

Products	Cat #	Pack Size
GeneScriptPlus™ Reverse Transcriptase	GC-066-5	5000 U
GeneScriptPlus™ Reverse Transcriptase	GC-066-10	10 000 U

DESCRIPTION

GeneScriptPlus™ Reverse Transcriptase is an optimized mutant of GeneScript™ Reverse Transcriptase that **is active at 55°C** and has a half-life of 15 minutes, providing increased specificity with Gene-Specific Primers (GSPs) and the **highest cDNA yield of all RTs**. It is ideal for RT-PCR of a specific gene or generating cDNA from total or poly (A)+ RNA sample. It synthesizes a complementary DNA strand from single-stranded RNA or DNA. GeneScriptPlus™ is genetically engineered by the introduction of point **mutations that reduce RNase activity and increase thermal stability**. The structural modifications provide:

- **Full activity at 55°C** for increased specificity with GSP
- Ability to increase RT units without inhibiting subsequent PCR

CONCENTRATION

200 units/μl

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 1 nmol of dTTP into 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 (v/v)

STORAGE TEMPERATURE

Store GeneScriptPlus™ Reverse Transcriptase below 0°C, preferably at -20°C, in a constant temperature freezer.

5X REACTION BUFFER

250 mM Tris-HCl pH 8.3, 15 mM MgCl₂, 400 mM KCl

Add to buffer: dNTPs (end concentration 2 mM), MnCl₂, (end concentration 2-4 mM) and DTT (end concentration 10 mM).

EXTRA SOLUTIONS

25 mM MnCl₂, 100 mM DTT

UNIT ASSAY CONDITIONS

20 mM Tris-HCl pH 8.0, 2 mM MnCl₂, 100 mM KCl, 1 mM DTT, 0.6 mM poly rA, 0.1 mM poly(dT)10-20, 0.5 mM dTTP(3H) – 0.5-5 units of enzyme

QUALITY ASSURANCE

GeneScriptPlus™ 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
Fill with water to the volume 15 ul, incubate at 80°C 3 min, put on ice and centrifuge briefly	
RNasin (10U/ul)	2 ul
25 mM MnCl ₂	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 MnCl₂ in the 1x RT buffer is optional.