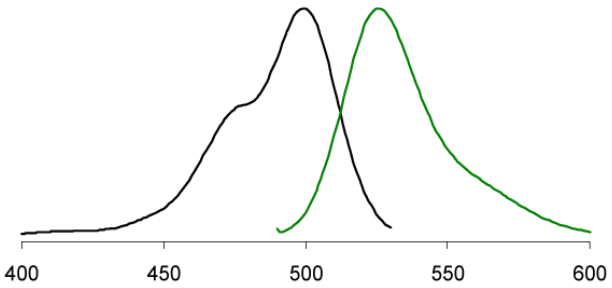
DATA SHEET

Jena Bioscience

SCRIPT RT-qPCR GreenMaster

RT-real-time-PCR mix with EvaGreen® fluorescent DNA stain

Cat. No.	Amount
PCR-514XS	500 µl (2x conc.)
PCR-514S	2 x 1,25 ml (2x conc.)
PCR-514L	10 x 1,25 ml (2x conc.)



Excitation (left) and emission (right) spectra of EvaGreen® bound to dsDNA in PBS buffer (pH 7.3).

For *in vitro* use only!

Shipping: shipped on blue ice

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} 500 nm (bound to DNA), λ_{em} 530 nm (bound to DNA)

Description:
SCRIPT RT-qPCR GreenMaster is designed for quantitative real-time analyses of RNA templates using the fluorescent DNA stain EvaGreen®. 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) in one box to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzymes and the optimized reaction buffer containing ultrapure dNTPs ensure superior real time PCR results.
RT-qPCR is used to amplify double-stranded DNA from single-stranded RNA templates to allow a rapid real-time quantification of RNA targets. In the reverse transcription step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step the hot-start DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. The hot-start polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.
One-step RT-qPCR offers tremendous convenience when applied to analysis of targets from multiple samples of RNA and minimizes the risk of contaminations.

Content:

Component	PCR-514XS	PCR-514S	PCR-514L
SCRIPT RT-qPCR GreenMaster* 2x conc. **red cap**	500 µl	2 x 1.25 ml	10 x 1.25 ml
RNase-free water **white cap**	1.2 ml	2 x 1.2 ml	2 x 6 ml

*Ready-to-use mix of SCRIPT Reverse Transcriptase, Hot Start Polymerase, RNase Inhibitor, dNTPs, reaction buffer, EvaGreen fluorescent DNA stain and stabilizers.

SCRIPT RT-qPCR GreenMaster

RT-real-time-PCR mix with EvaGreen® fluorescent DNA stain

EvaGreen® Fluorescent DNA Stain:
EvaGreen® Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR) and high-resolution DNA melting curve analysis (HRM). 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 both thermally and hydrolytically, providing convenience during routine handling. The high quantum yield, excellent stability and lowest inhibition toward PCR makes it the ideal fluorophore in real-time PCR applications and a superior replacement for the widely used SYBR® Green I dye. To perform the EvaGreen-based assay simply select the optical setting for SYBR® Green on the detection instrument.

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.

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

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

[1)] up to 100 ng polyA RNA or total RNA
[2)] SCRIPT RT-qPCR GreenMaster 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.

reverse transcription [3)]	50°C	10-15 min	1x
initial denaturation [4)]	95°C	5 min	1x
denaturation	95°C	15 sec	35-45x
annealing [5)]	55-65°C	20 sec	35-45x
elongation [6)]	72°C	30 sec	35-45x

[3)] 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.
[4)] An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase
[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 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.