

## High Fidelity Pol

### Thermostable DNA polymerase for high accuracy

Thermus species, recombinant, E. coli

	Cat. No.	Size	Conc.
	PCR-204S	100 units	2.5 units/ $\mu$ l
	PCR-204L	500 units	2.5 units/ $\mu$ l

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 $\mu$ l PCR assay				
component	stock conc.	final conc.	amount	cap
High Fidelity Buffer	10x	1x	5 $\mu$ l	green cap
dNTP Mix	10 mM each dNTP	200 $\mu$ M	1 $\mu$ l	
forward Primer	10 $\mu$ M	0.2-0.5 $\mu$ M	2-5 $\mu$ l	
reverse Primer	10 $\mu$ M	0.2-0.5 $\mu$ M	2-5 $\mu$ l	
Template DNA		1-100 ng		
High Fidelity Pol	2.5 units/ $\mu$ l	1.25 units/assay	0.5 $\mu$ l	red cap
PCR-grade water			fill up to 50 $\mu$ l	

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

#### High Fidelity Pol (red cap)

2.5 units/ $\mu$ l high fidelity polymerase in storage buffer

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

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#### 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)</sup>	68°C	1 min/kb	
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 primer-template pair.

#### Related products

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