Instructions for use

2x qPCR Probe MasterMix

1 ml, 100 reactions

Optimized 2x qPCR Probe MasterMix for all probe-based qPCR assays

1. Description

Our ***2x qPCR Probe MasterMix*** is an optimized ready-to-use mixture for probe-based assays such as TaqMan®, Beacons and MGBs. It contains a modified fast HotStart Taq DNA Polymerase, dNTPs and $MgCl_2$ combined in an optimized buffer system for Real-Time PCR / qPCR applications except primers and template DNA. The Master Mix can be used not only for expression analysis but also for genotyping, copy-number analyses and all sorts of probe-based quantitative PCRs.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

2. Applications

2x qPCR Probe MasterMix is recommended for use in all probe-based Real-Time PCR / qPCR applications such as Taqman, Beacons and MGBs. The optimized buffer system provides fast kinetics and highly efficient target amplification with low DNA template amounts and even for difficult templates. The ***2x qPCR Probe MasterMix*** contains all components, you just need to add primers and template DNA / cDNA. The ***2x qPCR Probe MasterMix*** is not only suitable for expression analysis, but also for genotyping, copy-number analysis and all sorts of probe-based quantitative PCR assays. Using our ***2x qPCR Probe MasterMix*** not only reduces contamination risks, but is also time saving, highly reproducible and very easy to prepare

3. Set contents

2x qPCR Probe MasterMix in **2x reaction buffer** containing the modified HotStart Taq polymerase, 0.4 mM each dNTP and an optimized buffer system containing $MgCl_2$.

Reagent	Amount	Lid colour
2x qPCR Probe MasterMix (100 reactions)	1 tube, 1 ml	white

Note: Some qPCR cyclers require the addition of ROX. The ***2x Probe qPCR MasterMix*** is also available with low or high concentrations of ROX.

4. Protocol

Before you start: Thaw the tube and invert the Master Mix 5-6 times to ensure mixing of the solution. Do not vortex! After thawing spin the tube briefly!

Note: Reactions can be conveniently set up at room temperature.

Recommended reaction mixture per well:

Components	Apply for 20 µl reaction	Apply for 10 µl reaction	Final concentration
2x qPCR Probe MM	10 µl	5 µl	1x
Forward primer	variable (e.g. 2 µl)	variable (e.g. 1 µl)	0.1 – 0.4 µM
Reverse primer	variable (e.g. 2 µl)	variable (e.g. 1 µl)	0.1 – 0.4 µM
Probe	variable (e.g. 2 µl)	variable (e.g. 1 µl)	0.2 – 0.4 µM
Template DNA	variable	variable	1 pg – 10 ng/reaction
Sterile dest. water	**adjust to 20 µl**	**adjust to 10 µl**	

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- **3-Step PCR protocol**

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
30 - 45 cycles		
Denaturation	5 seconds	92-95 °C
Annealing	5 seconds	60 °C (depends on primers)
Extension	5 – 20 seconds	72 °C

- **2-Step PCR protocol**

Step	Time	Temperature
Initial denaturation	2 minutes	92 - 95 °C
30 – 45 cycles		
Denaturation	5 seconds	92 - 95 °C
Annealing/Extension combined	5- 30 seconds	60 °C (depends on primers)

Note: For maximum efficiency and specificity, annealing temperatures, as well as extension time, primer/probe concentration and template DNA concentration may need to be optimized.

5. Storage conditions

Long-term storage: at -20°C (stable for about 24 months).
Short-term storage: at 4°C (stable for about 3 months).
However, short term storage for a few hours or up to 3 days at room temperature will not affect the performance of the master mix.

Product is not covered by pending or issued patents or may have certain limitations. To our best knowledge, this product does not provide any conflict with pending or issued patents.

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