

*n*Taq-HOT (*UDG plus*)

Cat.#	Size	Conc.
P725U	250 units	5 units/ μ l
P750U	500 units	5 units/ μ l

Store at -20°C

Supplied with: 10X *n*Taq-HOT Buffer
dNTP Mixture with dUTP (2 mM each)
Sterile water

India Contact:

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Product description

*n*Taq-HOT DNA Polymerase is a hot start PCR polymerase which remains inactive at temperatures lower than 75°C. 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 of target DNA amplification. Also, UDG and dUTP are included in the mixture to prevent the reamplification of carry-over PCR products between reactions.

Characteristics

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

Applications

- High specific amplification of DNA fragments shorter than 3kb.
- Amplification of cDNA and genomic DNA.

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

ISO9001 ISO14001 ISO13485

- 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

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°C in a 50- μ l reaction mixture (20 mM Tris-HCl/pH 8.8, 50 mM KCl, 2.5 mM MgCl₂, 10 mM β -mercaptoethanol, 12.5 μ g of calf thymus DNA).

Storage

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.

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Standard PCR conditions

- PCR mixture

10X <i>n</i> Taq-HOT Buffer	2 μ l
<i>n</i> Taq- Pure HOT(<i>UDG plus</i>) ^a (5 units/ μ l)	0.2 μ l
dNTP Mixture with dUTP (2 mM each)	2 μ l
Template DNA ^b (0.1–500 ng/ μ l)	1 μ l
Primer 1 (5 pmole/ μ l)	1 μ l
Primer 2 (5 pmole/ μ l)	1 μ l
Sterile water	up to 20 μ l

^aAdd the PCR polymerase at the final step
^bPlasmid DNA, 0.1 ng–30 ng; genomic DNA, 50 ng–500 ng

- PCR cycle

Pre-incubation (for UDG)	25°C	10 min
Initial denaturation ^a	95°C	10 min
Denaturation	95°C	30 sec
Annealing ^b	55°C–65°C	30–60 sec
Elongation	72°C	1 min/kb
Number of cycles	25–35 times	
Final elongation	72°C	5 min

When cycles are over, keep the reaction mixture at 4°C; may add 10 mM EDTA until use to prevent DNA degradation.
^aAt least 10 min of initial denaturation time is required to fully activate the chemically modified PCR DNA polymerase
^bRecommended annealing temperatures is 5 to 10°C below the lower T_m of the two primers used.

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