

NOVASCRIPT III RNase H minus Reverse Transcriptase

Cat # NS18064-10K

10000 Units

NovaScript III is a premium ultrapure, highly sensitive RNase H minus Reverse Transcriptase offering higher & superior cDNA yields with distinct stability over a wide range of temperatures.

Applications

- NovaScript III is suitable for first strand cDNA synthesis in cDNA library construction.
- For the production of templates for RT-PCR analysis.
- NovaScript III can be used with total RNA, mRNA or in vitro transcribed RNA.

DISCUSSIONS

A successful reverse transcription reaction is dependent on many factors, including the reverse transcriptase used in the reaction. The enzyme should be able to generate high quality full-length cDNA from different types of templates and under various conditions. Commercially available reverse transcriptases include the Moloney Murine Leukaemia Virus (MMLV) reverse transcriptase and the Avian Myeloblastosis Virus (AMV) reverse transcriptase. These retroviral enzymes exhibit RNA-dependent DNA polymerase activity and RNA-degrading RNase H activity. Many variations of these enzymes are currently available in the market, including the wild type MMLV and AMV, which display full RNase H activity, and variations of MMLV which either lack the RNase H domain or contain its modified version, which greatly reduces its basal activity.

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Description

NOVASCRIPT III is a Moloney Murine Leukaemia Virus **RNase H minus** Reverse Transcriptase that exhibits high stability and is active at a wide range of temperatures. NovaScript III is an RNA and DNA-dependent DNA Polymerase requiring a DNA primer for initiation of elongation. Unlike wild-type enzyme, NovaScript III possesses RNase H minus activity which results in enhanced yields. NovaScript III is highly sensitive even when the amount of template is a limiting factor and is also suitable for real-time PCR experiments.

→ NovaScript III **produces high yield of cDNA, which shows excellent performance in real-time RT-PCR experiments--**

Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique for mRNA detection and quantification currently available. Good quality cDNA is essential to this technique when high sensitivity is desired. The superior quality and performance of cDNA synthesized by NovaScript III is ratified wide end-point and real-time RT-PCR assays.

→ NovaScript III **shows excellent performance with genespecific primers, oligo (dT)18 as well as random hexamers, and synthesizes cDNA with more splice variants--**

→ NovaScript III **shows superior performance in reverse transcription of long templates**

For many applications full-length cDNA of long templates is required. To test the performance of NovaScript III for the synthesis of long transcripts a number of experiments were successfully carried out

→ NovaScript III **synthesizes high yield of cDNA over a wide range of temperatures**

Many RNA transcripts form stable secondary structures at lower temperatures, making them less suitable as templates for RT-PCR at those temperatures. Therefore, it is important that a reverse transcriptase is not only active at 37°C-42°C but also at higher temperatures without a loss of activity. The superior quality and performance of cDNA synthesized by NovaScript III is ratified at much higher temperatures. The Ct values for NovaScript III cDNA at higher temperatures is lower than the Ct values for the competing enzymes.

NovaScript III is suitable for first-strand cDNA synthesis, cDNA library construction and the production of templates for RT-PCR analysis of gene expression. NovaScript III can be used with total RNA, mRNA and *in-vitro* transcribed RNA and shows excellent performance with gene-specific primers, Oligo (dT) as well as random hexamers. It is highly sensitive, even for low amounts of RNA, delivers high yields and is superior to leading competitor enzymes in the production of full-length cDNA, especially for long templates. NovaScript III is robust, easy to use and works well under a wide range of temperatures. To conclude, NovaScript III is an all-purpose reverse transcriptase with excellent features and is superior than other reverse transcriptases available commercially.

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Storage Conditions:

To be Stored at -20°C .
 NovaScript III will remain stable if stored as specified.

Shipping Conditions:

On Dry Ice (-20°C)
Concentration: 200u/ μl

Components:

NOVASCRIPT III Storage Buffer	5x Reaction Buffer
25 mM Tris-Cl (pH 7.9)	250 mM Tris-cl (Ph 8.3)
100 mM NaCl	350 mM KCl
1mM EDTA	15 mM MgCl_2
5Mm DTT	50 mM DTT
0.1% Triton X-100	
50% glycerol	

Unit Definition:

One unit catalyses the incorporation of 1 nmole of dTTP into acid-insoluble material in 10 minutes at 37°C in 50 mM Tris-HCl, pH 8.6, 40mM KCl, 1 mM MnSO_4 , 1 mM DTT, and 0.5 mM $[^3\text{H}]\text{TTP}$, using 200 μM oligo(dT)₁₂₋₁₈-primed poly(A)_n as template.

Protocol for first-strand cDNA synthesis with NovaScript III RNase H minus Reverse Transcriptase

A. Prepare a mix, containing:

1. Template RNA:

Total RNA	0.5 – 5 μg
or mRNA	0.01 – 0.5 μg
or Specific RNA	Up to 0.5 μg

NB: The amount of total RNA or mRNA required for the reaction depends on the level of expression of the gene encoded.

