DATA SHEET

LAROVA
The **Nucleotide** Company

High Fidelity Hot Start Polymerase

Heat-activatable DNA polymerase for high accuracy and specificity
Thermus species, recombinant, *E. coli*

Cat. No.	Amount
HFHS_10KU	10 kunits
HFHS_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/µl

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. The enzyme is highly purified and free of bacterial DNA.

Activation step:
High Fidelity Hot Start Pol requires no prolonged heating or denaturing step. The polymerase inhibiting antibodies are released at the increased temperature of the initial denaturation.

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).

Content:
High Fidelity Hot Start Pol (red cap)
2.5 units/µl High Fidelity Hot Start 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 Hot Start 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.

IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
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