Product Information	
StableScript™	
Part Number	P7720L
Concentration	5,000 U/mL
Unit Size	250 units
Storage Temperature	-25°C to -15°C
Lot Number	(Shipment Specific)
Reference Number	(Shipment Specific)

Product Description:
StableScript™ is a versatile reverse transcriptase designed for use in one-step RT-qPCR and Long Range RT-PCR. It demonstrates high sensitivity for RNA detection, improved thermostability, processivity and inhibitor resistance over first generation M-MLV Reverse Transcriptase RNase H minus.

Product Specifications						
Assay	**SDS Purity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	**E. coli DNA Contamination**	**RNase Contamination**
Units Tested	**n/a**	**5,000**	**5,000**	**5,000**	**5,000**	**5,000**
Specification	>99%	<5.0% Released	<1.0% Released	No Conversion	<10 copies	No Detectable non-specific RNase

Product Specifications	
Assay	**RT-qPCR**
Specification	Amplification of Test Lot within 1Ct of Reference Lot in a one-step RT-qPCR assay

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.

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X StableScript Diluent and added to 50 µL reactions containing 20 µg/mL poly r(A) RNA, oligo (dT) DNA, 1X StableScript™ Reaction Buffer, 3H-dTTP and 250 µM dTTP and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).
The functionality of the RT-PCR Assay is evaluated by amplification of three mRNA transcripts in a one-step RT-qPCR assay. The amplification threshold (Ct) of the test lot is compared to a reference lot.
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.

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.

Polymerase Properties
Extension Temperature Range 50C°C to 65°C
Optimum: 55°C
Transcription Length: up to 12.3kB

Source of Protein: A recombinant *E. coli* strain carrying the engineered Reverse Transcriptase gene. This novel reverse transcriptase has no detectable RNase H activity and has increased thermostability.

Kit Contents

Part Number	P7720L
Concentration	5,000 U/mL
Unit Size	250 U
4X StableScript Reaction Buffer	1 X 1.5 mL

Supplied in: 20 mM KPO4, pH 7.0, 1 mM EDTA, 1 mM DTT, stabilizer, 50% glycerol

Protocol
General precautions against degradation of RNA template should be taken when setting up a reaction, including setting up the reaction with nuclease free water, RNase inhibitor, nuclease free PCR tubes and sterile pipette tips with filter, adding reverse transcriptase last, gentle pipetting, thorough mixing and a quick centrifugation. The following procedure can be used as a guideline. Reactions may need to be optimized individually.
Reaction setup (for 20 µL)*

1. Add the following components in a nuclease-free tube.

Component	Volume (µL)	Final Concentration
Nuclease Free H_2O	x	Up to 13µL
50 µM oligo dT(20) or Random hexamers or 10 µM gene-specific primer	1	1-2.5 µL
RNA template	x	1 ng to 1µg total RNA

2. Heat the reaction tube to 65°C for 5 minutes and then quickly cool down on ice for at least 2 minutes to anneal primer to the RNA template.
3. Add the following components to a reaction tube with annealed RNA:

Component	Volume (µL)	Final Concentration
4X StableScript Buffer (Include dNTP)	5	1X
Rnase Inhibitor	1	10U
StableScript	1	5 U

4. Incubate each 20µL reaction as follow:
   a. 25°C for 2 minutes (oligo dT or gene-specific primer) or 25°C for 10 minutes (random hexamers)
   b. 55°C for 10-30 minutes
   c. 80°C for 10 minutes

Use cDNA in the downstream PCR amplification or store at -20°C. For RT-qPCR, 1to 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 the cDNA:RNA hybrid, incubating at 37°C for 20 minutes and then at 65°C for 10 minutes (heat inactivation). RNase H treatment is recommended for amplification of long amplicons (> 5 kB).