

2.0X Taq RED Master Mix Kit

(1.5mM MgCl₂)

Cat #: 42-138

Contents: 500 Reactions

Storage: -20°C

Reagent for in-vitro laboratory use only

General Description

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

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 $^{\circ}$ 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 MgCl₂ concentration of this 2x Taq Master Mix RED is 1.5 mM. In some applications, more than 1.5 mM MgCl₂ is required for best results. Use 25 mM MgCl₂ (may be purchased separately) to adjust the Mg²⁺ concentration according to table

Table 1. Additional volume (μl) of MgCl₂ 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

- 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 μ L. If desired, the reaction size may be scaled down. Use 10 μ l of the Taq 2x Master Mix RED in a final volume of 20 μ l.

Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
Taq 2x Master Mix	25 μΙ	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 μΜ (0.1 – 1.0 μΜ)
Primer B (10 μM)	1 μl (0.5 – 5 μl)	0.2 μM (0.1 – 1.0 μM)
PCR-grade H₂O	ΧμΙ	-
Template DNA	Xμl	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 μΙ	-

^{*} 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.
- Add template DNA to the individual tubes containing the reaction mix.
- 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 ℃
25 - 35	20 – 30 seconds ^b	95 °C
	20 – 40 seconds ^c	50 – 65 °C
	30 seconds ^d	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.
- 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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NOTICE: In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license. Reagent for in vitro laboratory use only.

Apex General Purpose Agarose



- Low EEO (=0.12) This means biological macromolecules such as proteins or nucleic acids as well as larger particles such as viruses and subcellular fragments can migrate through the gel network virtually interference free due to the gel's neutral properties.
- Sharp, finely resolved banding resolution as well as excellent transparency for easy reading.
- · Extraordinarily low gel background after applying staining agents.
- · Superior mechanical resistance for more reliable and easier handling.



EtBr Dropper Bottle - Adding and removing Ethidium Bromide has never been easier! For the recommended final concentration of 0.5ug/ml, simply add one drop for every 50ml of solution.

Concentration: 0.625mg/ml



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Description	Cat. No.
Apex TAE 25X Buffer, 1.6L Ultra Pure	20-195
Apex TBE Buffer 10X Concentrate, 4L	20-196
Apex EtBr Dropper Bottle, 10ml, 0.625mg/ml	20-276
Apex EtBr Destaining Bags, 25 Bags/Unit Also Works Great w/ Coomassie Blue & Other Dyes!	20-277
Apex General Purpose Agarose, 500g	20-102
Apex Super Resolution Agarose, 100g	20-106
Apex Super Fine Resolution Agarose, 100g	20-239
Apex Low Melt Agarose, 100g	20-104
Apex Low Melt Sieve Agarose, 100g	20-245

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