerase at the final step
[b]Plasmid DNA, 0.1 ng~30 ng; genomic DNA, 50 ng~500 ng

- PCR cycle

Step	Temperature	Time
Initial denaturation[a]	95℃	10 min
Denaturation	95℃	30 sec
Annealing[b]	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]At least 10 min of initial denaturation time is required to fully activate the chemically modified PCR DNA polymerase
[b]Recommended annealing temperatures is 5 to 10℃ below the lower Tm of the two primers used.

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Denaturation 95℃ 30 sec
Annealing[b] 55℃~65℃ 30~60 sec
Elongation 72℃ 1 min/kb
Number of cycles 25~35 times
Final elongation 72℃ 5 min
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