



## Direct qPCR ProbesMaster highROX

Robust real-time PCR master mix for amplification directly from blood, swabs or tissue with highROX  
2 x conc. master mix

Cat. No.	Amount
PCR-397S	2 x 1,25 ml (250 reactions x 20 µl)
PCR-397L	10 x 1,25 ml (1250 reactions x 20 µl)

### For *in vitro* use only!

**Shipping:** shipped on gel packs

**Storage Conditions:** store at -20 °C

**Additional Storage Conditions:** avoid freeze/thaw cycles,  
stable at 4 °C for up to 4 weeks

**Shelf Life:** 12 months

**Form:** liquid

**Concentration:** 2 x

### Description:

Direct qPCR ProbesMaster highROX is designed for quantitative real-time analysis of target DNA directly from whole blood, swabs and animal- or plant tissue. The mix allows robust amplification avoiding the requirement of any prior DNA purification procedures. The mix is recommended for use with dual-labeled fluorescent probes, e.g. TaqMan®, Molecular Beacon or Scorpion probes. It provides a powerful tool for multiplex-quantification of sample DNA in a broad dynamic range with exceptional sensitivity and precision. The mix contains all reagents required for qPCR (except template, primer and labeled fluorescent probe) in a premixed 2 x concentrated ready-to-use solution. High robustness, reliability and sensitivity of the mix are based on an antibody-blocked hot start polymerase in combination with an optimized and well-balanced buffer system. The mix ensures fast and easy preparation with a minimum of pipetting steps and is specially recommended for:

- Direct detection of viral or bacterial DNA in nasal or throat swabs
- Direct PCR from whole blood samples
- Direct amplification of target DNA from various tissue samples
- Point-of-Care diagnostics.

### Multiplexing Capability

Real-time PCR technology based on dual-labeled DNA probes provides a high sensitive and specific PCR system with multiplexing capability. The simultaneous detection of multiple targets in a single tube requires a primer/probe set for each target amplification. Sequences and concentrations of primers and probes should be optimized to avoid mutual influence, secondary structures and primer-dimer formations. Amplification of each target is detected in a separate fluorescence channel.

### ROX reference dye

Direct qPCR ProbesMaster highROX contains 500 nM ROX passive reference dye in the final assay. The dye does not take part in the PCR reaction but allows to normalize for non-PCR related signal variation and provides a baseline in multiplex reactions.

The reaction chemistry of the kit is optimized for instruments that are compatible with the evaluation of a high ROX reference signal.

### Content:

#### Direct qPCR ProbesMaster highROX (red cap)

2x conc. mix of antibody-blocked Hot Start polymerase, dNTPs, reaction buffer, 1 µM ROX, additives and stabilizers

#### Extraction Buffer (yellow cap)

10x conc.

Please handle with care and wear personal protective equipment!

#### PCR-grade Water (white cap)



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### Procedure

Before starting, take reagents out from fridge and allow to thaw completely. Vortex all reagents briefly and spin down the liquids.

#### 1. Sample preparation

##### 1.a Whole Blood (not recommended for heparin-, EDTA- or citrate-treated whole blood)

- Add whole blood (1-2 µl for 20 µl or 2-5 µl for 50 µl total assay volume) without any pre-treatment directly to the qPCR assay.

##### 1.b Samples from nasal or throat swabs

- Dilute 10x Extraction buffer to 1x with PCR-grade water
- Transfer 200 µl 1x Extraction Buffer into a 1.5 ml microtube
- Cut off the cotton tip with the collected nasal or throat swab and place it in the micro tube
- Close the tube and vortex for 15 sec
- Incubate at room temperature (20-25 °C) for 2-3 min
- Remove the cotton tip and squeeze it out at the rim of the tube
- Centrifuge briefly and transfer 1-5 µl of the supernatant (1-2 µl for 20 µl or 2-5 µl for 50 µl total assay volume) to the qPCR assay.

##### 1.c Samples from Animal or Plant Tissue

- Prepare a small piece from animal or plant tissue not exceeding 8 mm in diameter
- Crack plant seeds to less than 1 mm in diameter using a Bead-Beater, Tissue Lyser or small hammer
- Place the sample in a 1.5 ml microtube
- Add Extraction Buffer to the tissue sample as following:

Sample size (diameter)	1-2 mm	3-4 mm	5-8 mm
PCR-grade water	45 µl	90 µl	180 µl
Extraction Buffer	5 µl	10 µl	20 µl

- Mix briefly by tapping or vortexing and make sure that the sample is soaked with Extraction Buffer
- Incubate at room temperature (20-25 °C) for 3 min
- Centrifuge briefly and transfer 1-5 µl of the supernatant (1-2 µl for 20 µl or 2-5 µl for 50 µl total assay volume) to the RT-qPCR assay
- If sample is liquid:** Dilute 10x Extraction buffer to 2x with PCR-grade water. Add 2x Extraction buffer to your sample in a ratio of 1:1.

#### 2. Preparation of the PCR Assay

Preparation of a master mix is crucial in quantitative PCR reactions

to reduce pipetting errors. Prepare a master mix of all components except template as specified below. A reaction volume of 20-50 µl is recommended for most real-time instruments. Pipet with sterile filter tips and minimize the exposure of the labeled DNA probe to light. Perform the setup in an area separate from DNA preparation or analysis. No-template controls should be included in all amplifications.

component	stock conc.	final conc.	20 µl assay	50 µl assay
Direct qPCR ProbesMaster highROX	2x	1x	10 µl	25 µl
Extracted Sample or whole blood	-	-	1-2 µl	2-5 µl
Forward Primer 1 <sup>1)</sup>	10 µM	300 nM	0.6 µl	1.5 µl
Reverse Primer 1 <sup>1)</sup>	10 µM	300 nM	0.6 µl	1.5 µl
TaqMan® / Dual Labeled Probe 1 <sup>1)</sup>	10 µM	200 nM	0.4 µl	1 µl
Forward Primer 2 <sup>2)</sup>	10 µM	300 nM	0.6 µl	1.5 µl
Reverse Primer 2 <sup>2)</sup>	10 µM	300 nM	0.6 µl	1.5 µl
TaqMan® / Dual Labeled Probe 2 <sup>2)</sup>	10 µM	200 nM	0.4 µl	1 µl
PCR-grade water	-	-	fill up to 20 µl	fill up to 50 µl

- <sup>1)</sup> The optimal concentration for primers and probe may vary from 100 to 500 nM and should be optimized for each new assay set-up  
<sup>2)</sup> Required only for multiplex PCR applications

Mix the tubes briefly and spin down to remove bubbles.



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### 3. PCR Cycling

Switch on the real-time PCR cycler and set all cycling parameters as recommended in the table below. Place the vials into the instrument and start the program.

Initial denaturation	95 °C	2 min	1x
Denaturation Annealing and Elongation	95 °C 60-65 °C <sup>3)</sup>	15 sec 30-60 sec <sup>4)</sup>	35-45x

<sup>3)</sup> The annealing temperature depends on the melting temperature of the primers

<sup>4)</sup> The elongation time depends on the length of the amplicon. A time of 30 sec is sufficient for fragments < 500 bp

To obtain optimal specificity and amplification results an individual optimization of the recommended parameters is recommended for each particular sample/primer pair.

### 4. Data Analysis

- Calculate ct-values and evaluate the data according to the instruction of the cycler and requirements of the experiment/application.