DATA SHEET

Jena Bioscience

SCRIPT RT-qPCR ProbesMaster UNG

RT-real-time-PCR mix for using DNA probes
2 x conc. master mix

Cat. No.	Amount
PCR-523S	2 x 1,25 ml (250 reactions x 20 µl)
PCR-523L	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

Shelf Life: 12 months

Form: liquid

Concentration: 2x conc.

Description:
SCRIPT RT-qPCR ProbesMaster UNG is designed for quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes. 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, primers and the dual labeled fluorescent probe) 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.

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.

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.
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 ProbesMaster UNG
Ready-to-use mix of SCRIPT Reverse Transcriptase, Hot Start Polymerase AB+, UNG, RNase Inhibitor, dNTPs incl. dUTP, reaction buffer and stabilizers.

PCR-grade Water

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Dual Labeled Fluorescent probes:
Real-time PCR technology based on dual labeled DNA probes provides a highly sensitive and specific PCR system with multiplexing capability. It requires two standard PCR primers and the DNA probe that hybridizes to an internal part of the amplicon. The sequence of the dual labeled DNA probe should avoid secondary structure and primer-dimer formation.

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
dual-labeled Probe [3)]	10 µM	200 nM	0.4 µl	1 µl
SCRIPT RT-qPCR Probes-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.
[3)]Optimal results may require a titration of DNA probe concentration beetween 50 and 800 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.

reverse transcription [5)]	50-55 °C	20-30 min	1x
initial denaturation [6)]	95°C	5 min	1x
denaturation	95°C	15 sec	35-45x
annealing and elongation	60-65 °C [7)]	40-60 sec [6)]	35-45x

[5)] A reverse transcription time of 20 min is recommended for optimal amplicon lengths between 100 and 200 bp. Longer amplicons up to 500 bp may require a prolonged incubation of 30 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 and DNA probe used.
[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.