DATA SHEET

Jena Bioscience

Direct qPCR ProbesMaster

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

Cat. No.	Amount
PCR-396S	2 x 1,25 ml (250 reactions x 20 µl)
PCR-396L	10 x 1,25 ml (1250 reactions x 20 µl)

For general laboratory use.

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: 2x

Description:
Direct qPCR ProbesMaster is designed for quantitative real-time analysis of target DNA directly from 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 a 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 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.

Interference of remaining components from sample matrix
Due to the fast and easy but relatively rough sample preparation remaining components from the sample matrix may be co-transferred into the PCR assay. These remains are mostly blocked by a combination of specially optimized additives. If inhibition of the PCR reaction occurs with higher volumes of transferred sample volume, please reduce the sample volumes or use a dilution of the sample in 1x Direct Extraction Buffer or water.
Remaining components of the sample matrix may also show fluorescence signals specially in the yellow and red spectral range. If using this fluorescence range for multiplex PCR assays or if using ROX, please take special attention that there is no interference between fluorescence from remaining matrix material and the used fluorescence channels for amplicon detection.

ROX Reference Dye
The mix can also be used in combination with ROX reference dye (#PCR-351) in PCR instruments that are compatible with the evaluation of the ROX signal.

Direct qPCR ProbesMaster

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

Content:
Direct qPCR ProbesMaster (red cap)
2x conc. mix of antibody-blocked Hot Start polymerase, dNTPs, reaction buffer, additives and stabilizers

Direct Extraction Buffer (yellow cap)
10x conc.
Please handle with care and wear personal protective equipment!

PCR-grade Water (white cap)

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 Blood Samples / Liquid Samples

- Dilute 10x Direct Extraction Buffer to 1x concentrated Buffer with PCR-grade water.
- Transfer 2 µl of the Blood/Liquid Sample into a tube containing 100 µl to 200 µl of 1x concentrated Direct Extraction Buffer (a dilution of Blood 1:50 to 1:100 in 1x Direct Extraction Buffer is recommended).
- Close the tube and vortex for 15 sec
- Incubate the tube at room temperature (20-25 °C) for 2-3 min.
- Transfer 1-2 µl of the supernatant into a 20 µl qPCR assay or 2-5 µl into a 50 µl qPCR assay (see table for Preparation of the PCR Assay below).

1.b Samples from nasal or throat swabs

- Dilute 10x Direct Extraction Buffer to 1x concentrated Buffer with PCR-grade water.
- Transfer 200 µl 1x Direct 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 1-2 µl of the supernatant into a 20 µl qPCR assay or 2-5 µl into a 50 µl qPCR assay (see table for Preparation of the PCR Assay below).

1.c Samples from Animal or Plant Tissue

- Dilute 10x Direct Extraction Buffer to 1x concentrated Buffer with PCR-grade water.
- 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 1x concentrated Direct Extraction Buffer to the tissue sample as following:

Sample size (diameter)	1-2 mm	3-4 mm	5-8 mm
1x Direct Extraction Buffer	50 µl	100 µl	200 µl

Direct qPCR ProbesMaster

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

- Mix briefly by tapping or vortexing and make sure that the sample is soaked with Direct Extraction Buffer
- Incubate at room temperature (20-25 °C) for 3 min
- Centrifuge briefly and transfer 1-2 µl of the supernatant into a 20 µl qPCR assay or 2-5 µl into a 50 µl qPCR assay (see table for Preparation of the PCR Assay below).

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	2x	1x	10 µl	25 µl
Extracted Sample	-	-	1-2 µl	2-5 µl
Forward Primer 1[1)]	10 µM	300 nM	0.6 µl	1.5 µl
Reverse Primer 1[1)]	10 µM	300 nM	0.6 µl	1.5 µl
TaqMan® / Dual Labeled Probe 1[1)]	10 µM	200 nM	0.4 µl	1 µl
Forward Primer 2[2)]	*10 µM*	*300 nM*	*0.6 µl*	*1.5 µl*
Reverse Primer 2[2)]	*10 µM*	*300 nM*	*0.6 µl*	*1.5 µl*
TaqMan® / Dual Labeled Probe 2[2)]	*10 µM*	*200 nM*	*0.4 µl*	*1 µl*
ROX Reference Dye #PCR-351 [3)]	*25 µM*	*500 nM*	*0.4 µl*	*1 µl*
PCR-grade water	-	-	fill up to 20 µl	fill up to 50 µl

[1)] The optimal concentration for primers and probe may vary from 100 to 500 nM and should be optimized for each new assay set-up
[2)] Required only for multiplex PCR applications
[3)] The mix can be used in combination with ROX reference dye

Mix the tubes briefly and spin down to remove bubbles.

Direct qPCR ProbesMaster

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

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 [4)]	15 sec 30-60 sec[5)]	35-45x

[4)] The annealing temperature depends on the melting temperature of the primers
[5)] 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.

Direct Extraction Buffer

Signal word: Danger

Hazard statements:
H314 Causes severe skin burns and eye damage.

Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection/....
P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P363 Wash contaminated clothing before reuse.
P405 Store locked up.

For further information see Safety Data Sheet.