

# High Fidelity Pol

## Thermostable DNA polymerase for high accuracy

*Thermus spezies*, recombinant, *E. coli*

	Cat. No.	Size	Conc.
	PCR-204S	100 units	2.5 units/μl
	PCR-204L	500 units	2.5 units/μl

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20 °C, avoid frequent thawing and freezing

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

### 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 <sub>2</sub> O	

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

### High Fidelity Pol (red cap)

2.5 units/μl High Fidelity Polymerase in storage buffer

### 10x High Fidelity Buffer (green cap)

**Recommended thermocycling conditions**

Initial denaturation	95°C	2 min	1x
Denaturation	95°C	20 sec	20-30x
Annealing <sup>1)</sup>	50-68°C	30 sec	
Elongation <sup>2,3)</sup>	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.