GENAXXON bioscience

fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

Lyophilized 5x qPCR Multiplex MasterMix

lyophilized ready-to-use qPCR master mix without ROX and without SybrGreen

	Cat#	M3026.0500	M3026.2500	Colour code of cap
Component				
Lyophilized 5x qPCR Multiplex MasterMix shipped in a foil bag.		2 tubes per bag	5x 2 tubes per bag	**Blue**
5X Rehydration buffer with 2mM MgCl2 final concentration.		2x 1.3mL	10x 1.3mL	**Black**

Product Description

The Genaxxon lyophilized 5x qPCR Multiplex MasterMix without ROX and without SybrGreen is offered as a dry master mix in 1.5mL reaction tubes. The 5x LyoMix contains the Genaxxon chemical inhibited Hotstart *Taq* DNA Polymerase together with our extremely high-quality dNTPs and an optimized PCR buffer, thus simply reconstitute the master mix by adding 1.25mL of the also supplied rehydration buffer and you can start to add your template and primers and you're all set!

The master mix formulation simplifies reaction set-up—saving time and reducing the risk of contamination and pipetting errors

The used Hotstart DNA-polymerase is activated by the first denaturation step for 15 minutes. Thus, during setup and the first PCR cycle, the enzyme is not active and misprimed primers are not extended. As a result specificity and yield are increased compared to standard *Taq* DNA-polymerase. Additionally, difficult targets with high GC-content can be amplified.

Sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial saving in time and reagents.

Product Specifications of used Hotstart DNA polymerase

Format:	Ready-to-use lyophilized qPCR master mix
Extension rate:	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

Key Features

- Lyophilized PCR master mix with aptamer inhibited Hotstart DNA Polymerase.
- Stable at ambient temperature
- Shipment: not cooled! Storage at +15°C to +30°C.
- reduced CO2 impact.

Important note

The lyophilized 2X LyoMix is susceptible to air humidity and has to be kept in the aluminium foil bag. If possible reconstitute all 4 tubes of one bag with the supplied rehydration buffer at once to avoid stability problems of the remainder tubes and store the reconstituted PCR mix at -20°C!

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

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PCR Protocol Part

Protocol using lyophilized 5x qPCR Multiplex MasterMix

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

- The lyophilized 2X LyoMix is susceptible to air humidity and has to be kept in the aluminium foil bag. If possible reconstitute all 4 tubes of one bag with the supplied rehydration buffer at once to avoid stability problems of the remainder tubes and store the reconstituted PCR mix at -20°C!
- Reconstitute each tube with the lyophilized 5x qPCR Multiplex MasterMix with 1.25mL of the supplied 5x Rehydration buffer.
  TIP: Add only 0.625mL of the rehydration buffer using reversed pipetting. Vortex for 30 seconds. Add the remainder 0.625mL and incubate at RT for 1 minute and finally vortex again.
  The rehydrated master mix does have a MgCl2 concentration of 2mM which will produce satisfactory results in most cases. However, if a higher Mg2+ concentration is required, you can add additional MgCl2.
  Important NOTE: After reconstitution the 5x qPCR Multiplex MasterMix should be stored best at -20°C for long term storage or at +2°C to +8°C for short term storage.
  NOTE: Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
  NOTE: 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 nuclease free water or a sterile buffer of 10mM Tris/HCl pH8.0-8.5. 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 5.0µL per reaction.
2. **Thaw (use) the rehydrated 5x qPCR Multiplex MasterMix**
   Subsequently invert the closed tube a few times or briefly vortex the mixture before use.
3. **NOTE:** It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts.
4. **Prepare PCR reaction mix.** Table 1 shows the reaction set up for a final volume of 25µL. If desired, the reaction size may be scaled down. Use 5µL of 5x qPCR Multiplex MasterMix for a final volume of 25µL. If desired, the reaction size may be scaled down.
5. Mix PCR reaction mixes thoroughly and dispense appropriate volumes into each reaction tube. Mix gently, e.g., by pipetting the reaction mix up and down a few times.
6. Add template DNA to the individual tubes containing the reaction mix.

