enzymatics a QIAGEN company

***Product Specifications P7600L** Rev 02*

Product Information	
EnzScript™	
Part Number	P7600L
Concentration	200,000 U/mL
Unit Size	10,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: EnzScript™ (M-MLV Reverse Transcriptase RNase H minus) is an RNA-dependent DNA polymerase with no detectable RNase H activity. EnzScript™ can be used to generate first-strand cDNA from polyA mRNA or total RNA for use in downstream applications such as RT-PCR, cDNA cloning or library construction for RNA-Seq. Point mutations in the RNase H domain increase the thermostability of the enzyme and support greater cDNA yield of full-length transcripts than wild type M-MLV Reverse Transcriptase (1).

Product Specifications								
P7600								
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**	**RNase Contamination**	**Functional RT-PCR Assay**
Units Tested	**n/a**	**n/a**	**2,000**	**2,000**	**2,000**	**2,000**	**2,000**	**n/a**
Specification	>95%	180,000 U/mg	<5.0%	<1.0%	No Conversion	<10 copies	No Detectable non-specific RNase	Synthesis of 9.4 kb cDNA transcript

Source of Protein: A recombinant E. coli strain carrying the Moloney-Murine Leukemia Virus Reverse Transcriptase gene with 3 point mutations in the RNase H domain that eliminate detectable RNase H activity.
Unit Definition: 1 unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid insoluble material in 10 minutes at 37°C using poly r(A)/oligo (dT) as a substrate.
Molecular weight: 75,938 Daltons

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X M-MuLV RT RNAse H-Buffer and added to 50 µL reactions containing 20 µg/mL poly r(A) RNA, oligo (dT) DNA, 1X RT Buffer, 3H-dTTP and 250 µM dTTP. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).
Reverse Transcriptase function was determined by the enzyme's ability to generate a 9.4 kB cDNA transcript. Following 2-Step RT-PCR the 9.4Kb amplicon was visualized by agarose gel electrophoresis
Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.
Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.
Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.
E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.
Non-Specific RNAse contamination is assessed using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.
Supplied in: 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA , 1 mM DTT, <0.01% NP-40 Alternative, 50% glycerol, pH 7.5 @ 25°C.
Supplied with: 5X M-MLV Reverse Transcriptase RNAse H- Reaction Buffer (B7601) and 100mM DTT (B9060)
References: Gerard G.F. et al., *Nucl. Acids Res*. (2002) 30 (14): 3118-3129

Kit Contents

Part	Part Number
EnzScript™	P7600
5X M-MLV Reverse Transcriptase RNase H- Buffer	B7601
100 mM DTT	B9060

Polymerase Properties

Molecular Weight: 75.9 kDa
Optimum Extension Temperature: 42°C
RNase H activity: none detected
Unit Concentration: 200 U/µl
Transcript Length: 12.3 kB

Common Applications

EnzScript™ (M-MLV Reverse Transcriptase RNase H minus) is an RNA-dependent DNA polymerase commonly used to synthesize First-Strand cDNA for RT-PCR amplification, cDNA cloning or RNASeq. Reduced RNase H activity enables greater yield of full-length cDNA transcripts (> 5 kb) and increased thermal stability over standard M-MLV RT.

First Strand Reaction Protocol

General precaution against RNase degradation of template RNA should be taken when setting up First-Strand reactions such as use of nuclease-free water, RNase inhibitor, RNase-free tubes and sterile pipet tips with filters. The following procedure can be used as a guideline for preparing a 20 µl First-Strand cDNA reaction.

Materials to be provided by User:

- Sterile, nuclease-free water
- Primer (oligo $dT_{(15-20)}$ *or* random hexamers *or* gene-specific)
- dNTP mix (dATP, dCTP, dGTP, dTTP)
- RNA template
- RNase Inhibitor

1. Add the following components to an RNase-free microcentrifuge tube on ice. For more than one reaction, prepare a mastermix.

Component	Volume
50 µM oligo $dT_{(15-20)}$ *or* 50 µM random hexamers *or* 10 µM gene-specific primer 10 1x	1 to 2 µl
5 mM dNTP mix	2 µl
RNA template*	X µl
Sterile, nuclease-free water	to 12 µl

* 1 ng to 1 µg total RNA *or* 1 to 250 ng mRNA

2. Heat microcentrifuge tube to 65°C for 5 minutes and quickly cool on ice for 2 minutes to anneal primer to RNA template. Spin tube briefly to collect condensate.

3. Add the following components (to each First-Strand reaction) to the microcentrifuge tube on ice:

Component	Volume
5X M-MLV Reverse Transcriptase RNase H- Buffer 10 1x	4 µl
100 mM DTT	2 µl
RNase Inhibitor (optional) or nuclease-free water	1 µl
200 U EnzScript™	1 µl

4. Incubate each 20 µl First Strand reaction as follows:
   a. 25°C for 2 minutes (oligo dT(15-20), gene-specific primer) or 25°C for 10 minutes (random hexamer)
   b. 42°C for 30 to 60 minutes
   c. Heat kill at 70°C for 15 minutes

5. Use cDNA in downstream application or store at -20°C. For RT-PCR, 1 to 2 µl of cDNA from First-Strand reaction is typically added as template to PCR. Optional: Remove RNA strand prior to PCR by adding 1 µl RNase H (5 U) to cDNA:RNA hybrid, incubate at 37°C for 20 minutes and 65°C for 10 minutes (heat kill). RNase H treatment is recommended for amplification of long amplicons (> 5 kB).

Related Enzymatics Products

Part	Part Number
25 mM dNTP mix	N2050
RNase Inhibitor	Y9240
RNase H	Y9220

Frequently Asked Questions and Troubleshooting

For Frequently Asked Questions (FAQ) and troubleshooting please visit www.enzymatics.com

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.