www.mypols.de
support@mypols.de
T +49(0)7531 122 965 00

qPCR Probe 2x Master Mix

#9801
Store at -20°C

Contents

qPCR Probe 2x 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.

Description

qPCR Probe 2x 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 fluorescence-based hydrolysis 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 including human-, mammal-, and plant-derived samples. The qPCR Probe 2x Master Mix ensures reproducible results, significantly reduces set-up times and the risk of pipetting mistakes.

Quality Control Assays

qPCR Probe 2x 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.

myPOLS Biotec GmbH, Blarerstraße 56, 78465 Konstanz, Germany T +49(0)7531 122 965 00

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
qPCR Probe 2x 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	x 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**	25-40 cycles
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.

Please notice that an initial denaturation of more than 2 min is not needed. Nevertheless, an extended initial denaturation of up to 15 min has no negative effect on the PCR performance.
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.

Related Products

This product is also available with a passive reference dye.

Recommendations for sample handling

- Thaw and keep all reagents on ice.
- 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 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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