

SciRed™ Taq Mix

2 mM MgCl₂ final concentration, 50 rxn (1250 µL)

Cat. No.: SciRed-1250.2

Key Features

SciRed™ Taq Mix is a ready-to-use 2X reaction mix with the Debna BioGene Taq DNA polymerase, the NH₄⁺ buffer system, dNTPs and magnesium chloride present. Each reaction requires 25 µl of the SciRed™ Taq Mix. The primers, template and water are added to a total reaction volume of 50 µl to carry out primer extensions and other molecular biology applications.

SciRed™ Taq Mix offers several advantages. For example the set up time is significantly reduced. And the risk of contaminating component stocks is removed. Also the reduction of reagent handling steps leads to a better reproducibility.

It is noted that there is no need to purchase and use separate loading dyes. A portion of the reaction product is loaded onto an agarose gel for electrophoresis and the follow-up visualization. The red dye front runs at 300 – 1000 bp on a 0.5 – 1.5% agarose gel.

Composition of the SciRed™ Taq Mix

- Tris-HCl pH 8.5, (NH₄)₂SO₄, 4 mM MgCl₂, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Debna BioGene Taq DNA polymerase
- Inert red dye and stabilizer

Recommended Storage and Stability

Long term storage can be achieved at -20 °C. The product expiration date at -20 °C is stated on the label.

Storage at +4 °C can be done for up to 6 months.

Quality Control

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

Protocol

The protocol serves as guidance for ensuring optimal PCR results when using SciRed™ Taq Mix. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA must be determined individually.

1. First, thaw SciRed™ Taq Mix 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 1 shows the reaction set-up for a final volume of 50 µL. If needed, the reaction size may be scaled down. Use 10 µl of the SciRed™ Taq Mix in a final volume of 20 µl.

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

Component	Vol./reaction*	Final concentration*
SciRed™ Taq Mix	25 µl	1x
25 mM MgCl ₂	0 µl (0 – 5 µl)	2 mM (2 – 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 ₂ O	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 parenthesis

3. Mix the reaction mix vigorously and dispense appropriate volumes into the 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 that contain the reaction mix.
5. Program the thermal cycler by following the manufacturer's instructions.

Temperatures and cycling times should be optimized for each new template target or primer pair for achieving optimum yield and specificity.

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 2. Three-step PCR Program

Cycles	Duration of cycle	Temperature
1	2-5 minutes	95 °C
25-35	20-30 seconds ^a 20-40 seconds ^b 30 seconds ^c	95 °C 50-65 °C 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.

Table 3. Two-step PCR Program

Cycler step	Temperature	Duration	Cycles
Initial heating	98 °C	40 seconds	1
Denaturation	92 °C	2 seconds	30
extension ^a	60 °C	2 seconds	
Final extension	72 °C	20 seconds	1

^a The extension temperature depends on the primer set. For fast PCR choose highest possible T_m values.

Notes:

The final MgCl₂ concentration of this SciRed™ Taq Mix is 2 mM. In some applications, more than 2 mM MgCl₂ is required for best results. Use 25 mM MgCl₂ to adjust the Mg²⁺ concentration according to Table 4.

Table 4. Additional volume (µl) of MgCl₂ per 50 µl reaction:

Final MgCl ₂ conc. in reaction (mM)	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM MgCl ₂	0	1	2	3	4	5