DATA SHEET

Jena Bioscience

SCRIPT RT-qPCR GreenMaster UNG

RT-real-time-PCR mix with green-fluorescent DNA stain and UNG

Cat. No.	Amount
PCR-526S	2 x 1,25 ml (2x conc.)
PCR-526L	10 x 1,25 ml (2x conc.)

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 conc.

Spectroscopic Properties: λ_{exc} 495 nm, λ_{em} 520 nm (dye bound to DNA)

Description:
SCRIPT RT-qPCR GreenMaster is designed for quantitative real-time analyses of RNA templates using a green-fluorescent DNA stain that is structurally similar to SYBR® GREEN and has been specially developed for DNA analysis applications including real-time PCR (qPCR). The ready-to-use mix is based on a genetically engineered reverse transcriptase with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments.
The 2x conc. mix contains all reagents required for RT-qPCR (except template and primers) allow fast and easy preparation with a minimum of pipetting steps. The premium quality enzymes and the optimized reaction buffer ensure superior real time PCR results.

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.

SCRIPT RT-qPCR GreenMaster is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR, the Hot Start Taq polymerase synthesizes DNA molecules that are complementary to the cDNA, thus generating a double-stranded DNA template. In the following cycle rounds, the DNA polymerase amplifies this double-stranded DNA template exponentially.
In one-step RT-qPCR, all components for reverse transcription and PCR are combined in one tube so that both reactions take place one after the other without opening the tube. This offers enormous convenience when analyzing targets from multiple RNA samples and minimizes the risk of contamination.
The master mix already contains an optimized amount of RNase inhibitor to prevent a decrease in sensitivity due to the degradation of RNA by RNase contamination when using small amounts of templete material.

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 RT-qPCR GreenMaster
Ready-to-use mix of SCRIPT Reverse Transcriptase, Hot Start Polymerase AB+, UNG, RNase Inhibitor, dNTPs, reaction buffer, green-fluorescent DNA stain, additives and stabilizers.

PCR-grade Water

Green-fluorescent DNA stain:
Green-fluorescent DNA stain is a superior DNA intercalator dye specially developed for DNA analysis applications like real-time

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PCR (qPCR). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing only lowest inhibition to the PCR process. The dye is stable both thermally and hydrolytically, providing convenience during routine handling.

Sensitivity:
Targets can generally be detected from <1 pg to 20 ng poly(A) RNA (mRNA) or 10 pg to 1 µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

Preparation of the RT-qPCR assay:
Add the following components to a nuclease-free microtube and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before adding the master mix.

component	stock conc.	final conc.	20 µl assay	50 µl assay
PCR-grade water	-	-	fill up to 20 µl	fill up to 50 µl
RNA template [1)]	-	<100 ng	x µl	x µl
forward Primer [2)]	10 µM	400 nM	0.8 µl	2 µl
reverse Primer [2)]	10 µM	400 nM	0.8 µl	2 µl
SCRIPT RT-qPCR Green-Master UNG [4)]	2x	1x	10 µl	25 µl

[1)] up to 100 ng polyA RNA or total RNA
[2)]The optimal concentration for primers and probe may vary from100 to 500 nm.
[4)] The Mix already contains RNase inhibitor that may be essential when working with low amounts of starting RNA.

Continue with reverse transcription and thermal cycling as recommended.

Reverse transcription and thermal cycling:
Place the vials in a PCR cycler and start the following program. Select SYBR® GREEN as fluorescence dye and collect the signal in the corresponding channel.

reverse transcription [5)]	50-55 °C	10-15 min	1x
initial denaturation [6)]	95°C	5 min	1x
denaturation	95°C	15 sec	35-45x
annealing [7)]	55-65°C	20 sec	35-45x
elongation [8)]	72°C	30 sec	35-45x

[5)] 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.
[6)] An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase
[7)] The annealing temperature depends on the melting temperature of the primers.
[8)] The elongation time depends on the length of the amplicon. A time of 1 min for a fragment of 1,000 bp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary. Note that optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.

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