GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

HH Scriptase III - reverse Transcriptase

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (RNA-directed); EC 2.7.7.49

	Cat#	M3030.1000	M3030.1010	M3030.5010	Colour code of cap
Component					
HH Scriptase III - reverse Transcriptase		1000 units (5-10 rxn)	10000 units (50-100 rxn	50000 unit (10x (50-100 rxn))	
Complete 5X HH Scriptase III buffer		0.5mL	5mL	5x 5mL	

Product description

HH Scriptase III reverse transcriptase (RNase H–) is a genetically engineered M-MLV–derived RNA dependent DNA polymerase that lacks both RNase H and 3′→5′ exonuclease activity, enabling high-yield, full-length cDNA synthesis. The enzyme exhibits enhanced thermostability, maintaining robust activity at temperatures up to +55°C. This allows efficient reverse transcription of RNA templates with complex secondary structures by improving primer annealing and template accessibility.

HH Scriptase III is ideal for challenging samples where sensitivity, reproducibility, and resistance to structural barriers are critical.

Supplied buffers/solutions

HH Scriptase III:	200 units/µL in storage buffer containing 50% glycerol (v/v).
5X Reaction Buffer:	Tris/HCl (pH8.3), KCl, MgCl2, stabilize.r

Unit definition

One unit of M-MuLV RT 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.

Application

- RT PCR / RT-qPCR
- Synthesis of first-strand cDNA at temperatures up to 55°C, enabling increased specificity and improved performance with GC-rich RNA templates.
- Suitable for the analysis of long RNA transcripts, generating of cDNA with lengths ranging from 100 bp to >12 kb.
- More efficient reverse transcription with higher yields of cDNA and full-length products.
- Easy use in challenging RNA analyses by improved efficiency with GC-rich target RNAs.

Stability and Storage

HH Scriptase III, including buffers and reagents, should be stored immediately upon receipt at -20°C.
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.

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).

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

Quality Control

Nuclease activity:	50ng of radio labelled DNA or RNA is incubated with 200 units of the enzyme in 1X reaction buffer for one hour at 37°C, resulting in <1% release of free measurable radio activity in the supernatant.
Endonuclease Activity:	Incubation of 200 U of enzyme with 1µg λ DNA for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
Exonuclease Activity:	Incubation of 200 U of enzyme with 1 µg λ-Hind III digest DNA for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
Nickase Activity:	Incubation of 200 U of enzyme with 1 µg pBR322 for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
RNase Activity:	Incubation of 200 U of the enzyme with 1.6µg MS2 RNA for 16 hours at 37°C yields no degradation as determined agarose gel electrophoresis.
***E.coli* DNA:**	The *E.coli* DNA content found by TaqMan qPCR is less than 1 copy if >200 U of the enzyme are used as DNA template in the qPCR assay.
Purity:	>98% as determined by SDS-polyacrylamide gel analysis.

Product Use Limitations

The Genaxxon biosience M-MuLV 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.

Safety information

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)

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

RT Protocol

Important notes before getting started

Things to do before starting

- **Thaw 5X buffer, dNTPs or 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.

RT- Reverse Transcription Protocol / cDNA synthesis

Add the below mentioned components to a nuclease-free microtube. Pipette on ice and mix the components by pipetting gently up and down.
Note: In general, water, RNA and primers should be mixed before the remaining components are added.

Primer-Template Annealing

Combine RNA (10pg–5µg), primer (Oligo(dT), random hexamers, or gene-specific primer) in a PCR tube (Mix 1)

RNA Denaturation Mix 1:

Component	Stock conc.	Final amount /concentration	10µL assay
RNA template	-	Total RNA: 10pg up to 5µg or mRNA: 10pg up to 500ng	x µL
Primer	10µM (each) 50µM 50ng/µL	-gene-specific primer (0.5µM – 1µM) -oligo-dT20 primer (5µM); cat#: M3039 -random primer (5ng/µL); cat#: M3038	1µL - 2µL 1µL 1µL
Nuclease-free water	-	-	fill up to 10µL

Incubate at 65°C to 70°C for 5 minutes to melt secondary structures.

Place the tube at room temperature (if using specific primer) or on ice (if using oligo-dT or random primer) for at least 1 minute, then spin down briefly.

Prepare Reaction Mix 2 containing RT buffer, dNTPs, DTT (optional), RNase inhibitor, and HH Scriptase III enzyme.

Preparation of Reaction Mix 2:

Component	Stock conc.	Final amount /concentration	10µL assay
HH Scriptase III buffer complete	5X	1X	4µL
dNTP-Mix	10mM each	500µM each	1µL
RNase Inhibitor (optional) [1)]	40 units/µL	20 - 40 units (cat#: M3034)	0.5µL – 1µL
HH Scriptase III [2)]	200 units/µL	100 – 200 units	0.5µL – 1µL
Nuclease-free water	-	-	fill up to 10µL

1) Addition of 20 – 40 units RNase Inhibitor is recommended and may be essential if working with low amounts of starting RNA (RNase Inhibitor has to be ordered separately).
2) 100 units of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units may result in even higher transcription yields under selected assay conditions.

cDNA synthesis

Combine Mix 1 and Mix 2 resulting in 20µL total volume.

Mix gently and centrifuge.

Place tube in a heating block / thermos cycler following the time and temperature recommendation given in the tables below.

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

cDNA synthesis protocol using gene specific primers

Step	Time	Temperature
cDNA synthesis (for gene specific primers)	5 - 30 min [1)]	50°C to 55°C)
Inactivation of HH Scriptase III	2 min	85°C

cDNA synthesis protocol using oligo-dT or random primers

Step	Time	Temperature
cDNA synthesis (for Random Hexamers)	5 min followed by 5 - 30 min	25°C 50°C to 55°C
cDNA synthesis ((for Oligo dT Primers)	10 min followed by 5 - 30 min	42°C 50°C to 55°C
Inactivation of HH Scriptase III	2 min	85°C

1) The optimal time depends on the length of cDNA. Incubation of 5 min for cDNA with 500bp. 30 min for cDNA with >1.5 kbp length.
2) 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: The optimal reaction time and temperature should be adjusted for each particular RNA.
Note: For downstream PCR applications, the volume of cDNA should not exceed 1/10 of the total PCR reaction volume.

After the termination step (85°C) chill the tube on ice.

Optional:

Add RNase H and incubate at 37°C for 20 minutes to remove the RNA template before downstream PCR. *

* The Genaxxon HH Scriptase III together with the supplied buffer are free of RNase activity, meanwhile we recommend adding RNase Inhibitor into the mixture to inhibit possible RNase contaminations of the sample.

Storage of cDNA

For long term storage of the cDNA, store Mix at -20°C or at -80°C, if the cDNA is not used immediately in PCR / qPCR,

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658