2.0X Taq RED Master Mix Kit

(1.5mM $MgCl_2$)

Cat #: 42-137

Contents: 100 Reactions

Storage: -20°C

Reagent for in-vitro laboratory use only

General Description

Apex Taq RED DNA Polymerase Master Mix from Genesee Scientific is a ready-to-use 2.0X reaction mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications.

Apex Taq RED DNA Polymerase, the NH_4^+ buffer system, dNTPs and magnesium chloride are present in the Taq RED DNA Polymerase Master Mix. Each reaction requires 25 µl of the 2.0X reaction mix. Simply add primers, template and water to a total reaction volume of 50 µl.

Taq RED DNA Polymerase Master Mix 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.

Storage and Stability

The unopened kit is stable at -20 °C for 2 years after the production date.

Quality Control

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

Suggested Protocol Using Taq 2x Master Mix RED

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.
- The final $MgCl_2$ concentration of this 2x Taq Master Mix RED is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ (may be purchased separately) to adjust the Mg^{2+} concentration according to table 1.

Table 1. 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

1. Thaw Taq 2x Master Mix RED 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. Prepare a reaction mix. Table 2 shows the reaction set up for a final volume of 50 µL. If desired, the reaction size may be scaled down. Use 10 µl of the Taq 2x Master Mix RED in a final volume of 20 µl.

Table 2. 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 – 7 µl)	1.5 mM (0.5 – 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) plasmid DNA: 0.5 ng (0.1 – 1 ng) 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. See table 3 for an example.
   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.

7. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

Table 3. Three-step PCR program

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