DATA SHEET

Jena Bioscience

# SCRIPT Direct RT-qPCR ProbesMaster UNG

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

| Cat. No. | Amount |
|---|---|
| PCR-530S | 2 x 1,25 ml (250 reactions x 20 µl) |
| PCR-530L | 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:**
SCRIPT Direct RT-qPCR ProbesMaster UNG 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 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 RNA in a broad dynamic range with exceptional sensitivity and precision.
The mix contains all reagents required for RT-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 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 blood samples
- Point-of-Care diagnostics.

**Multiplexing Capability**
Real-time RT-PCR technology based on dual-labeled DNA probes provides a high sensitive and specific RT-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.

**UNG (Uracil-N-Glycosylase)**
The mix contains UNG (Uracil-N-Glycosylase) and dUTP instead of dTTP to eliminate carry-over contamination of DNA from previous PCR reactions. The UNG treatment at the onset of thermal cycling removes uracil residues from dU-containing DNA and prevents it from serving as template.

**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.

**Content:**
**SCRIPT Direct RT-qPCR ProbesMaster UNG**
2x conc. mix of Reverse Transcriptase, antibody-blocked Hot Start polymerase, UNG, dNTPs, reaction buffer, additives and stabilizers

**Extraction Buffer (Please handle with care and wear personal protective equipment!)**

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10x conc.

**PCR-grade Water**

**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 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 Extraction Buffer (a dilution of Blood 1:50 to 1:100 in 1x 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 Extraction Buffer to 1x concentrated Buffer 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 1-2 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 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 Extraction Buffer to the tissue sample as following:

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

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| component | stock conc. | final conc. | 20 µl assay | 50 µl assay |
|---|---|---|---|---|
| SCRIPT Direct RT-qPCR ProbesMaster UNG | 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 optionally be used in combination with ROX reference dye

Mix the tubes briefly and spin down to remove bubbles.

**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[4)] | 50-55 °C | 10-15 min | 1x |
| Initial denaturation | 95 °C | 5 min | 1x |
| Denaturation<br>Annealing and elongation | 95 °C<br>60-65 °C [5)] | 15 sec<br>30-60 sec[6)] | 35-45x |

[4)] 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.
[5)] The annealing temperature depends on the melting temperature of the primers.
[6)] 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.

**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/....

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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.