DATA SHEET

Jena Bioscience

SCRIPT RT-qPCR SybrMaster Lyophilisate

Lyophilised RT-qPCR Master Mix with SYBR®Green fluorescent DNA stain

Cat. No.	Amount
PCR-169S	192 reactions x 20 µl
PCR-169L	960 reactions x 20 µl

For general laboratory use.

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Additional Storage Conditions: store in an aluminium-coated bag or in a dry place

lyophilisates may hydrate at humidity levels >70 % when sealing is opened.

Shelf Life: 6 months in sealed package

Spectroscopic Properties: λ_{exc} 494 nm, λ_{em} 521 nm (SYBR®Green bound to DNA)

Description:
SCRIPT RT-qPCR SybrMaster Lyophilisate is designed for quantitative real-time analyses of RNA templates using the fluorescent DNA stain SYBR®Green. The enzyme mix is based on a genetically engineered reverse transcriptase with enhanced thermal stability resulting in an increased specificity, higher cDNA yield and an improved efficiency for highly structured and long cDNA fragments.
The lyophilisate contains all reagents required for RT-qPCR (except template and primer) in one tube to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzyme mix, ultrapure dNTPs and the optimized complete reaction buffer ensure superior amplification results.
SCRIPT RT-qPCR SybrMaster Lyophilisate 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.

Content:
SCRIPT RT-qPCR SybrMaster Lyophilisate
Preloaded lyophilisates containing SCRIPT Reverse Transcriptase, RNase Inhibitor, Hot Start Polymerase Ab+, dNTPs, Reaction Buffer, $MgCl_2$, SYBR®Green DNA intercalotor dye and stabilizers

PCR grade water

Handling
SCRIPT RT-qPCR SybrMaster Lyophilisate is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete RT-qPCR master mix in a dry, room temperature stable format. The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.
Each vial contains all components (except primers and template) required for a 20 µl RT-qPCR assay.
To perform the assay, only fill up the vials with a mix of primers and RNA template.

SCRIPT RT-qPCR SybrMaster Lyophilisate

Lyophilised RT-qPCR Master Mix with SYBR®Green fluorescent DNA stain

SYBR®Green fluorescent DNA stain:
SYBR® Green fluorescent DNA stain is a superior DNA intercalator dye specially developed for DNA analysis applications like real-time 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 10 pg to 500 ng polyA RNA or 10 pg to 1 µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

RT-qPCR assay preparation
1. Preparation of the RNA/Primer Mix
Add the following components to a nuclease-free microtube and mix by pipetting gently up and down:

Component	stock conc.	final conc.	1 assay
RNA template		10 pg - 1 µg	
forward Primer	10 µM	400 - 600 nM	0.8 - 1.2 µl
reverse Primer	10 µM	400 - 600 nM	0.8 - 1.2 µl
RNase-free water			fill up to 20 µl

2. Denaturation and primer annealing (optional)
Please note: Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure, for self- or cross-complementary primers and for initial experiments with new targets.

Incubate the mixture at 70°C for 5 min and place it at room temperature for 5 min.

For many standard combinations of RNA and primers, the heat treatment can be omitted without any negative effect on results. In this case, the primers can be added to the lyophilisate before the template is added.

3. Dispensing the master mix
Dispense 20 µl of the RNA/Primer Mix to each lyophilisate containing tube or well of the plate.

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 [1)]	50-55 °C	10-15 min	1x
initial denaturation [2)]	95°C	5 min	1x
denaturation	95°C	15 sec	35-45x
annealing [3)]	55-65°C	20 sec	35-45x
elongation [4)]	72°C	30 sec	35-45x

[1)] 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.
[2)] An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase
[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 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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