

DATA QUALITY SHEET



GeneScript™ Reverse Transcriptase

FOR RESEARCH USE ONLY

GC-016-0500, GC-016-1000, GC-016-10000, GC-016-5000, GC-016-50000

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	E.coli strain that carries a plasmid with the cloned and modified M-MuLVRT gene with deleted RNaseH coding part.
CONCENTRATION	100 units/μl.
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 ₂ , 400 mM KCl Add to buffer: dNTPs (end concentration 2 mM), MnCl ₂ (end concentration 2-4 mM) and DTT (end concentration 10 mM). Incubate at 37°C.
EXTRA SOLUTION	25 mM MnCl ₂ , 100 mM DTT
UNIT ASSAY CONDITION	20 mM Tris-HCl pH 8.0, 2 mM MnCl ₂ , 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: 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 ₂ in the 1x RT buffer is optional.