DATA SHEET

Jena Bioscience

SCRIPT High Fidelity RT-PCR Kit

One-Step RT-PCR Kit for highly precise and fast amplification

Cat. No.	Amount
PCR-510S	100 reactions x 50 µl
PCR-510L	5 x 100 reactions x 50 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

Description:
SCRIPT High Fidelity RT-PCR Kit is the ideal choice for applications where highly sensitive reverse transcription and high fidelity PCR at high amplification speed in single tubes are required. The enzyme 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. Additionally, a genetically engineered proof-reading enzyme is implemented as polymerase. It provides a 50-fold higher accuracy and an increased processivity compared to Taq, resulting in 2-fold shorter elongation times.
The kit contains all reagents required for RT-PCR (except template and primer) in one box to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzyme mix and the optimized complete reaction buffer containing ultrapure dNTPs ensure superior amplification results.
RT-PCR 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 step Taq DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. During subsequent rounds of cycling the DNA polymerase exponentially amplifies this double-stranded DNA template.
In one-step RT-PCR all components of RT and PCR are mixed in one tube prior to starting the reaction and thus carried out sequentially without opening the tube. This offers tremendous convenience when applied to analysis of single targets from multiple samples of RNA and minimizes the risk of contaminations.

Content:
SCRIPT High Fidelity RT-PCR Enzyme Mix (red cap)
Mix of SCRIPT Reverse Transcriptase, Hot Start High Fidelity Polymerase and RNase Inhibitor in storage buffer with 50 % glycerol (v/v)

SCRIPT High Fidelity RT-PCR Reaction Mix (green cap)
2x conc. Reaction Buffer containing dNTPs

PCR-grade Water (white cap)

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.

RT-PCR assay without sample denaturation:
(standard RNA/primer combinations)
Preparation of the RT-PCR Assay
[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. For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on

SCRIPT High Fidelity RT-PCR Kit

One-Step RT-PCR Kit for highly precise and fast amplification

results.]
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.	1 assay
SCRIPT High Fidelity RT-PCR Reaction Mix	2x	1x	25 µl
RNA template[1)]	-	1 pg - 1 µg	x µl
forward Primer	10 µM	200-400 nM	1-2 µl
reverse Primer	10 µM	200-400 nM	1-2 µl
SCRIPT High Fidelity RT-PCR Enzyme Mix[2)]	-	-	2 µl
PCR-grade water	-	-	fill up to 50 µl

[1)]10 pg to 200 ng polyA RNA or 100 pg to 2 µg total RNA

[2)]SCRIPT High Fidelity RT-PCR Enzyme Mix already contains RNase inhibitor that is recommended and may be essential when working with low amounts of starting RNA.

Continue with reverse transcription and thermal cycling as recommended.

RT-PCR assay with sample denaturation:
(RNA/primer with a high degree of secondary structure)
[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. For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results.]

Preparation of the RNA Template / 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[1)]	-	1 pg - 1 µg	-
forward Primer	10 µM	200-400 nM	- 1-2 µl
reverse Primer	10 µM	200-400 nM	- 1-2 µl
PCR-grade water	-	-	fill up to 10 µl

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

Preparation of the RT-PCR Mix
Add the following components to a further nuclease-free microtube and mix by pipetting gently up and down.

component	stock conc.	final conc.	1 assay
SCRIPT High Fidelity RT-PCR Reaction Mix	2x	1x	25 µl
SCRIPT High Fidelity RT-PCR Enzyme Mix[2)]	-	-	2 µl
PCR-grade water	-	-	fill up to 40 µl

[1)]10 pg to 200 ng polyA RNA or 100 pg to 2 µg total RNA

[2)]SCRIPT High Fidelity RT-PCR Enzyme Mix already contains RNase inhibitor that is recommended and may be essential when working with low amounts of starting RNA.

Complete RT-PCR Mix
Add 40 µl RT-PCR Mix to 10 µl RNA Template / Primer Mix to complete the 50 µl assays. Pipett on ice and mix by pipetting gently up and down.

Reverse transcription and thermal cycling:
Place the vials in a PCR cycler and start the following program.

reverse transcription[3)]	50 °C	30-60 min	1x
initial denaturation[4)]	95 °C	5 min	1x
denaturation	95 °C	10 sec	30-40x
annealing[5)]	55-65 °C	20 sec	30-40x
elongation[6)]	72 °C	30 sec/kb	30-40x
final elongation	72 °C	2 min	1x

[3)]The optimal time depends on the length of cDNA. Incubation of 60 min is recommended for cDNA fragments of more than 2,000 bp length. 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)]A prolonged initial denaturation time of up to 5 min is recommended to inactivate the reverse transcriptase
[5)]The annealing temperature depends on the melting temperature of

SCRIPT High Fidelity RT-PCR Kit

One-Step RT-PCR Kit for highly precise and fast amplification

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.