StellarScript HT Reverse Transcriptase Kit (200 U/µL)

Product Description

StellarScript HT Reverse Transcriptase (RT) is a M-MLV RT variant with improved thermostability and reduced RNase H activity. This enzyme has optimal activity at 50°C, providing increased specificity, higher cDNA yields, and longer cDNA products than wild type M-MLV RT. This enzyme has minimal template switching activity.

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Kit Contents

Kit	Kit Code	Description	Component Volume	
			50 µL kit (10 kU)	200 µL kit (40 kU)
StellarScript Multi Reverse Transcriptase Sample Pack	**7K0083-50UL**	StellarScript RT (200 U/µL)	50 µL	N/A
		StellarScript HT RT (200 U/µL)	50 µL	N/A
		StellarScript HT+ RT (200 U/µL)	50 µL	N/A
		10X RT Reaction Buffer	1 mL (x3)	N/A
		100 mM DTT	1 mL	N/A
		10 mM dNTP Mix	200 µL	N/A
StellarScript HT Reverse Transcriptase Kit (200 U/µL)	**7K0070-50UL** (50 µL) **7K0070-200UL** (200 µL)	StellarScript HT RT (200 U/µL)	50 µL	50 µL (x4)
		10X RT Reaction Buffer	1 mL	1 mL (x4)
		100 mM DTT	500 µL	1 mL
		10 mM dNTP Mix	50 µL	200 µL

For larger volume and higher concentration products, contact the **Sales Team** at sales@watchmakergenomics.com.

Product Applications

StellarScript HT works well in several applications including, but not limited to:

- First strand cDNA synthesis at elevated temperatures
- One- and two-step RT-qPCR
- Primer extension
- RNA-sequencing

Unit Definition and Buffer Compositions

- One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using poly(A)/oligo (dT) as a substrate
- Enzyme Storage Buffer: 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Tween 20, 50% glycerol
- 10X RT Reaction Buffer: 500 mM Tris-HCl (pH 8.3), 750 mM KCl, 30 mM $MgCl_2$

Storage and Handling

StellarScript HT Reverse Transcriptase Kits are shipped on ice packs. Upon receipt, store all kit components at -20°C ± 5°C.

Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to homogenize solutions thoroughly before use and during reaction setup. Do not vortex the RT enzyme.

DTT is sensitive to freeze/thaw cycling. The generation of single-use aliquots can prevent loss of potency.

When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Required Materials not Included

- RNase Inhibitor (optional; see **Prior to Starting** for more detail)
- Primers (random hexamers, oligo$(dT)_{18\text{-}20}$ or gene-specific; see **Prior to Starting** for more detail)
- PCR-grade water
- Thermocycler

Prior to Starting

Input RNA

StellarScript HT is appropriate for use with both degraded RNA, such as that derived from FFPE, as well as intact material with template inputs ranging from 10 pg to 1 µg.

To avoid contamination, work in a laminar flow hood, if available, and keep all sample and reagent tubes closed unless in use. Wear gloves when handling reagents and preparing libraries. Change gloves and pipette tips if they come into contact with non-sterile surfaces. Use RNase-free consumables where possible.

To avoid RNA degradation, store RNA in an RNase-free diluent, avoid vortex mixing, and limit the number of sample freeze-thaw cycles.

RT concentration

For certain applications, reducing the amount of RT in a reaction can improve yield. It is recommended to perform a titration from 5 to 200 U per reaction (0.25 to 10 U/µL final concentration) to determine the optimal RT input for a given application.

RNase inhibitor

RNase Inhibitor is not included in this kit and is not required for first strand synthesis. However, if the RNA input is from a crude sample, an RNase Inhibitor is recommended.

DTT

A minimum final concentration of 1 mM DTT is recommended in first strand synthesis. Certain RNase Inhibitors require additional DTT for optimal activity. Refer to manufacturer's instructions for DTT requirements.

DTT oxidizes quickly. The generation of single-use aliquots can prevent loss of potency.

Reverse transcription time

Reverse transcription time will depend on the application. Below are recommendations by target transcript length:

Table 1. Reverse transcription time recommendations based on desired cDNA length

Target cDNA length	Reverse transcription time
Less than 300 nt	10 minutes or less
300 to 800 nt	10 to 30 minutes
Greater than 800 nt	40 to 60 minutes

Primer selection and concentration

Primer selection depends on multiple factors. Any of the following priming strategies should be appropriate for shorter RNAs with minimal secondary structure. For longer RNAs with a higher degree of secondary structure, consider the following:

Table 2. Primer selection and concentration information

Primer type	Recommended final concentration	Pre-incubation at 25°C	Additional information
Oligo(dT)$_{18\text{-}20}$	1 to 5 µM	No	Full-length reverse transcription of eukaryotic mRNAs and retroviruses with polyadenylated tails.
Random hexamers	2 to 6 µM[1]	Yes	Reverse transcription of most RNA species, not just those that are polyadenylated. Appropriate for degraded RNA templates. Can improve reverse transcription efficiency, especially for long transcripts with a high degree of secondary structure.
Oligo(dT)$_{18\text{-}20}$ and random hexamers	1 to 6 µM each	Yes	Provides benefits of both oligo(dT)$_{18\text{-}20}$ and random hexamer primers.
Gene-specific	0.5 to 1 µM	No	Reverse transcript of a specific region. Knowledge of the target sequence is required.

[1]For short products (300 nt or less), final concentrations up to 80 µM can increase yield.

Protocol

The following example protocol is for a single reaction. When preparing multiple reactions, prepare master mixes with an appropriate overage to improve inter-sample consistency.

1. First strand cDNA synthesis

1.1 Thaw the 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT, and primers on ice. Vortex for 5 to 10 seconds and briefly centrifuge.

1.2 Briefly centrifuge StellarScript HT RT (200 U/µL).

1.3 On ice, combine components as specified:

Component	Final Concentration	Volume (per 20 µL reaction)
RNA template (10 pg to 1 µg)	Variable	Variable
10X RT Reaction Buffer	1X	2 µL
dNTP Mix	0.5 mM	1 µL
DTT	1 – 5 mM[1]	Variable
Primers[1]	0.5 – 6 µM	Variable
RNase Inhibitor (optional)[1]	Variable	Variable
StellarScript HT RT (200 U/µL)	5 – 200 U/ reaction[1]	1 µL
PCR-grade water	–	Up to 20 µL

[1]See **Prior to Starting**.

1.4 Mix thoroughly by pipetting and centrifuge briefly.

1.5 Incubate reactions for first strand cDNA synthesis as follows:

Step	Temperature (°C)	Time (min)
Lid temperature	85	N/A
Pre-incubation (for random hexamers only)[1]	25	10
Reverse transcription	50	15 – 60[2]
Heat inactivation	80	10

[1]Omit this step if random hexamers are not being used.
[2]See **Prior to Starting**.

For Technical Support, please contact the **Scientific Support Team** at support@watchmakergenomics.com.

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