Taq DNA Polymerase
With 5x PCR Buffer RED (7.5 mM MgCl₂)

Concentration: 5 units/µl

Cat. No.: A111803

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Cap colour</th>
<th>Size (µl)</th>
<th>Taq Polymerase 5 U/µl</th>
<th>5x PCR Buffer RED, 7.5 mM MgCl₂</th>
<th>MgCl₂ 25 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A111803</td>
<td>Purple</td>
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<td>5100100</td>
<td>5575801</td>
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</tr>
</tbody>
</table>

Cat. No.: A111803

Key Features
- Direct loading of PCR products onto agarose gel with 5x PCR Buffer RED
- Time and cost saving
- No proofreading – lacks a 3'→5' exonuclease activity
- Ideal for TA cloning – leaves an A' overhang

Ampliqon Taq DNA Polymerase is a thermostable, recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. The enzyme is isolated from Thermus aquaticus and has a molecular weight of approximately 94 kDa. Ampliqon Taq DNA Polymerase has a 5'→3' DNA polymerase and a 3'→5' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity (no proofreading activity). Taq DNA Polymerase leaves an A' overhang, which makes the enzyme ideal for TA cloning.

The PCR Buffer RED is a 5x PCR buffer that consists of the Ampliqon Ammonium Buffer, a red tracking dye and a density agent. Thus, when the PCR is finished, PCR products can be directly loaded onto a DNA gel for gel electrophoresis and subsequent visualization. There is no need to buy and use separate loading buffers. The red dye front runs at 1000 – 2000 bp on a 0.5 – 1.5 % agarose gel.

The red dye of 5x PCR Buffer RED may interfere with downstream applications that apply absorbance- or fluorescence measurements. However, treatment with PCR clean up products such as ExoSAP-IT and spin columns followed by DNA sequencing is compatible with Taq OptiMix GREEN.

Kit Components

**Taq DNA Polymerase in Storage Buffer**
5 U/µl Taq, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween® 20, 0.5 % NP40, 50 % glycerol.

**5x PCR Buffer RED**
Tris-HCl pH 8.5, (NH₄)₂SO₄, 7.5 mM MgCl₂, 1 % Tween® 20, red tracking dye, density agent.

**MgCl₂**
25 mM MgCl₂.

**Recommended Storage and Stability**
Long term storage at -20 °C. Product expiry at -20 °C is stated on the label. Option: Store at +4 °C for up to 6 months.

**Quality Control**
Taq DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity, exonuclease activity.

**Unit definition**
One unit is defined as the amount of polymerase that incorporates 10 nmoles of dNTPs into acid-precipitable DNA in 30 minutes at 72 °C under standard assay conditions.

**Protocol**

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

**Notes:**
- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.
- 7.5 mM MgCl₂ is present in the Ampliqon 5x PCR Buffer RED. The 1x concentration is 1.5 mM MgCl₂. In some applications, more than 1.5 mM MgCl₂ is required for best results. For this reason, 25 mM MgCl₂ is included in the kit. Table 1 provides the volume of 25 mM MgCl₂ to be added to the reaction mix if a higher MgCl₂ concentration than 1.5 mM is required.

**Table 1. Additional volume of MgCl₂ per 50 µl reaction**

<table>
<thead>
<tr>
<th>Final MgCl₂ conc. in reaction (mM)</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of 25 mM MgCl₂ (µl)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

1. Thaw buffer, dNTP mix and primer solutions. It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts. The polymerase is provided in glycerol and does not need thawing.

**Important:** Spin vials briefly before use.

2. Prepare a reaction mix according to table 2. The reaction mix typically contains all the components needed for primer extension except the template DNA.

**Table 2. Reaction mix and template DNA**

<table>
<thead>
<tr>
<th>Component</th>
<th>Vol./reaction*</th>
<th>Final concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x Buffer</td>
<td>10 µl</td>
<td>1x</td>
</tr>
<tr>
<td>25 mM MgCl₂</td>
<td>0 µl (0 – 6.5 µl)</td>
<td>1.5 mM (0.5 – 5 mM)</td>
</tr>
<tr>
<td>dNTP mix</td>
<td>1 µl (10 mM each)</td>
<td>0.2 mM of each dNTP</td>
</tr>
<tr>
<td>Primer A (10 µM)</td>
<td>1 µl (0.5 – 5 µl)</td>
<td>0.2 µM (0.1 – 1.0 µM)</td>
</tr>
<tr>
<td>Primer B (10 µM)</td>
<td>1 µl (0.5 – 5 µl)</td>
<td>0.2 µM (0.1 – 1.0 µM)</td>
</tr>
<tr>
<td>Taq DNA Pol.</td>
<td>0.2 µl (0.2 – 1 µl)</td>
<td>1 unit (1 – 5 units)</td>
</tr>
<tr>
<td>PCR-grade H₂O</td>
<td>X µl</td>
<td>-</td>
</tr>
<tr>
<td>Template DNA</td>
<td>X µl</td>
<td>genomic DNA: 20 ng (1 – 200 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)</td>
</tr>
</tbody>
</table>

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, eg. 0.1 µl Taq instead of 0.2 µl Taq.
3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master mix up and down a few times.

4. Add template DNA to the individual tubes containing the master mix.

5. Program the thermal cycler according to the manufacturer’s instructions. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

6. Place the tubes in the thermal cycler and start the reaction.

### Table 3. Three-step PCR program

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Duration of cycle</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 – 5 minutes(^a)</td>
<td>95 °C</td>
</tr>
<tr>
<td>25 – 35</td>
<td>20 – 30 seconds(^b)</td>
<td>95 °C</td>
</tr>
<tr>
<td></td>
<td>30 – 90 seconds(^c)</td>
<td>50 – 65 °C</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>72 °C</td>
</tr>
</tbody>
</table>

\(^a\) Initial denaturation step (optional).

\(^b\) Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

\(^c\) Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the Tm (melting temperature) of the primers used.

\(^d\) Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

\(^e\) Final elongation: This step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

### Related Products

- **Taq DNA Polymerase (500 units)\(^*\)**
  - A110003
  - A111103
  - A111803

- **Hot Start Polymerase (500 units)\(^*\)**
  - A220003
  - A221103
  - A221803

- **High Fidelity - Proof reading (500 units)\(^**\)**
  - A210003
  - A221103

**Buffers for DNA polymerases**

- 10x Ammonium Buffer, 3 x 1.5 ml
  - A301103
  - A302103
  - A303103
  - A301810

**Special Master Mixes (500 x 50 µl reactions)**

- 2x Master Mix, 1.5 mM MgCl\(_2\) final concentration
  - A140303
  - A180303

- GC TEMPLEase Hot Start Master Mixes (500 x 50 µl reactions)\(^*\)

**Real-time PCR Master Mixes (400 x 25 µl reactions)**

- RealQ Plus 2x Master Mix for probe,
  - A313402
  - A314402
  - A315402

- Ultra Pure dNTPs\(^*\)

**Loading Buffers and Ladders**

- 5x Loading Buffer Red, 5 x 1 ml
  - A600104
  - A610341

**Loading Buffers and Ladders**

- PCR DNA Ladder, 100 – 3000 bp, 1 x 0.5 ml
  - A610341

*Other concentrations and single dNTPs are available.

Reagents for in vitro laboratory use only.

Other product sizes, combinations and customized solutions are available. Please look at www.ampliqon.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

Issued 06/2017