DATA SHEET

Jena Bioscience

SCRIPT Direct RT-qPCR SybrMaster highROX

Robust real-time RT-PCR master mix with SYBR Green and ROX
for highly sensitive and specific amplification directly from tissue, swabs or blood

Cat. No.	Amount
PCR-533S	2 x 1,25 ml (250 reactions x 20 µl)
PCR-533L	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, store dark stable at 4 °C for up to 4 weeks

Shelf Life: 12 months

Form: liquid

Concentration: 2x

Description:
SCRIPT Direct RT-qPCR SybrMaster highROX is designed for quantitative real-time analysis of target RNA directly from animal- or plant tissue, swabs and blood. The mix allows robust amplification avoiding the requirement of any prior RNA purification procedures.
The mix contains SYBR® Green Fluorescent DNA Stain. It allows fast and easy quantification of sample RNA over a wide dynamic range with exceptional sensitivity and precision.
The mix contains all reagents required for RT-qPCR (except template and primers) in a premixed 2 x concentrated ready-to-use solution. High robustness, reliability and sensitivity of the mix are based on a genetically engineered reverse transcriptase and 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 amplification of target RNA from various tissues samples
- Direct detection of viral or bacterial RNA in nasal or throat swabs
- Direct PCR from whole samples
- Point-of-Care diagnostics.

SYBR® Green Fluorescent DNA Stain
SYBR® Green Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing no detectable inhibition to the PCR process. The dye is extremely stable, providing convenience during routine handling.
SYBR® Green is in contrast to EvaGreen® not recommended for high-resolution melting curve analysis (HRM).
To perform the SYBR® Green-based assay simply select the optical setting for SYBR® Green on the detection instrument.

ROX Reference Dye
The SCRIPT Direct RT-qPCR SybrMaster 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 variations in instruments that are compatible with the evaluation of the ROX reference signal.

Content:
SCRIPT Direct RT-qPCR SybrMaster highROX
2 x conc. mix of Reverse Transcriptase, antibody-blocked Hot Start polymerase, dNTPs, SYBR® Green DNA intercalator dye, ROX, reaction buffer, additives and stabilizer

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

PCR-grade Water

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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 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 RT-qPCR assay or 2-5 µl into a 50 µl RT-qPCR assay (see table for Preparation of the RT-qPCR 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 RT-qPCR assay or 2-5 µl into a 50 µl RT-qPCR assay (see table for Preparation of the RT-qPCR 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

- 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 RT-qPCR assay or 2-5 µl into a 50 µl RT-qPCR assay (see table for Preparation of the RT-qPCR 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
SCRIPT Direct RT-qPCR SybrMaster	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
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

Mix the tubes briefly and spin down to remove bubbles.

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3. RT-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.

Reverse transcription[2)]	50-55 °C	10-15 min	1x
Initial denaturation	95 °C	5 min	1x
Denaturation Annealing Elongation	95 °C 60-65 °C [3)] 72 °C	15 sec 20-30 sec 30-60 sec[4)]	35-45x

[2)] A reverse transcription time of 10 min is recommended for optimal amplicon lengths between 100 and 200 bp. Longer amplicons up to 500 bp may require a prolonged incubation of 15 min. Add 3 min for each additional 100 bp. The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.
[3)] The annealing temperature depends on the melting temperature of the primers.
[4)] 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.