DATA SHEET

Jena Bioscience

SCRIPT Reverse Transcriptase

Reverse Transcriptase with increased thermal stability without buffer

Cat. No.	Amount
PCR-425-100KU	100 kilo units
PCR-425-1MU	1 mio units
PCR-425-10MU	10 mio units

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37 °C.

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: 200 units/µl

Description:
SCRIPT Reverse Transcriptase is a genetically engineered version of M-MLV Reverse Transcriptase (M-MLV RT) with eliminated RNase H activity and increased thermal stability. The enzyme is a RNA-directed DNA polymerase that synthesizes a complementary DNA strand initiating from a primer using single-stranded RNA or DNA as template. Its enhanced thermal stability in combination with the deactivated RNase H activity results in an increased specificity, higher cDNA yield and an improved efficiency for full length cDNA synthesis compared with standard M-MLV RT. The enzyme is recommended for synthesis of cDNA from 100 bp up to 10 kb length.

Content:
Reaction Buffer and Nucleotides are not included. Please refer to our our sections Buffers & Components and Nucleotides.

SCRIPT Reverse Transcriptase
200 units/µl in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50 % Glycerol (v/v), pH 7.5

Recommended protocol for cDNA synthesis:
Sample denaturation prior to the assay set-up is only 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. In this case incubate the mixture of RNA and primers at 65-70 °C for 5 min and place it at room temperature (if using a specific primer) or on ice (if using oligo-dT or random primer).

Assay set-up
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	final conc.
SCRIPT RT Buffer complete	1 x
dNTP Mix	500 µM each dNTP
SCRIPT Reverse Transcriptase [1)]	100-200 units/assay
RNase Inhibitor [2)]	20 units/assay
Primer	-gene-specific primer: 10-20 pmol (50-100 ng) -oligo-dT_{15-25} primer: 50 pmol (300 ng) -random primer: 50 pmol (100 ng)
PCR-grade Water	fill up to final volume

Use 10 pg - 5 µg of total RNA or 10 pg-500 ng of mRNA per assay.

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[1] 100 units (0.5 µl) of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units (1 µl) per assay may show even higher transcription yields under selected assay conditions.
[2] Addition of 20-40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA.

First-strand cDNA synthesis

Incubate the reaction mix at 50 °C for 30-60 min if using a gene-specific primer. If using oligo-dT or random primer incubate at 42°C for
10 min followed by incubation at 50°C for 30-60 min.
Please note: 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.

Optional: Heat inactivation

Heat the mixture to 70°C for 10 min to inactivate the Reverse Transcriptase.

Optional: RNA removal

Add 2 units DNase-free RNase and incubate at 37°C for 20 min.
The cDNA can now be used as template in PCR or be stored at -20°C.
Apply 2-5 µl of the RT assay for further amplification in PCR.
However, some specific DNA applications may require prior inactivation of the remaining RTase or the enzymatic removal of RNA.