

# Genaxxon BioScience

## RedTaq Polymerase PCR Master Mix (2X)

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Product	Cat#	Package size
Premixed Taq Polymerase, dNTPs, buffer and red-dye for reliable PCR. Mix for 50 µL reaction volume.	M3029.0100	100 reactions
Premixed Taq Polymerase, dNTPs, buffer and red-dye for reliable PCR. Mix for 50 µL reaction volume.	M3029.0500	500 reactions

### Description

The Genaxxon Taq PCR Master Mix (2X) is a 2-fold concentrated ready-to-use PCR mixture of RedTaq DNA Polymerase, PCR buffer, MgCl<sub>2</sub> and dNTPs. The 2X Mix contains all components for PCR, except DNA template and primers. To facilitate handling and downstream applications like gel electrophoresis, RedTaq-Mastermix can be applied directly on an agarose gel without further addition of loading buffer. RedTaq Mastermix does not only contain the robust Genaxxon Taq-polymerase, it also contains a special mixture of components stabilising the polymerase and nucleotides, e.g. during storage with repeated freeze/thaw steps and during cycling reaction. The mixture was shown to be effective for high through put applications.

### Supplied Material

RedTaq Mastermix (2-times): RedTaq DNA Polymerase (0.05 units/µL), Tris-HCl (pH 8.8), 0.02% Tween-20, 3 mM MgCl<sub>2</sub>, 0.4 mM of each dNTP (dATP, dCTP, dGTP, dTTP)

Magnesium stock solution: 25 mM MgCl<sub>2</sub>

### Performance and purity tests

Tested for the absence of endodeoxyribonucleases and exodeoxyribonucleases (2 hours with 100 µL of MasterMix with 0.22 µg EcoR I digested lambda DNA at 72°C).

The 2-times RedTaq PCR Master Mix is tested in the amplification of a single-copy gene of mouse genomic DNA.

### Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25µL with the 2-times RedTaq PCR Master Mix with 1µg of pUC19 DNA in 50µl for 4 hours neither at 37°C nor at 70°C

### Properties and application

The Genaxxon RedTaq DNA Polymerase is a thermostable DNA polymerase from *T. aquaticus* of high purity with good fidelity and high processivity in the DNA chain elongation reaction. Using this enzyme, amplification of DNA fragments ranging from 100 bp to 10 000 bp can be achieved under standard assay conditions described below.

**Application:** PCR • Primer extension • Multiplex PCR • Low-copy targets PCR • Real-time PCR

### Storage and Stability

Store at -20°C.

The mixture is stable for more than 12 months if stored at -20°C. Avoid repeated freeze-thaw cycles, as this will harm the components of the mixture.

## Standard DNA amplification assay

### Usage (PCR protocol)

To prevent from unwanted (unspecific) product or primer-dimers formation, it is recommended to set up the pipetting procedure on ice, or to use the Genaxxon HotStart master mixes.

### Add to your PCR tube

The protocol below serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA or primer may vary and must be determined individually.

Pipette the following into a PCR tube, mix and make up to a final volume of 50  $\mu$ L (25  $\mu$ L).

We recommend dispensing all reagents on ice. It is important to vortex all solutions (incl.  $MgCl_2$ ) before use to remove any gradients that may result from repeated freeze/thaw steps.

If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

Component	50 $\mu$ L reaction		25 $\mu$ L reaction	
	Volume	Final concentration	Volume	Final concentration
2X <i>RedTaq</i> Mastermix	25 $\mu$ L	1X	12,5 $\mu$ L	1X
Forward Primer	Variable	0.1 – 1 $\mu$ M *	Variable	0.1 – 1 $\mu$ M *
Reverse Primer	Variable	0.1 – 1 $\mu$ M *	Variable	0.1 – 1 $\mu$ M *
Template DNA	Variable	100 pg – 500 ng **	Variable	100 pg – 500 ng **
Sterile deionized water	Up to 50 $\mu$ L	---	Up to 25 $\mu$ L	---

Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube. Overlay the sample with mineral oil or add an appropriate amount of wax if the thermal cycler is not equipped with a heated lid. Place the samples in a thermocycler and start a PCR program.

\* 1  $\mu$ M primer final concentration corresponds with 4-7  $\mu$ L of 3  $\mu$ M stock solution (10-20 pmol absolute).

\*\* 10 pg – 500 ng template DNA mean as a rule of thumb: 0.1 ng – 10 ng of plasmid DNA and 5 ng – 500 ng genomic DNA

**Table 1:**  $MgCl_2$  concentration in a 50  $\mu$ L reaction (*RedTaq* Mastermix)

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM $MgCl_2$ per reaction ( $\mu$ L)	0	1	2	3	4	5	6

### Notes:

- Drops should be collected by centrifugation and 50  $\mu$ L of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. Each programme should start with an initial heat incubation step at 94°C for 3-5 minutes!
- **Recommended elongation time is 1 minute per 1kb of target!**
- For maximum yield and specificity, temperatures (annealing) and cycling times should be optimised for each new template target or primer pair.