



High Fidelity Polymerase

Thermostable DNA polymerase for high accuracy
 Thermus species, recombinant, *E. coli*

Cat. No.	Amount
HF_10KU	10 kunits
HF_100KU	100 kunits

Unit Definition: One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 74 °C.

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid

Concentration: 2.5 units/ μ l

Description:

High Fidelity Pol is based on a blend of Taq DNA polymerase and a proofreading enzyme specially designed for highly accurate and efficient amplification. It shows excellent results with extremely long (up to 30 kb), GC-rich or other difficult templates.

The enzyme blend includes a highly processive 5'→3' DNA polymerase and possesses a 5'→3' polymerization-dependent exonuclease replacement activity. Its inherent 3'→5' exonuclease proofreading activity results in a greatly increased fidelity of DNA synthesis compared to Taq polymerase.

The enzyme is highly purified and free of bacterial DNA.

Fidelity of the enzyme:

High Fidelity Pol is characterized by a 4-fold higher fidelity compared to Taq polymerase.

$$ER_{\text{High Fidelity Pol}} = 3.4 \times 10^{-6}$$

The error rate (ER) of a PCR reaction is calculated using the equation $ER = MF/(bp \times d)$, where MF is the mutation frequency, bp is the number of base pairs of the fragment and d is the number of doublings

($2^d = \text{amount of product} / \text{amount of template}$).

Content:

High Fidelity Pol (red cap)

2.5 units/ μ l High Fidelity Polymerase in storage buffer

High Fidelity Buffer (green cap)

10x conc.



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Recommended 50 µl PCR assay:

5 µl	10x High Fidelity Buffer	green cap
200 µM	each dNTP	-
0.2 - 0.5 µM	each Primer	-
1 - 100 ng	template DNA	-
0.5 µl (1.25 units)	High Fidelity Pol	red cap
Fill up to 50 µl	PCR-grade water	-

Please note that it is essential to add the polymerase as last component.

Recommended cycling conditions:

initial denaturation	95 °C	2 min	1x
denaturation	95 °C	20 sec	20-30x
annealing ¹⁾	50 - 68 °C	30 sec	20-30x
elongation ²⁾	68 °C	1 min/kb	20-30x
final elongation	68 °C	1 min/kb	1x

¹⁾The annealing temperature depends on the melting temperature of the primers used.

²⁾The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.