



## High Fidelity Hot Start Core Kit

Kit of heat-activatable DNA polymerase for high accuracy and specificity, dNTPs and reaction buffer

Cat. No.	Amount
PCR-235S	100 units
PCR-235L	500 units

**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/ $\mu$ l

### Description:

High Fidelity Hot Start Core Kit contains all reagents required for PCR (except template and primer) in one box combining simple handling with high flexibility. The premium quality polymerase, ultrapure dNTPs and the optimized complete reaction buffer ensure superior amplification results.

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.

### Content:

Component	PCR-235S	PCR-235L
High Fidelity Hot Start Pol 2.5 units/ $\mu$ l in storage buffer <b>red cap</b>	40 $\mu$ l 100 units	200 $\mu$ l 500 units
dNTP Mix 10 mM each dATP, dCTP, dGTP, dTTP <b>white cap</b>	100 $\mu$ l	500 $\mu$ l
High Fidelity Buffer 10x conc. <b>green cap</b>	500 $\mu$ l	2 x 1.2 ml

### 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.



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

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

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

### Recommended 50 µl PCR assay:

5 µl	10x High Fidelity Buffer	green cap
1 µl	dNTP Mix	white cap
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 <sup>1)</sup>	50 - 68 °C	30 sec	20-30x
elongation <sup>2)</sup>	68 °C	1 min/kb	20-30x
final elongation	68 °C	1 min/kb	1x

<sup>1)</sup>The annealing temperature depends on the melting temperature of the primers used.

<sup>2)</sup>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.

### Related Products:

Ready-to-Use Mixes / direct gel loading  
 Ready-to-Use Mixes  
 Thermophilic Polymerases  
 Deoxynucleotides (dNTPs)  
 Supplements  
 Primers and Oligonucleotides  
 DNA Ladders

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