MANUAL

PCR RedMastermix (2X)

with Red Dye and Taq DNA polymerase

Component	Cat#	M3029.0100	M3029.0500	M3029.2500	Colour code of cap
Premixed 2-time PCR Mastermix for standard PCR.		2x 1.25mL for 200x 25µL reactions	10x 1.25mL for 1000x 25µL reactions	50x 1.25mL for 5000x 25µL reactions	red
25mM MgCl2 solution		1mL	1mL	5 x 1mL	green

Description

The **Genaxxon PCR RedMastermix (2X)** is a 2-fold concentrated, ready-to-use PCR master mix containing Taq DNA polymerase, PCR buffer, $MgCl_2$, dNTPs, and a red dye for **easy handling**. The 2-fold master mix contains all components for PCR, except DNA template and primers. To facilitate handling and downstream applications like gel electrophoresis or pipetting, the PCR RedMastermix (2X) allows **direct loading on an agarose gel** without addition of a loading buffer.

The Genaxxon PCR RedMastermix (2X) does not only contain our robust Taq DNA polymerase, it also contains a special formulation that stabilizes the polymerase and nucleotides, **ensuring reliability** even after repeated freeze-thaw cycles. The mastermix is also ideal for **high-throughput applications.**

The PCR RedMastermix (2X) comes with extra $MgCl_2$ and in a **convenient 1.25 mL aliquot** for secure handling.

Product specifications

Concentration:	2 time ready-to-use master mix
Substrate analogues:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	1-2 kb/min. at 72°C
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No

Advantages

Clear visualization – Easy tracking of pipetting steps
Robust performance – Highly stable PCR master mix
Colony screening – Optimized for rapid screening applications
No extra steps – Loading dye already included
Convenient format – 1.25 mL aliquot size for easy handling

Shipment: on wet ice but full activity at RT (+15°C to +25°C) for at least 1 week

Short-Term Storage: Can be stored at +2°C to +8°C for up to 8 weeks
Long-Term Storage: Stored at -20°C immediately upon receipt

Shelf-life: Stable until expiration date when stored and handled correctly

Unit definition

One unit of Taq DNA Polymerase E used for the Genaxxon bioscience PCR RedMastermix (2X) is defined as the amount of enzyme that incorporates 10nmol of dNTP's into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

Important notes before getting started

- This protocol is a guideline; optimize conditions as needed.
- The mix provides 1.5 mM $MgCl_2$, suitable for most applications. Extra 25 mM $MgCl_2$ is included if needed.
- Prepare reactions away from DNA prep/PCR analysis areas.
- Use filtered tips to prevent cross-contamination.

MANUAL

PCR Protocol

Thaw primer solutions

Keep on ice after complete thawing and mix well before use.

Optional:

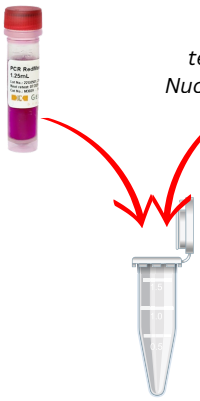
Prepare a primer mix of an appropriate concentration using sterile, nuclease-free water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA should not exceed 25µL per reaction.

Thaw PCR RedMastermix (2X) at RT or on ice.

Keep on ice after thawing and mix well before use.

It is very important to mix the PCR RedMastermix well before use to avoid local differences in salt concentration.

The Genaxxon bioscience PCR RedMastermix is provided as a 2X concentrated (i.e. a 12.5µL volume of PCR RedMastermix is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of PCR RedMastermix to diluted primer mix, template DNA and water should be maintained. A negative control (PCR without template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

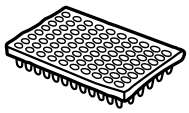


Distribute 25 µl reaction mix into each tube.

PCR protocol

Components	Quantities
PCR RedMastermix (2X)	12.5 µL
Diluted primer mix:	
primer 1:	variable volume: 0.1 - 0.25 µM (5 – 12 pmol absolute)
primer 2:	variable volume: 0.1 - 0.25 µM (5 – 12 pmol absolute)
Template DNA*	variable volume: < 5ng plasmid DNA variable volume: < 250ng gDNA
Nuclease-free water	up to 25µL

*For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume should not exceed 10% of the final PCR volume.



Place PCR tubes in the thermal cycler* and start program.

A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

*When using a thermal cycler with a heated lid, do not use mineral oil. Otherwise, overlay with app. 50µL mineral oil

PCR conditions

Step	Time	Temperature
Initial denaturation	3 min	95 °C
Denaturation	0.5 - 1 min	95 °C
Annealing	0.5 - 1 min	50 - 68 °C*
Extension	0.5 - 1 min**	72 °C
Number of Cycles	25 - 35	
Final Extension	10 min	72 °C

*Approximately 5°C below Tm of primers
**For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.

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Additional information

Applications

- **Standard PCR**
- **Detection of low target copy number**
- **2-step RT-PCR**

The PCR RedMastermix (2X) can also be used for Sanger Sequencing - Just dilute the PCR reaction 1:8 after PCR, or purify using spin columns and then apply.

Quality Control

Amplification efficiency:	Amplification efficiency is tested in parallel amplification reactions and additionally against competitors products.
PCR reproducibility:	PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity:	Linearized DNA is incubated with PCR RedMastermix (2X).
Endonuclease activity:	Plasmid DNA is incubated with PCR RedMastermix (2X).
RNase activity:	RNA is incubated with PCR RedMastermix (2X).
Protease activity:	PCR RedMastermix (2X) is incubated in storage buffer.
Self-priming activity:	PCR is performed under standard conditions, without primers, using PCR RedMastermix (2X) and human genomic DNA.

Recommendations for Standard PCR-Primers

Length	18-30 nucleotides
GC content	40-60%
Tm	Design primer pairs with similar Tm values. Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence	Avoid complementarities of two or more bases at the 3' ends of primer pairs. Avoid runs of 3 or more Gs or Cs at the 3' end. Avoid a 3'-end T. Avoid complementary sequences within primer and between primer pairs.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

Product Use Limitations

PCR RedMastermix is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Further information

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