2. Primer:

Oligo (dT) ₁₈	0.5 μg
or Random hexamer	0.2 μg
or Specific oligo	5-20pmol

3. Deionized water (nuclease-free) up to 12 μl totalB. Incubate the mix for 5 min at 70°C , and then chill on ice.

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C. Add the following:

RNase inhibitor (optional)	10 units
dNTP Mix <ul style="list-style-type: none"> • 40mM dNTP mix (10mM each) • or 100mM dNTP mix (25mM each) 	1 μ l 0.4 μ l
5x Reaction Buffer (provided)	4 μ l
Deionized water (nuclease-free)	*up to 19.75 μ l total when using NovaScript III at 200u/ μ l

NB: We recommend a total dNTP concentration of 2 mM but the concentration can be decreased to 0.5 mM during the optimization of the reaction.

D. Mix by pipetting. Add 0.25-1.0 μ l of NovaScriptIII at 200 u/ μ l.

E. Incubate at 37-42°C for 60 min

NB: 1) If random hexamer oligos are used, an initial 10-minute incubation at 25°C is recommended.

2) Incubation at 37-42°C is desirable for generating longer(>1000 bases) cDNAs, however, the use of higher incubation temperatures up to 50°C can increase the yield of cDNA synthesised (<1000 bases), especially in cases of complex RNA secondary structures

F. Stop the reaction by heating at 70°C for 10 minutes, or by adding of an equal volume of 10 mM EDTA (pH 7.0). Chill on ice.

G. Use the cDNA synthesized in subsequent amplification reactions without any additional purification

<u>Other AuPreP™ DNA/RNA Kits</u>	<u>Other Related Products</u>
AuPreP™ Plasmid Maxi Kit	AuPreP Oligos (High Affinity Purified Oligo synthesis available in different scales, purifications & modifications)
AuPreP™ Plasmid Midi Kit	AuPreP TaQ DNA Polymerase (Ultrapure, Ultra-stable & Ultra-sensitive Taq DNA Polymerase)
AuPreP™ SPIN™ SPIN Miniprep Kit	AuPreP Hotstart TaQ DNA Polymerase (Robust Polymerase for Hotstart PCR assays)
AuPreP™ Blood Genomic DNA Maxi	AuPreP Super Fidelity TaQ DNA Polymerase (High fidelity Polymerase produces blunt ended amplicons upto 5Kb)
AuPreP™ Blood Genomic DNA Extraction Midi Kit	PCR Doctor - (PCR enhancer for AuPreP Hotstart Taq or Super Fidelity Taq especially designed for GC/AT/Dirty/Difficult Templates)
AuPreP™ GEN^{bt} DNA Extraction Kit	AuPreP Longjump Polymerase (Robust Long Polymerase for templates > 4kb to 18kb+ for challenging PCRs)
AuPreP™ DNA easy Plant Maxi kit	AuPreP Red PCR Master Mix (2x Master mix with Red Dye without Enhancer)
AuPreP™ DNA easy Plant Mini Kit	AuPreP DIAMOND MASTER-MIX (2x Mastermix with PCR Enhancer & Stabilizer without tracking dyes)
AuPreP™ PCR Purification Kit	AuPreP DIAMOND DOUBLE DYE MASTERMIX (2x Mastermix with PCR Enhancer, Stabilizer & tracking dyes)
	AuPreP DNA Extraction System (A fast Reagent for pure genomic DNA isolation for down stream applications)

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<p>AuPreP™ Plant RNA Maxi Kit</p> <p>AuPreP™ Plasmid Maxi Kit</p> <p>AuPreP™ RNA Easy Midi Kit</p> <p>AuPreP™ RNA™ Mini Kit</p> <p>AuPreP™ RNV™ Viral RNA Extraction Miniprep Kit</p>	<p>AuPreP RNA Extraction System (for Purest & High Quality RNA extraction with simple cost effective protocol)</p> <p>AuPreP Gold cDNA Synthesis Kit (Highly Cost effective cDNA Synthesis Kit using RT with reduce Rnase H activity)</p> <p>AuPreP Gold RT-PCR Combo Kit (2 step RT-PCR protocol with tracking Dye)</p> <p>AuPreP Extra Mile First Strand cDNA System (Premium cDNA Synthesis Kit using RT with point mutant Rnase H minus activity)</p> <p>Novascript III RNase H⁻ RT (Premium Ultra-stable Rnase H minus RT for long high quality cDNA construction)</p> <p>Novascript III single step RT-PCR System (Premium 1step RT-PCR system using Novascript & AuPreP Hotstart DNA Polymerase)</p> <p>AuPreP Random Primer labeling Mix System (Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides)</p>
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