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qPCR Probe 2x LyoCake Master Mix

#9801lyo

Freeze-dried qPCR Probe 2x LyoCake Master Mix can be stored at room-temperature or at -20°C once it has been rehydrated.

Please store the included rehydration buffer upon arrival at -20°C.

Before use:

Please rehydrate the lyocake by adding exactly **218µl** of the respective **Rehydration Buffer** to each of the PCR mixes, resulting in 250µl of ready-to-use Master Mix.
Subsequently invert the closed tube a few times or briefly vortex and spin down the mixture before use.
The rehydrated 2x PCR Master Mix is now ready to be used for setting up a PCR experiment or can be stored at -20°C.

Contents

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.

Quality Control Assays

qPCR Probe 2x LyoCake Master Mix is tested in standard qPCR. The product demonstrates linearity of amplification over a specified serial dilution of human genomic DNA.
DNA polymerase activity: DNA polymerase activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme-concentration has been determined by protein-specific staining. Please inquire more information at info@mypols.de for the lot-specific concentration.
No contamination has been detected in standard test reactions.

Safety

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 the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLS Biotec takes no liability for damage resulting from handling or contact with this product. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

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Description

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 realtime 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 wide range of templates.

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
qPCR Probe 2x LyoCake Master Mix	12.5 µl	1x
Primer forward (10 µM)*	0.5 µl	0.2 µM (0.05-1 µM)
Primer reverse (10 µM)*	0.5 µl	0.2 µM (0.05-1 µM)
Probe	x µl	0.2 µM (0.05-0.3 µM)
Template	y µl	<300 ng** DNA
Nuclease-free water		up to 25 µl total volume

* 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 ng – 1 pg (plasmid/viral DNA).

Typical 2-step PCR protocol

Initial denaturation	95°C	2 min	
Denaturation	95°C	15 sec**	
Annealing/Extension*	60°C	60 sec**	25-40 cycles

* 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.

Please notice that an initial denaturation of more than 2 min is not needed. PCR protocol times and temperatures may vary depending on the used cycler, the nature of template and the amplicon length.

Cycler and Probe compatibility

This product is compatible for the use with any probe system and qPCR cycler not requiring a passive reference dye.

Recommendations for sample handling

- 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.
- Minimize the number of freeze-thaw cycles by storing the rehydrated product in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.
- We recommend the use of disposable gloves, DNase and RNase free filter tips and plastics.

Troubleshooting

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.

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