Data sheet

High Fidelity Pol

Thermostable DNA polymerase for high accuracy

Thermus spezies, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	HF_10KU	10 kU	2.5 units/µl
	HF _100KU	100 kU	2.5 units/µl

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C, avoid frequent thawing and freezing

High Fidelity Pol (red cap)
2.5 units/µl High Fidelity Polymerase in storage buffer

10x High Fidelity Buffer (green cap)

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 x 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 = amount of product / amount of template).

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.

Recommended PCR assay

50 µl PCR assay		
5 µl	10x High fidelity buffer	green cap
200 µM	each dNTP	
0.2-0.5 µM	forward Primer	
0.2-0.5 µM	reverse Primer	
1-100 ng	Template DNA	
0.5 µl (1.25 units)	High Fidelity Pol	red cap
Fill up to 50 µl	PCR grade H_2O	

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

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Recommended thermocycling conditions

Initial denaturation	95°C	2 min	1x
Denaturation	95°C	20 sec	20-30x
Annealing [1)]	50-68°C	30 sec	
Elongation [2,3)]	72°C	1 min / kbp	
Final elongation	72°C	1 min / kbp	1x

1) The annealing temperature depends on the melting temperature of the primers used.
2) For amplification of fragments longer than 5 kb the elongation temperature should be set to 68°C.
3) The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kbp is recommended.

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

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