

## *nTaq-multi* HOT (UDG plus)

Cat.#	Size	Conc.
P725HCU	250 units	5 units/μl
P750HCU	500 units	5 units/μl

Store at -20°C

Supplied with: 10X *nTaq-multi* HOT Buffer  
dNTP Mixture with UDG (2 mM each)  
Sterile water

### India Contact:

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

ISO9001 ISO14001 ISO13485

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### Product description

*nTaq-multi* HOT (UDG plus) is a hot start PCR polymerase which remains inactive at temperatures lower than 75°C as a result of the chemical modifications. Therefore, a heat activation step is required to activate *nTaq-multi* HOT. Activation is accomplished at denaturation steps of PCR cycles and, thus, *nTaq-multi* HOT is active only after initiation PCR cycles. This product can be used in general multiplex PCR and genotyping experiment related to genetic diagnostics because it produces up to 20 different amplified products within a single tube reaction. 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:  $2.4 \times 10^{-5}$
- 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.
- Carry-over contamination control: contains UDG

### Applications

- High specific amplification of DNA fragments shorter than 3kb.
- Amplification of cDNA and genomic DNA.
- Amplification of template DNA with secondary or higher ordered structure that is resistant to PCR amplification

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- 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-μl reaction mixture (20 mM Tris-HCl/pH 8.8, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 12.5 μg of calf thymus DNA).

### Storage

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 *nTaq-multi* HOT Buffer

Containing 15 mM MgCl<sub>2</sub>

### Cautions

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

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### Standard PCR conditions

- PCR mixture

10X <i>nTaq-multi</i> HOT Buffer	2 μl
<i>nTaq-multi</i> HOT <sup>a</sup> (5 units/μl)	0.2 μl
dNTP Mixture with dUTP (2 mM each)	2 μl
Template DNA <sup>b</sup> (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
<sup>a</sup> Add the PCR polymerase at the final step	
<sup>b</sup> Plasmid DNA, 0.1 ng–30 ng; genomic DNA, 50 ng–500 ng	

- PCR cycle

Pre-incubation (for UDG)	25°C	10 min
Initial denaturation <sup>a</sup>	95°C	10 min
Denaturation	95°C	30 sec
Annealing <sup>b</sup>	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.		
<sup>a</sup> At least 10 min of initial denaturation time is required to fully activate the chemically modified PCR DNA polymerase		
<sup>b</sup> Recommended annealing temperatures is 5 to 10°C below the lower T <sub>m</sub> of the two primers used.		

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