

## Instructions for use

### 2x qPCR Probe BLUE MasterMix (low ROX)

1 ml, 100 reactions

Optimized 2x PCR MasterMix for all probe-based qPCR assays, including ROX reference dye (low concentration), blue coloured

#### 1. Description

Our **2x qPCR Probe BLUE Master Mix** is an optimized ready-to-use mixture for probe-based assays such as TaqMan<sup>®</sup>, Beacons and MGBs. It contains a modified fast HotStart Taq DNA Polymerase, dNTPs and MgCl<sub>2</sub> combined in an optimized buffer system for Real-Time PCR / qPCR applications except primers, probe 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. The **2x qPCR Probe BLUE MasterMix** contains ROX reference dye at a concentration of 100 nM (in its final concentration 50 nM) and a blue dye for convenient pipetting.

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

#### 2. Applications

**2x qPCR Probe BLUE MasterMix** is recommended for use in all probe-based Real-Time PCR / qPCR applications such as TaqMan<sup>®</sup>, 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 BLUE MasterMix** contains all components, you just need to add primers, probe and template DNA / cDNA. The **2x qPCR Probe BLUE 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 BLUE MasterMix** not only reduces contamination risks, but is also time saving, highly reproducible and very easy to prepare.

#### 3. Set contents

**2x qPCR Probe BLUE MasterMix** in **2x reaction buffer** containing the modified HotStart Taq polymerase, 0.4 mM each dNTP and an optimized buffer system containing MgCl<sub>2</sub> and a blue dye.

Reagent	Amount	Lid colour
<b>2x qPCR Probe BLUE MasterMix</b> (100 reactions)	1 tube, 1 ml	brown

**Note:** The **2x qPCR Probe BLUE MasterMix** is also available without or with high concentrations of ROX.

#### 4. Protocol

**Before you start:** Thaw the tube and invert the MasterMix 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
<b>2x qPCR Probe BLUE MM</b>	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	<b>adjust to 20 µl</b>	<b>adjust to 10 µl</b>	

- **3-Step PCR protocol**

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
<b>30 - 45 cycles</b>		
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
<b>30 – 45 cycles</b>		
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 MasterMix.

*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.*