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Scriptase RT - cDNA Synthesis Kit

Component	Cat#	SM3134.0100	SM3134.0500	SM3134.1000	Colour code of cap
Scriptase RT (200 units/µL)		100µL	5x 100µL	10x 100µL	orange
5X Scriptase RT Buffer complete		450µL	5x 450µL	10x 450µL	brown
dNTP-Mix (10mM each)		100µL	5x 100µL	10x 100µL	transparent
Oligo-(dT)20 primer (100µM)		100µL	5x 100µL	10x 100µL	white
Random Hexamers (100µM)		100µL	5x 100µL	10x 100µL	pink
RNase Inhibitor (40 units/µL)		50µL	5x 50µL	10x 50µL	purple
RNase-free water		1.5mL	5x 1.5mL	10x 1.5mL	blue
10X DNase Incubation Buffer		300µL	5x 300µL	10x 300µL	yellow
DNase (5 units/µL)		100µL	5x 100µL	10x 100µL	green

Product description

The Genaxxon bioscience Scriptase RT – 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.

All reagents required for cDNA synthesis from RNA/mRNA are provided with this kit (except template and specific primers) combining simple handling with high flexibility. The high quality Scriptase Reverse Transcriptase is a genetically engineered version of M-MLV Reverse Transcriptase (M-MLV RT) with eliminated RNase H activity and increased thermal stability, ultrapure dNTPs and the optimized complete reaction buffers ensure superior amplification results.

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.

Supplied buffers/solutions

Scriptase RT:	200 units/µL in storage buffer containing 50% glycerol (v/v).
5X RT Reaction Buffer:	250mM Tris/HCl (pH8.3), 375mM KCl, 15mM MgCl2, 50mM DTT
dNTP-Mix	10 mM each dNTP (dATP, dCTP, dGTP, dTTP)
Oligo-(dT)20 primer	100µM
Random Hexamers	100µM
RNase Inhibitor	40 units/µL RNase inhibitor in storage buffer with 50 % glycerol (v/v)
RNase-free water	
Random Hexamers	100µM
10X DNase buffer	40 units/µL RNase inhibitor in storage buffer with 50 % glycerol (v/v)
DNase	5 units/µL

Unit definition

One unit of Scriptase is defined as the amount of enzyme that incorporates 1nmol of dTTP's into acid-insoluble fraction in 10 minutes at 37°C using poly(A) oligo dT as a template primer.

Quality Control

The quality of this product is ensured to be more than 98 % for enzyme purity and homogeneity.

Scriptase RT is free of detectable exo- and endonuclease activity.

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Stability and Storage

Scriptase RT, including buffers and reagents, should be stored at -20°C immediately upon receipt.
When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

Please avoid frequent freeze/thaw cycles of the reagents.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
For more information, please consult the appropriate material safety data sheets (MSDSs).
These are available online as pdf-file or on request (info@genaxxon.com).

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com).

Product Use Limitations

The Genaxxon bioscience Scriptase RT is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

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RT Protocol Part

Important notes before getting started

- **Thaw 5X buffer, dNTP-mix, primer solutions** at RT or on ice.
  Keep the solutions on ice after complete thawing.
- Mix well before use to avoid localized differences in salt concentration.

Optional: gDNA removal by DNase digest:

If subsequent analysis after reverse transcription focusses on RNA only, it is recommended to remove gDNA in order to improve the quality of the cDNA. This is especially true for PCR/qPCR assays with primers/probes that are exon-spanning. The removal of gDNA is performed in three steps:

1. Preparation of a 10 µL reaction assay including the RNA template (see table below, please keep all reagents on ice))
2. DNase digestion for 5 – 10 min at 37°C
3. Heat Inactivation for 2 min at 95°C
4. Spin down and use immediately for cDNA synthesis or place on ice

Table 1. DNase-Assay Preparation Table (Please keep all reagents on ice)

Component	Stock Concentration	10 µL Reaction Assay	Final Concentration
10X DNase Incubation Buffer	10X	1µL	1X
DNase Enzyme Mix	5 units/µL	1µL	0.5 units/µL
RNA Sample	variable	0.1 - 2µg	variable
RNase-free Water	–	Adjust to 10µL	–

Table 2. DNase digestion and Heat Inactivation

Step	Time	Temperature
Digestion	5-10 min	37°C
Inactivation	2 min	95°C

Spin down and use immediately for cDNA synthesis or place on ice

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Recommended protocols for cDNA synthesis:

Standard Protocol:

The standard protocol is performed in two steps:

1. Preparation of a 20 µL reaction assay including the RNA template (see table below, please keep all reagents on ice)
2. Reverse Transcription

Table 3. Reaction Assay Preparation Table Note: Keep all reagents on ice!

