GENAXXON bioscience

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PCR Mastermix (2X)

with *Taq* DNA polymerase

Component	Cat#	M3014.0100	M3014.0500	Colour code of cap
Premixed 2-time PCR Mastermix for standard PCR.		2x 1.25mL for 200x 25µL PCR reactions	10x 1.25mL for 1000x 25µL PCR reactions	green
25mM MgCl2 solution		1mL	1mL	green

Product Description

The Genaxxon bioscience PCR Mastermix (2X) is a 2-fold concentrated ready-to-use PCR mixture of *Taq* DNA polymerase, PCR buffer, $MgCl_2$ and dNTPs. The 2X Mix contains all components for PCR, except DNA template and primers. The Genaxxon PCR Mastermix does not only contain the robust Genaxxon bioscience *Taq* DNA polymerase, it also contains a special mixture of components stabilizing 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 throughput applications.

Product Specifications

Concentration:	2 time ready-to-use PCR master mix
Substrate analogues:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	2-4 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

Unit definition

One unit of *Taq* DNA Polymerase E used for the Genaxxon bioscience PCR Mastermix (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.

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 Mastermix (2X).
Endonuclease activity: Plasmid DNA is incubated with PCR Mastermix (2X).
RNase activity: RNA is incubated with PCR Mastermix (2X).
Protease activity: PCR Mastermix (2X) is incubated in storage buffer.
Self-priming activity: PCR is performed under standard conditions, without primers, using PCR Mastermix (2X) and human genomic DNA.

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Application

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

Stability

The Genaxxon bioscience PCR Mastermix (2X) is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 1 week.

The Genaxxon bioscience PCR Mastermix (2X), including buffers and reagents, should be stored immediately upon receipt at -20°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience PCR Mastermix (2X) can also be stored at +2°C to +8°C up to 8 weeks.

Product Use Limitations

PCR Mastermix (2X) 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.

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).

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PCR Protocol Part

Protocol using PCR Mastermix (2X)

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Important notes before getting started

- PCR Mastermix (2X) provides a final concentration of 1.5mM MgCl2 which will produce satisfactory results in most cases.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **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, bidest 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, added at step 4, should not exceed 25µL per reaction.
2. **Thaw PCR Mastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the PCR Mastermix (2X) well before use to avoid local differences in salt concentration. The Genaxxon bioscience PCR Mastermix (2X) is provided as a 2X concentrated (i.e. a 12.5µL volume of PCR Mastermix (2X) is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of PCR Mastermix (2X) 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.
3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the PCR Mastermix (2X).**
4. **Add template DNA** (<1µg/reaction) to the individual PCR tubes.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.

Table 1: PCR reaction components using PCR Mastermix (2X) (**25µL PCR reaction**)

Components	Quantities
PCR Mastermix	12.5µL
Diluted primer mix	
primer 1:	variable volume: 0.1 - 0.25µM (5 – 12 pmol absolute)
primer 2:	variable volume: 0.1 - 0.25µM (5 – 12 pmol absolute)
Template DNA	variable volume: < 5ng plasmid DNA variable volume: < 250ng genomic DNA
sterile, bidestilled water	up to 25µL

5. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
7. **Place PCR tubes in the thermal cycler and start program.**

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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	3 min.	95°C	
3-step cycling			
Denaturation:	0.5 - 1 min.	95°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C below Tm of primers.
Extension:	0.5 - 1 min.	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	25 - 35		
Final extension	10 min.	72°C	

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.

Table 3: 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.

Table 4: Migration Chart of some Gel Tracking Dyes

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	10000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	

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