Table 1: Recommendations for PCR / Reaction Setup (25µL PCR reaction)

Components	Volume	Final concentration
rehydrated 5x qPCR Multiplex MasterMix	5µL	1X
primer 1*:	0.5µL (0.25 – 2.5µL)	0.2µM (0.1-1µM)
primer 2*:	0.5µL (0.25 – 2.5µL)	0.2µM (0.1-1µM)
Probe (10µM)** (optional)	x µL	0.05-1µM
Template DNA / sample extract***	y µL	Genomic DNA: 50 ng (10 – 500ng) Plasmid DNA: 0.5 ng (0.1 – 1ng) Bacterial DNA: 5 ng (1 – 10ng)
nuclease free water	up to 25µL	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Primers should ideally have a GC content of 40-60%. For optimal results we recommend amplicon lengths in the range of 60 to 300bp.
** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!
*** Recommended template concentration should be 1ng – 300ng (genomic DNA) or 1ng – 1pg plasmid/viral DNA.

7. **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.
8. **Place PCR tubes in the thermal cycler and start program.**

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Table 2: Typical PCR/qPCR protocol for amplification of DNA

Step	Temperature	Time
Initial denaturation	95°C	15 min.
2-step PCR Protocol in case of TM >60°C		
Denaturation	95°C	20 sec.
Annealing/Extension*	>60°C - 75°C	20 - 30 sec. (25 - 40 cycles)
Alternatively use a 3-step PCR Protocol in case of TM <60°C		
Denaturation	95°C	20 sec.
Annealing	50°C - 60°C	20 - 30 sec. (25 - 40 cycles)
Extension*	68°C - 75°C	30 sec. (25 - 40 cycles)
Optional: Final extension	68°C - 75°C	5 minutes
Hold	<10°C	hold

NOTE: A two-step as well as a three-step PCR protocol can be used.

NOTE: Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used. It is highly recommended to establish a new RT-PCR by running a temperature gradient in order to find the best annealing/extension temperature for each new primer pair! Also a three-step PCR protocol can be applied with separate annealing and extension steps.

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

The final concentration of the 5x qPCR Multiplex MasterMix is 2mM. Some applications may require higher concentrations for best results. Use 25mM stock MgCl2 solution to adjust Mg2+ concentration according to table 3.

Table 3: Additional volume (µL) of MgCl2 per 25µL PCR reaction

Final MgCl2 conc. in reaction (mM) **complete buffer S**	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl2 per 50µL reaction (µL)	0	0.5	1	1.5	2	2.5

Table 4: 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 5: 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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Trouble shooting:

How can I optimize the PCR conditions and prevent false amplification?

- The annealing/extension temperature can usually be optimized.
  Try a **temperature gradient** and determine the best temperature, which results in a high amplification signal.
- Shorten the extension and annealing time. Too long and too many cycles may lead to over-amplification and side products.

Additional information

Quality Control

Lyophilized 5x qPCR Multiplex MasterMix is tested for contaminating activities, with no traces of endonuclease or exonuclease activity, and no nicking activity.

Applications

Automated Hotstart PCR
PCR with high specificity (Real time PCR / quantitative PCR)
Detection of low target copy number
2-step PCR
easy use for diagnostic kits

Storage and Stability

The Genaxxon bioscience qPCR Probe 2X LyoMix is shipped at ambient temperature and will retain full activity if stored at +15°C to +30°C) for at least 12 months if delivered aluminium foil bags are not opened (details are printed on the product label).

The rehydration buffer shipped together with the LyoMix has to be stored at -20°C to preserve its full function for 24 months.

After rehydration not used rehydrated 5x qPCR Multiplex MasterMix should be stored at -20°C to retain full activity for 24 months.

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.

NOTE: After addition of nuclease free water, template and primers the mix has to be used within the normal procedure time of a PCR.

Product Use Limitations

The lyophilized 5x qPCR Multiplex MasterMix 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.

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

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