Data sheet

High Fidelity Hot Start Pol

Heat-activatable DNA polymerase for high specificity and accuracy

Thermus spezies, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	HFHS_10KU	10 kU	2.5 units/µl
	HFHS_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 Hot Start Polymerase in storage buffer

10x High Fidelity Buffer (green cap)

Description
High Fidelity Hot Start Pol is based on a blend of Taq DNA polymerase and a proofreading enzyme specially designed for highly accurate and efficient amplification. The additional hot-start function provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The enzyme 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.

Fidelity of the enzyme
High Fidelity Pol is characterized by a 4-fold higher fidelity compared to Taq polymerase.
$ER_{High\ Fidelity\ Pol} = 3.4x10^{-6}$
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	

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Thermostable DNA polymerase for high accuracy

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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.

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)]	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.

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