AMPLIQON
PCR ENZYMES & REAGENTS

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# Taq OptiMix CLEAR 2x Master Mix

MADE IN DENMARK

| Cat. No. | Reactions (50 µl) | Taq OptiMix CLEAR 2x Master Mix 1.5 mM $MgCl_2$ ID: 5200800 |
|---|---|---|
| A370501 | 100 | 2 x 1.25 ml |
| A370503 | 500 | 10 x 1.25 mL |
| A370506 | 2500 | 50 x 1.25 ml |
| A370507 | 5000 | 25 x 5 ml |
| A370599 | Sample - 20 | 1 x 0.5 ml |

## Key Features

Taq OptiMix CLEAR is a ready-to-use 2x master mix with the Ampliqon Taq DNA polymerase, the $NH_4^+$ buffer system, dNTPs and magnesium chloride present. Each reaction requires 25 µl of the Taq OptiMix CLEAR. Simply add primers, template and water to a total reaction volume of 50 µl.

Taq OptiMix CLEAR offers several advantages: Reduced set up time and risk of contamination. Fewer reagent handling steps resulting in higher reproducibility. Optimized for increased specificity of DNA targets up to 4 kb.

### Composition of the Taq OptiMix CLEAR (2x)

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Ampliqon Taq DNA polymerase

### Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

### Quality Control

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

## Protocol

This protocol serves as a guideline to ensure optimal PCR results when using Taq OptiMix CLEAR 2x Master Mix. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw Taq OptiMix CLEAR and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice.

2. Set up each reaction. Table 1 shows the reaction set up for a final volume of 50 µL. If desired, the reaction size may be scaled down. Use 12.5 µl Taq OptiMix CLEAR in a final volume of 25 µl.

**Table 1. Reaction components (reaction mix and template DNA)**

| Component | Vol./reaction* | Final concentration* |
|---|---|---|
| Taq 2x Master Mix | 25 µl | 1x |
| 25 mM $MgCl_2$ | 0 µl (0-6 µl) | 1.5 mM (1.5 – 4.5 mM) |
| Primer A (10 µM) | 1 µl (0.5 – 5 µl) | 0.2 µM (0.1 – 1.0 µM) |
| Primer B (10 µM) | 1 µl (0.5 – 5 µl) | 0.2 µM (0.1 – 1.0 µM) |
| PCR-grade $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 50 ng (10 – 500 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>bacterial DNA: 5 ng (1 – 10 ng) |
| **TOTAL volume** | 50 µl | - |

* Suggested starting conditions; theoretically used conditions in brackets

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the reaction mix up and down a few times.

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

5. Program the thermal cycler according to the manufacturer´s instructions and recommendations in table 2. (DNA targets < 1kb) or table 3. (DNA targets 1 – 4 kb)
   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 2. Three-step PCR program for targets < 1kb**

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| 1 | 2 – 5 minutes | 95 °C |
| 25 - 35 | 20 – 30 seconds[a]<br>20 – 40 seconds[b]<br>30 seconds[c] | 95 °C<br>50 – 65 °C<br>72 °C |
| 1 | 5 minutes[d] | 72 °C |

**Table 3. Three-step PCR program for targets 1kb – 4kb**

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| 1 | 2 – 5 minutes | 95 °C |
| 25 - 35 | 20 – 30 seconds[a]<br>20 – 40 seconds[b]<br>60 – 300 seconds[c] | 95 °C<br>50 – 65 °C<br>72 °C |
| 1 | 5 minutes[d] | 72 °C |

[a] 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.

[b] 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 $T_m$ (melting temperature) of the primers used.

[c] 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.

[d] Final elongation: This single 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.

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## Two-step PCR program

Fast 2-step PCR protocols are available using this link:
https://ampliqon.com/en/pcr-technology/application-notes/

### Notes:

- The final $MgCl_2$ concentration of Taq OptiMix CLEAR is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ to adjust the $Mg^{2+}$ concentration according to table 4.

**Table 4. Additional volume (µl) of $MgCl_2$ per 50 µl reaction:**

| Final $MgCl_2$ conc. in reaction (mM) | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
|---|---|---|---|---|---|---|---|
| Volume of 25 mM $MgCl_2$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

For Research Use Only. Not for use in diagnostics procedures.

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**

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