



# GeneScript™ RTase



Products	Cat #	Pack Size
GeneScript™ Reverse Transcriptase (100U/ul)	GC-016-5	5000 U
GeneScript™ Reverse Transcriptase (100U/ul)	GC-016-10	10 000 U

## DESCRIPTION

Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase is a RNA-dependent DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either single-stranded RNA or DNA template. **The enzyme lacks RNaseH activity.** Source of GeneScript™ Reverse Transcriptase is *E.coli* strain + plasmid with the cloned and modified M-MuLVRT gene with deleted RNaseH coding part.

## UNIT DEFINITION

One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into an acid insoluble form in 10 minutes at 37°C using poly(rA)-oligo(dT) 10-20 as template primer.

## STORAGE BUFFER

50 mM Tris-HCl pH 8.3, 1 mM EDTA, 0.1 mM DTT, 0.1 mM NaCl, 0.1% Triton X-100, 50% glycerol

## 5X REACTION BUFFER

250 mM Tris-HCl pH 8.3, 15 mM MgCl<sub>2</sub>, 400 mM KCl

**Add to buffer: dNTPs (end concentration 2 mM), MnCl<sub>2</sub> (end concentration 2-4 mM) and DTT (end concentration 10 mM). Incubate at 37°C.**

## EXTRA SOLUTION

25 mM MnCl<sub>2</sub> 100 mM DTT

## UNIT ASSAY CONDITION

20 mM Tris-HCl pH 8.0, 2 mM MnCl<sub>2</sub>, 100 mM KCl, 1 mM DTT, 0.6 mM poly rA, 0.1 mM poly(dT)10-20; 0.5 mM dTTP( 3 H) - 0.5-5 units of enzyme

## QUALITY ASSURANCE

GeneScript™ reverse transcriptase is tested for its ability to synthesize full length cDNA from 4kb RNA.

## PROTOCOL

Set up a 20 µl reaction mixture as follows:

5 x GeneScript Buffer	4 ul
Primers	1 ul (random hexamers 50 ng/ul; oligo-dT 0,5 ug/ul or 25 pM of gene specific)
DNA Polymerization Mix 10 (10 mM each dNTP)	2 ul (1 mM end-concentration of each dNTP)
100 mM DTT	2 ul
RNA Template	Total RNA - 1-5 ug; poly(A) mRNA - 1-500 ng
<b>Fill with water to the volume 15 ul, incubate at 80°C 3 min, put on ice and centrifuge briefly</b>	

RNasin (10U/ul)	2 ul
25 mM MnCl <sub>2</sub>	2 ul

Add **GeneScript** or **GeneScriptPlus** 1 ul



Incubation:

**GeneScript** 30 min at 42°C

**GeneScriptPlus** 30 min at 52°C



**10 min at 80°C**



**centrifuge, fill with water to the final volume 100 ul („cDNA pool”) and use 1 - 5 ul for PCR with BioTherm, BioThermAB or BioThermStar DNA Polymerase**

**Optional protocol:**

total RNA 2-5 µg; RT-buffer; primer; 1 mM each dNTP; optional: 1 U/µl RNasin

- incubate at 70°C for 2 min, chill to 23°C to anneal primer to RNA
- add 200 units GeneScript™ and incubate 10 min at 23°C followed by 30 min at 42°C
- optional: incubate with RNaseH
- heat the reaction at 95°C for 5 min and chill on ice

Store the RT-reaction by -20°C and **use 2 µl for subsequent PCR.**

The addition of 2 mM MnCl<sub>2</sub> in the 1x RT buffer is optional.