2X Taq DNA Polymerase Master Mix RED

Master Mix Kit (1.5 mM MgCl₂)

Size :1.5 ML

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| Cat. no. | Size | Taq DNA Polymerase Master | MgCl ₂ |
|----------|-----------|---------------------------|-------------------|
| | reactions | Mix <mark>RED</mark> | Conc. |
| 160301-1 | 120 | Master Mix RED | 1.5 mM |

Store at -20°C. Reagent for in-vitro laboratory use only

General Description

Taq DNA Polymerase Master Mix RED is a ready-to-use 2X reaction mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications.

Bionovas Taq polymerase, the NH_4 buffer system, dNTPs and magnesium chloride are conveniently present in the Taq DNA Polymerase Master Mix RED. An inert red dye and a stabilizer are also present to allow direct loading of the final products onto an agarose gel for analysis.

Taq DNA Polymerase Master Mix RED offers several advantages. Set up time is significantly reduced. There is no need to buy and use separate loading dyes to load reaction products onto agarose gels for electrophoresis and subsequent visualization. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

Composition of Taq Master Mix RED

- 82.5 mM Tris-HCl pH 8.5, 22 mM (NH₄)₂SO₄, 1.65 mM MgCl₂*, 0.11% Tween 20
- 0.22 mM dNTPs
- 0.11 units/µL Bionovas Taq DNA polymerase
- Inert red dye and a stabilizer

*Taq DNA Polymerase Master Mix RED is offered in final MgCl₂ concentrations: 1.5 mM.

Suggested Protocol using Taq Master Mix RED

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

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1. Set up each reaction as follows:

| Component | Vol./reaction | Vol./reaction | Final Conc. |
|-----------------|---------------|---------------|---------------------|
| Master Mix RED | 25 µL | 12.5 µL | 1X |
| Primer A | Variable | Variable | 0.1 <i>-</i> 1.0 µM |
| Primer B | Variable | Variable | 0.1 <i>-</i> 1.0 µM |
| Distilled Water | Variable | Variable | |
| Template DNA | Variable | Variable | Variable |
| TOTAL volume | 50 µL | 25 µL | |

- After primer extension, a sample (10 to 30% of the reaction) can be loaded directly on a gel for analysis.
- Important: Spin Taq Master Mix RED vials briefly before use.
- 2. Mix gently by pipetting the solution up and down a few times.
- 3. 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.

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

| Cycles | Duration of cycle | Temperature | |
|--------|------------------------------|-------------|--|
| 25-35 | 20 – 30 seconds ^a | 95 °C | |
| | 20 -40 seconds ^b | 50-65 °C | |
| | 30 seconds ^c | 72 °C | |
| 1 | 5 minutes | 72 °C | |

^aDenaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 -30 seconds. It causes DNA melting of the DNA template by disrupting the

hydrogen bonds between complementary bases, yielding singlestranded 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 degrees Celsius below the Tm of the primers used.

[°] 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.