High Fidelity Polymerase
Thermostable DNA polymerase for high accuracy
Thermus species, recombinant, E. coli

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-204S</td>
<td>100 units</td>
</tr>
<tr>
<td>PCR-204L</td>
<td>500 units</td>
</tr>
</tbody>
</table>

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 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.

\[ \text{ER}_{\text{High Fidelity Pol}} = 3.4 \times 10^{-6} \]

The error rate (ER) of a PCR reaction is calculated using the equation

\[ \text{ER} = \frac{\text{MF}}{(\text{bp} \times d)} \]

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 = amount of product / amount of template).

Content:
High Fidelity Pol (red cap)
2.5 units/µl High Fidelity Polymerase in storage buffer

High Fidelity Buffer (green cap)
10x conc.

Recommended 50 µl PCR assay:

<table>
<thead>
<tr>
<th>5 µl</th>
<th>10x High Fidelity Buffer</th>
<th>green cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 µM</td>
<td>each dNTP</td>
<td>-</td>
</tr>
<tr>
<td>0.2 - 0.5 µM</td>
<td>each Primer</td>
<td>-</td>
</tr>
<tr>
<td>1 - 100 ng</td>
<td>template DNA</td>
<td>-</td>
</tr>
<tr>
<td>0.5 µl (1.25 units)</td>
<td>High Fidelity Pol</td>
<td>red cap</td>
</tr>
<tr>
<td>Fill up to 50 µl</td>
<td>PCR-grade water</td>
<td>-</td>
</tr>
</tbody>
</table>

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

Recommended cycling conditions:

<table>
<thead>
<tr>
<th>initial denaturation</th>
<th>95 °C</th>
<th>2 min</th>
<th>1x</th>
</tr>
</thead>
<tbody>
<tr>
<td>denaturation</td>
<td>95 °C</td>
<td>20 sec</td>
<td>20-30x</td>
</tr>
<tr>
<td>annealing(^1)</td>
<td>50 - 68 °C</td>
<td>30 sec</td>
<td>20-30x</td>
</tr>
<tr>
<td>elongation(^1)</td>
<td>68 °C</td>
<td>1 min/kb</td>
<td>20-30x</td>
</tr>
<tr>
<td>final elongation</td>
<td>68 °C</td>
<td>1 min/kb</td>
<td>1x</td>
</tr>
</tbody>
</table>

\(^1\)The annealing temperature depends on the melting temperature of the primers.
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The primers used.

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