

EcoTaq Ultra High-Fidelity DNA Polymerase, 5U/ μ L

100U, 500U

Cat No: ETHF10, ETHF50

Shipping : Ship with blue ice.

Storage : Store at -20°C.

General Information

EcoTaq Ultra High-Fidelity DNA Polymerase is a next-generation, ultra high-fidelity DNA polymerase engineered from Pfu DNA Polymerase, offering significantly enhanced amplification efficiency, specificity, and yield. The enzyme is optimized for robust performance across a wide range of templates, including both simple and complex DNA sources.

With an optimized reaction buffer system, EcoTaq Ultra High-Fidelity DNA Polymerase enables efficient amplification of long DNA fragments. It can amplify fragments up to 40 kb from simple templates such as lambda DNA and plasmids, up to 20 kb from complex templates such as genomic DNA, and up to 10 kb from cDNA templates.

The enzyme exhibits an exceptionally low mismatch rate, approximately 1/53 that of standard Taq DNA polymerase and 1/6 that of conventional Pfu DNA polymerase, ensuring superior accuracy in DNA amplification. In addition, it features a rapid extension rate of approximately 15–30 seconds per kilobase, enabling faster PCR workflows without compromising fidelity.

EcoTaq Ultra High-Fidelity DNA Polymerase generates blunt-ended PCR products and is suitable for applications requiring high precision and reliability. It is particularly well-suited for demanding applications such as long-range PCR, cloning, sequencing, and mutagenesis etc.

EcoTaq DNA Polymerase is supplied together with 5x Reaction Buffer. 5x Reaction Buffer contains 10 mM MgCl₂.

PCR Setup

Component	Amount
EcoTaq Ultra High-Fidelity DNA Polymerase, 5U/ μ l	1 μ L
5x Reaction Buffer	10 μ l
Forward primer, 10 μ M	2 μ l
Reverse primer, 10 μ M	2 μ l
dNTP Mix, 10 mM each	1 μ l
Template DNA*	<0.5 μ g
ddH ₂ O	up to 50 μ l
Total	50 μ l

*The optimal reaction concentration varies among different templates and can be adjusted according to the sample type.

Important Note: PCR reaction conditions might need optimization with additional MgCl₂ (not provided) depending on the primer binding properties.

PCR Reaction Condition

Temperature	Time	Cycles
95°C*	3 min	
95°C**	15 sec	
55-65°C#	15 sec	25-35
72°C	30-60 sec	
72°C	5-10 min	
4°C	∞	

* The recommended initial denaturation temperature for most templates is 95°C. For amplicons longer than 10 kb, the temperature may be reduced to 92°C with a maximum incubation time of 2 minutes.

** For most templates, a denaturation step at 95°C for 5–10 seconds is sufficient. For amplicons longer than 10 kb, the temperature can be reduced to 92 °C, and the denaturation time may be extended to 15 seconds.

The annealing temperature should be optimized according to the primers used, with a recommended annealing time of 10 seconds. For challenging templates, the annealing time can be extended to 10–30 seconds.

Important Notes:

1. Use high-quality template DNA for optimal results.
2. Avoid using dUTP or primers and templates containing uracil.
3. If required, the amount of *Eco*Taq Ultra High-Fidelity DNA Polymerase may be increased; however, it is recommended not to exceed 2 U per 50 µL reaction.
4. Due to the strong proofreading activity of *Eco*Taq Ultra High-Fidelity DNA Polymerase, PCR products should be purified before adding A-overhangs for TA cloning.
5. To minimize primer degradation caused by proofreading activity, add the polymerase as the final component when assembling the reaction mixture.