Add all components according to table 3 to a nuclease-free microtube and fill up with RNase-free water to a final volume of 20µL. 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 Concentration	20µL Reaction Assay	Final Concentration
RNA template or DNase-digested sample	–	10µL	10pg to 5µg/reaction*
Primer	100µM	1µL	5µM** Gene specific Primer: 500nM***
5X Scriptase RT Buffer complete	5X	4µL	1X
dNTP Mix	10mM	1µL	500µM
RNase Inhibitor (optional)	40 units/µL	0.5µL	1 unit/µL
Scriptase RT	200 units/µL	1µL	10 units/µL
RNase-free Water	–	Adjust to 20 µL	–

* Total RNA: 10pg - 5µg; purified mRNA: 10pg - 500ng; Sample from DNase Digestion Step
** either Oligo-(dT)20 primer or random hexamer or a 1:1 mixture of these primers
*** For gene-specific primers we recommend using 500nM final primer concentration

Table 4. Reverse Transcription

Step	Time	Temperature
Reverse Transcription	10 min *	50°C **
Optional: Heat Inactivation	2 min	95°C

* Time for the reverse transcription reaction may be adapted to the cDNA length.
** The temperature can be increased to 55°C for difficult templates.

Please store the resulting cDNA at -20°C and avoid frequent freeze/thaw cycles.

Do not use more than 5µL for downstream applications such as qPCR.

Difficult and long templates can be run for 30-60 min, to increase yield and cDNA length.

Optional: Extended Protocol including additional RNA denaturation

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.

The extended protocol is performed in five steps:

1. Preparation of 10µL **Primer Assay** including the RNA template (mix gently after preparation)
2. Incubation for 5 min at 70°C (Denaturation)
3. Preparation of 10µL **Final Reaction Assay** and addition to the **Primer Assay**
4. Reverse transcription for 10 min at 50°C
5. Heat Inactivation for 2 min at 95°C

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Table 5. Preparation of the Primer Assay

Component	Stock Concentration	10 µL Primer Assay	Final Concentration in 20µL mix
RNA template or DNase-digested sample	–	variable (up to 9µL)	10pg to 5µg/reaction*
Primer**	100 µM	1µL	5µM** Gene specific Primer: 500nM***
RNase-free water	–	fill up to 10µL	–

* Total RNA: 10 pg - 5 µg; purified mRNA: 10pg - 500ng; Sample from DNase Digestion Step
** either Oligo-(dT)20 primer or random hexamer or a 1:1 mixture of these primers
*** For gene-specific primers we recommend using 500nM final primer concentration

Table 6. Primer annealing - Incubation for 5 min at 70°C (Denaturation)

The denaturation step is performed with the **Primer Assay** of water, RNA, and primers described in Table 5.

Step	Time	Temperature
Incubation	5 min	70°C

Place reaction mixture on ice after incubation!

Proceed with the preparation of the final mix (Table 7)

Table 7. Preparation of final reaction mixture for 10µL Final Reaction Assay

Component	Stock Concentration	10 µL Final Reaction Assay	Final Concentration in 20 µL mix
5X Scriptase RT Buffer complete	5X	4µL	1X
dNTP Mix	10mM	1µL	500µM
RNase Inhibitor (optional)	40 units/µL	0.5µL	1 unit/µL
Scriptase RT	200 units/µL	1µL	10 units/µL
RNase-free Water	–	fill up to 10µL	–

Add 10µL of the **Final Reaction Assay** to the **Primer Assay (Table 5/Table6).**

Mix gently.

Table 8. Reverse Transcription and Heat Inactivation

Step	Time	Temperature
Reverse Transcription	10 min	50°C
Heat Inactivation	2 min	95°C

* Time for the reverse transcription reaction may be adapted to the cDNA length.
** The temperature can be increased to 55°C for difficult templates.

Please store the resulting cDNA at -20°C and avoid frequent freeze/thaw cycles.
Do not use more than 5µL for downstream applications such as qPCR.
Difficult and long templates can be run for 30-60 min, to increase yield and cDNA length.

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