

## SCRIPT cDNA Synthesis Kit protein

**Cat#: orb533402 (User Manual)**

### SCRIPT cDNA Synthesis Kit

First strand cDNA synthesis with high sensitivity and efficiency

#### For *in vitro* use only!

**Shipping:** shipped on gel packs

**Storage Conditions:** store at -20 °C

**Additional Storage Conditions:** avoid freeze/thaw cycles

**Shelf Life:** 12 months

**Form:** liquid

#### Applications:

Synthesis of highly structured and long cDNA fragments, extremely sensitive and highly specific RT-PCR, DNA labeling.

#### Description:

SCRIPT cDNA Synthesis Kit contains all reagents required for first strand cDNA synthesis in one box combining simple handling with high flexibility. The premium quality Reverse Transcriptase, ultrapure dNTPs and an optimized reaction buffer ensure superior results with highest reproducibility. The kit is optimized for high efficiency in a broad range of primer-template combinations.

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. Activity and stability has been tested in first strand cDNA synthesis.

**Content:**

Component	Cap	S	L
SCRIPT Re-verse Tran-scriptase 200 units/ $\mu$ l	red	100 $\mu$ l	500 $\mu$ l
SCRIPT RT Buffer com-plete 5x conc.	green	400 $\mu$ l	2x 1 ml
dNTP Mix 10 mM each	white	100 $\mu$ l	500 $\mu$ l
Oligo-(dT)20 primer 100 $\mu$ M	white	50 $\mu$ l	250 $\mu$ l
Random Hexamers 100 $\mu$ M	white	50 $\mu$ l	250 $\mu$ l
RNase Inhibitor 40 units/ $\mu$ l	yellow	50 $\mu$ l	250 $\mu$ l
Positive Control RNA 10 ng/ $\mu$ l	blue	10 $\mu$ l	50 $\mu$ l
RNase-free water	white	1.2 ml	6 ml

**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. Pipette 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
RNase-free water	-	-	fill up to 20 µl
RNA template	-	total RNA: 10 pg - 5 µg or mRNA: 10pg-500ng	x µl
Primer	100 µM	-gene-specific primer: 10-20 pmol (50-100 ng) -oligo-dT <sub>15-25</sub> primer: 50 pmol (300 ng) -random primer: 50 pmol (100 ng)	- 0.1-0.2 µl - 0.5 µl - 0.5 µl
SCRIPT RT Buffer complete	5x	1x	4 µl
dNTP Mix	10 mM each	500 µM each	1 µl
RNase Inhibitor <sup>1)</sup>	40 units/µl	20 units	0.5 µl
SCRIPT Reverse Transcriptase <sup>2)</sup>	200 units/µl	100 units	0.5 µl

1) Addition of 20-40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA.

2) 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.

Continue with step **2 First-strand cDNA synthesis**

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

*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.	20 $\mu$ l assay
RNase-free water	-	-	fill up to 10 $\mu$ l
RNA template	-	total RNA: 10 pg - 5 $\mu$ g or mRNA: 10pg-500ng	x $\mu$ l
Primer	100 $\mu$ M	-gene-specific primer: 10-20 pmol (50-100 ng) -oligo-dT <sub>15-25</sub> primer: 50 pmol (300 ng) -random primer: 50 pmol (100 ng)	- 0.1-0.2 $\mu$ l - 0.5 $\mu$ l - 0.5 $\mu$ l

*Denaturation and primer annealing*

Incubate the mixture at 65-70 °C for 5 min and place it at room temperature (if using specific primer) or on ice (if using oligo-dT or random primer).

*Preparation of the Reaction Mix*

Add the following components to a further nuclease-free microtube and mix by pipetting gently up and down:

component	stock conc.	final conc.	20 $\mu$ l assay
RNase-free water	-	-	fill up to 10 $\mu$ l
SCRIPT RT Buffer complete	5x	1x	4 $\mu$ l
dNTP Mix	10 mM each	500 $\mu$ M each	1 $\mu$ l
RNase Inhibitor <sup>1)</sup>	40 units/ $\mu$ l	20 units	0.5 $\mu$ l
SCRIPT Reverse Transcriptase <sup>2)</sup>	200 units/ $\mu$ l	100 units	0.5 $\mu$ l

1) Addition of 20-40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA.

2) 100 units (0.5  $\mu$ l) of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units (1  $\mu$ l) per assay may show even higher transcription yields under selected assay conditions.

*Complete Reaction Mix* Add 10  $\mu$ l Reaction Mix to 10  $\mu$ l RNA Template / Primer Mix to complete the 20  $\mu$ l Reaction Mix. Pipette on ice and mix by pipetting gently up and down.

## 2 First-strand cDNA synthesis

### Incubation

Incubate the reaction mix at 50 °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 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  $\mu$ l 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.