



Instructions for use

ROTI®Lux Probe qPCR Master Mix

For ultra-fast qPCR protocols and probe-based real-time PCR assays. Also perfectly suited for multiplexing assays.

25KP (no ROX)
25KN (low ROX)

1. Description

Our 2x ROTI®Lux Probe qPCR Master Mixes are suitable for all common types of probe-based assays, for probe-based genotyping using multiplexing and for the analysis of gene expression in quantitative real-time PCR. The qPCR Master Mixes contain an optimised DNA polymerase that enables ultra-fast runs in less than 20 minutes. Due to the high sensitivity, even the smallest amounts of cDNA (<1pg) can be detected. The Master Mixes are also characterised by a particularly high temperature stability, which enables pipetting at room temperature and which means that they remain stable even after several Freeze and Thaw cycles (>10x).

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

2. Applications

ROTI®Lux Probe qPCR Master Mix 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. Using our ROTI®Lux Probe qPCR Master Mix not only reduces contamination risks, but is also time saving, highly reproducible and very easy to prepare. The master mix can be used for the following applications:

- Probe-based qPCR analyses
- Multiplexing
- Gene expression analyses
- Genotyping

3. Content

The ROTI®Lux Probe qPCR Master Mix (25KP) contains master mix contains a modified HotStart Taq polymerase, 0.4 mM each dNTP and an optimized buffer system containing MgCl₂ and a blue dye. ROTI®Lux Probe qPCR Master Mix, low ROX (25KN) additionally contains ROX reference dye at a concentration of 100 nM and in its final concentration 50 nM. 1 ml Master Mix is sufficient for one 96-well plate.

4. Protocol

4.1 Setting up the reaction

The 2x ROTI®Lux Probe qPCR Master Mix has been optimised for 10 µl, as wells as 20 µl reactions.

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.

Components	Apply for 20 µl reaction	Apply for 10 µl reaction	Final concentration
ROTI®Lux Probe qPCR Master Mix	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	

4.2 Basic qPCR protocols

- **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 – 10 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- 20 seconds	60 °C (depends on primers)

Note: For maximum efficiency and specificity, annealing temperatures, as well as extension time, primer 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).

Note: short term storage for a few hours or up to 3 days at room temperature will not affect the performance of the MasterMix.

ROTI®Lux Probe qPCR Master Mix, no ROX	1 ml	25KP.1
	5 ml	25KP.2
	10 ml	25KP.3
ROTI®Lux Probe qPCR Master Mix, low ROX	1 ml	25KN.1
	5 ml	25KN.2
	10 ml	25KN.3

Carl Roth GmbH + Co. KG

Schoemperlenstraße 3-5 • 76185 Karlsruhe • P.O. Box 100121 • 76231 Karlsruhe
 Phone: +49 (0) 721/ 5606-0 • Fax: +49 (0) 721/ 5606-149 • info@carlroth.com • www.carlroth.com

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