



## High Fidelity Polymerase

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

Cat. No.	Amount
PCR-204S	100 units
PCR-204L	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 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/ $\mu$ l High Fidelity Polymerase in storage buffer

#### High Fidelity Buffer (green cap)

10x conc.

### Recommended 50 $\mu$ l PCR assay:

5 $\mu$ l	10x High Fidelity Buffer	green cap
200 $\mu$ M	each dNTP	-
0.2 - 0.5 $\mu$ M	each Primer	-
1 - 100 ng	template DNA	-
0.5 $\mu$ l (1.25 units)	High Fidelity Pol	red cap
Fill up to 50 $\mu$ l	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



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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:

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