DATA SHEET

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cDNA Synthesis Kit with RNase Inhibitor

First strand cDNA synthesis with high sensitivity and efficiency

Cat. Nº.	Amount
☐ PRT- 110XS	20 reactions x 20 µl
■ PRT- 110S	100 reactions x 20 µl
☐ PRT- 110L	200 reactions x 20 µl
☐ PRT- 110XL	500 reactions x 20 µl

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

Form:
Liquid

Additional Storage Conditions:
Avoid freeze/thaw cycles

Shelf Life:
12 months

For *in vitro* use only!

Kit contents:

SCRIPT Reverse Transcriptase #PRT-102 (blue cap)
200 units/µl in 20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 5 mM DTT and stabilizers

SCRIPT Reverse Transcriptase buffer 5x (red cap)
250 mM Tris-HCl (pH 8.3), 500 mM KCl, 30 mM MgCl2, 25 mM DTT

DTT stock solution (purple cap)
100 mM DTT

dNTP Mix #NUC-100 (white cap)
10 mM each dNTP

Random Hexamers (white cap)
100 µM

Rnase Inhibitor #PCK-122 (blue cap)
40 units/µl

RNAse-free water (white cap)

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.

Component	PRT-110XS 20 reactions	PRT-110S 100 reactions	PRT-110L 200 reactions	PRT-110XL 500 reactions
SCRIPT Reverse Transcriptase 200 U/µl	10 µl	50 µl	100 µl	250 µl
SCRIPT RT - Buffer complete 5x	80 µl	400 µl	800 µl	2 x 1 ml
DTT stock solution	20 µl	100 µl	200 µl	500 µl
dNTP Mix	20 µl	100 µl	200 µl	500 µl
Random Hexamers	10 µl	50 µl	100 µl	250 µl
RNAse Inhibitor 40 U/µl	10 µl	50 µl	100 µl	250 µl
RNAse-free water	1,2 ml	1,2 ml	2,4 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.

cDNA Synthesis Kit with RNAse Inhibitor

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

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 reaction
RNA template	-	**total RNA:** 10 pg - 5 µg **mRNA:** 10 pg - 500 ng	x µl
Primer	100 µM	**Specific primer:** 10-20 pmol (50-100 ng) **Random hexamer:** 50 pmol (100 ng) **oligo-dT$_{15-25}$** 50 pmol(300 ng)	0,1 - 0,2 µl 0,5 µl 0,5 µl
SCRIPT RT Buffer 5x	5x	1x	4 µL
dNTP Mix	10 mM each	500 µM each	1 µl
DTT stock solution[1]	100 mM	5 mM	1 µL
Rnase Inhibitor[2]	40 U/µl	20 U	0,5 µl
SCRIPT Reverse Transcriptase[3]	200 U/µl	100 U	0,5 µl
Nuclease-free water	-	-	fill up to 20 µl

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

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

[3] 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)

Assay preparation:

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

Component	Stock conc.	Final conc.	20 µl reaction
RNA template	-	**total RNA:** 10 pg - 5 µg **mRNA:** 10 pg - 500 ng	x µl
Primer	100 µM	**Specific primer:** 10-20 pmol (50-100 ng) **Random hexamer:** 50 pmol (100 ng) **oligo-dT$_{15-25}$** 50 pmol(300 ng)	0,1 - 0,2 µl 0,5 µl 0,5 µl
Nuclease-free water	-	-	fill up to 20 µl

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 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 µl reaction
SCRIPT RT Buffer 5x	5x	1x	4 µL
dNTP Mix	10 mM each	500 µM each	1 µl
DTT stock solution[1]	100 mM	5 mM	1 µL
Rnase Inhibitor[2]	40 U/µl	20 U	0,5 µl
SCRIPT Reverse Transcriptase[3]	200 U/µl	100 U	0,5 µl
Nuclease-free water	-	-	fill up to 20 µl

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

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

[3] 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.

Complete Reaction Mix

Add 10 µl Reaction Mix to 10 µl RNA Template / Primer Mix to complete the 20 µl Reaction Mix. Pipett on ice and mix by pipetting gently up and down.

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2 First-strand cDNA synthesis

Incubation

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.

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 up to 65 °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 the prior inactivation of the remaining RTase or the enzymatic removal of RNA.

Activity:

Activity and stability tested in first strand cDNA synthesis.