Medix Biochemica

Product Manual
Cat. No: #9801lyo

qPCR Probe 2x LyoCake Master Mix

Description

qPCR Probe 2x LyoCake Master Mix contains all components necessary for rapid, sensitive, and reproducible quantification of DNA and cDNA. An engineered DNA polymerase and an optimized buffer including ultrapure dNTPs are key components of the ready-to-use mix. A hot-start formulation of the included DNA polymerase prevents false amplification during the reaction set-up.

qPCR Probe 2x LyoCake Master Mix is a ready to use reaction mix. It contains all components necessary for a successful and reliable probe-based qPCR in all standard real-time PCR cyclers. Only primers, template and a probe need to be added.

This mix provides robust PCR performance for a wide range of qPCR applications. The buffer is optimized to function with a great variety of templates.

Kit components

Component	S pack	M pack
qPCR Probe 2x LyoCake Master Mix	1 x bag (containing 4 x LyoCakes)	5 x bags (each containing 4 x LyoCakes)
Rehydration Buffer	1 x 1.25 mL	5 x 1.25 mL

*Other pack sizes, bulk orders and customization are available upon request.

Storage and shipment

The freeze-dried qPCR Probe 2x LyoCake Master Mix can be stored at room-temperature. Once rehydrated, it should be stored at -20°C. Please store the included rehydration buffer upon arrival at -20°C. The reagents are stable until the expiration date if stored correctly.

Preparations before use

1. Rehydrate the LyoCake by adding exactly **218 µL** of the respective **Rehydration Buffer** onto the Lyocake, resulting in 250 µL of ready-to-use 2x Master Mix.

2. Subsequently invert the closed tube a few times, briefly vortex and spin down the mixture before use. The tube should be placed on ice after rehydration.

3. The rehydrated 2x PCR Master Mix is then ready to be used for setting up a PCR experiment or can be stored at -20°C.

Reaction Master Mix set-up

The recommended master mix set-up for a 25 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
qPCR Probe 2x LyoCake Master Mix	12.5	1x
∞Forward primer (10 µM)	0.5	0.2 µM (0.05–1 µM)
∞Reverse primer (10 µM)	0.5	0.2 µM (0.05–1 µM)
Probe	x	0.2 µM (0.05–0.3 µM)
Template/Sample extract	y	<300 ng* DNA
Nuclease-free water	Up to 25 µL final volume	

Spin down and mix all solutions carefully before use.

Always include a control without template.

∞Primers should ideally have a GC content of 40–60% typically. For optimal results we recommend amplicon lengths in the range of 60 to 300 bp.

Suggested template concentration should be about 1 ng – 300 ng (genomic DNA) or 1 pg – 1 ng (plasmid/viral DNA).

myPOLS Biotec GmbH
Byk-Gulden-Strasse 2
78467 Konstanz, Germany
VAT no. DE294545185

www.medixbiochemica.com
www.mypols.de
mdx@medixbiochemica.com

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Instrument and program set-up

Cycles	Steps	Temperature	Time
1	Initial denaturation	95°C	2 min
25–40	Denaturation	95°C	15 sec**
	Annealing / Extension*	60°C	60 sec**

* Typically, the annealing temperature is about 3–5°C below the calculated melting temperature of the primers used.

** Suggested cycling times depend strongly on the cycler, template, and amplicon length. For some probe systems a separate annealing and extension steps may be necessary.

Technical information and support

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.

myPOLS Biotec GmbH
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