DATA SHEET

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Script 3.0 Reverse Transcriptase

M-MLV Reverse Trascriptase with increased thermal stability

Cat. Nº.	Amount
☐ PRT- 102XS	10.000 units
☐ PRT- 102S	20.000 units
☐ PRT- 102M	40.000 units
☐ PRT- 102L	100.000 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.

Shipping:
Shipped on blue ice
Storage Conditions:
Store at -20 °C
Additional Storage Conditions:
Avoid freeze/thaw cycles
Shelf Life:
12 months

For *in vitro* use only!

Purity: free of endo- and exodeoxyribonucleases, phosphatases and ribonuclease

Form: liquid

Concentration: 200 units/µL

Kit contents:

SCRIPT 3.0 Reverse Transcriptase (blue cap)
200 units/µL in 20 mM Tris-HCl pH 7.5 (25°C), 5 mM DTT, 50% glycerol (v/v) and stabilizers.

5x SCRIPT 3.0 RT Buffer complete (red cap)
Tris-HCl (pH 8.3), KCl, $MgCl_2$ and DTT.

DTT stock solution (purple cap)
100 mM DTT

Applications:
Extremely sensitive and highly specific RT-PCR, synthesis of highly structured cDNA fragments, DNA labelling.

Description:
SCRIPT Reverse Transcriptase is a genetically engineered version of M-MLV Reverse Transcriptase (M-MLV RT) with reduced 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. 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.

Recommended protocols for cDNA synthesis:
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.

1a Assay set-up without sample denaturation (standard RNA/primer combinations)

Assay preparation

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
SCRIPT 3.0 RT buffer complete	5x	1 x	4 µL
RNA template[1]	-		x µL
Primer Fw and Rv[2]	10 µM		1 - 5 µL
SCRIPT 3.0 RT	200 units/ µl	100 units	0,5 µL
RNAse-free water	-	-	up to 20
DTT	100 mM	5 mM	1 µL
RNAse Inhibitor[3] (*optional*)	40 units / µl	40 units	1 µL
dNTP mix	10 mM	500 µM	1 µL

1) Total RNA: 10 pg - 5 µg or mRNA: 10 pg - 500 ng
2) Gene-specific primer: 10-20 pmol (50-100 ng) or Oligo-dT_{15-25} primer: 50 pmol (300 ng) or Random-primer: 50 pmol (100 ng)
3) Addition of 20-40 units of RNAse Inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA. NOT PROVIDED in this kit.

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NOTE:

- Adding of up to 5 mM DTT may increase the yield and is recommended for individual optimization
- 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.

1b. Assay set-up with sample denaturation: (RNA/primer with a high degree of secondary structure)

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.	10 µL assay
RNA template[1]	-	10 pg - 5 µg	x µL
Primer forward[2]	10 µM	10 - 50 pmol	1 - 5 µL
Primer reverse[2]	10 µM	10 - 50 pmol	1 - 5 µL
RNase-free water	-	-	up to 10 µL

1) Total RNA: 10 pg - 5 µg or mRNA: 10 pg - 500 ng

2) Gene specific primer: 10-20 pmol (50-100 ng) - Oligo-dT_{15-25} primer: 50 pmol (300 ng) - Random primer: 50 pmol (100 ng)

Denaturation and primer annealing

Incubate the mixture at 70 °C for 5 min and place it at room temperature for 5 min if using specific primer or on ice if using Oligo-dT or Random primer.

Preparation of the Reaction Mix

Add the following components to the RNA template and primer mix and mix by pipetting gently up and down.

Component	Stock conc.	Final conc.	20 µl assay
Rnase-free water	-	-	up to 20 µL
RNA template/primer	-	-	10 µL
SCRIPT RT Buffer	5x	1x	4 µL
DTT stock solution	100 mM	5 mM	1 µL
dNTP Mix	10 mM each	500 µM each	1 µL
Rnase Inhibitor	40 units/µL	40 units	1 µL
SCRIPT RT Enzyme	200 units/µL	100 units	0,5 µL

NOTE:

- Adding of up to 5 mM DTT may increase the yield and is recommended for individual optimization.
- Addition of 20-40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA. NOT PROVIDED in this kit.
- 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. First-strand cDNA synthesis:

- Place the vials in a PCR cycler and start the following program.
- Incubate the reaction mix at 55 °C for 30-60 min if using gene-specific primers. If using Oligo-dT or Random primers incubate at 42 °C for 10 min followed by incubation at 55 °C for 30-60 min.

Step	Temp.	Time
Reverse transcription[1]	55 °C	30-60 min
Inactivation of RT[2]	70 °C	10 min

1) 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 60 °C for difficult templates with high secondary structure.

2) Optional to heat inactivate the Reverse Transcriptase.

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.

3. RNA removal *(optional)*

- 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 the prior inactivation of the remaining RTase or the enzymatic removal of RNA.

Activity:
Activity and stability tested in first strand cDNA synthesis.

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.