EnzScript™ Reverse Transcriptase

Key benefits

- Thermostable and optimized for a wide range of reaction temperatures from 37°C to 55°C
- Accommodates GC-rich and challenging RNA templates with significant secondary structure
- Outperforms wild-type M-MLV RT by generating high yields of more full-length cDNAs
- Point mutations to eliminate detectable RNase H activity enhance performance
- Achieves picogram level sensitivity in end point RT-PCR
- Supports a wide detection range (down to 50 molecules)
- Template switching activity, making it the ideal choice for RNA-sequencing workflows
- Manufactured under an ISO 13485 system for purity and lot-to-lot consistency

Robust reverse transcriptase

EnzScript Reverse Transcriptase answers the need for robust, specific first-strand cDNA production from a variety of RNA templates to drive PCR and sequencing applications in laboratories where throughput, consistency and cost per sample are major drivers of success. EnzScript Reverse Transcriptase offers enhanced thermostability over M-MLV reverse transcriptases enabling successful DNA synthesis from a wide range of RNA templates. The enzyme has no detectable RNase H activity allowing production of more full-length cDNAs.

Increased thermostability

EnzScript generates high cDNA yields over a wide range of temperatures allowing for flexibility in reaction setup for specific RNA transcripts (Figure 1).

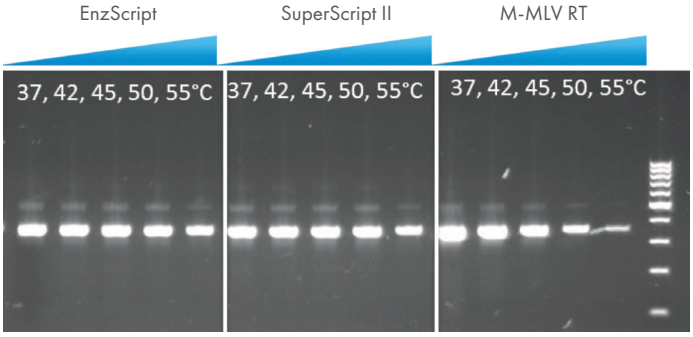

Figure 1
RT-PCR using various RTs (EnzScript, SuperScript™ II, or QIAGEN M-MLV RT) over a range of reaction temperatures from 37°C up to 55°C. First-strand reactions were set up in-house at the indicated temperatures with total human RNA (100 ng). QIAGEN Phoenix™ Hot Start Taq (P7590L) was then used to amplify a 350 bp target (POLR2A). PCR products shown were visualized on 2% agarose gel.

Ready for RNA-Seq workflows

EnzScript produces cDNA yields better than, or equivalent to, leading competitor enzymes commonly used for RNA-Seq applications (Figure 2). As an M-MLV Reverse Transcriptase, EnzScript exhibits inherent template switching activity, making it the ideal choice for RNA-seq applications.

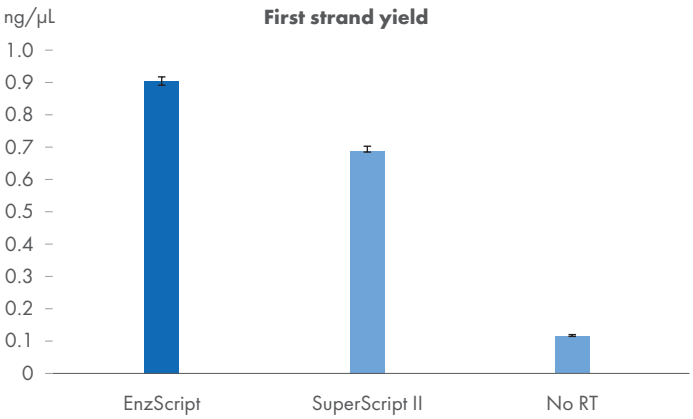

Figure 2
First strand yield following reaction clean-up with QIAquick® PCR spin columns. First-strand reactions were set up in-house with fragmented yeast RNA primed with random hexamers.

RT-qPCR performance

EnzScript displays a wide detection range for RT-qPCR ranging from 5 x 10^8 copies down to 50 copies (Figure 4).

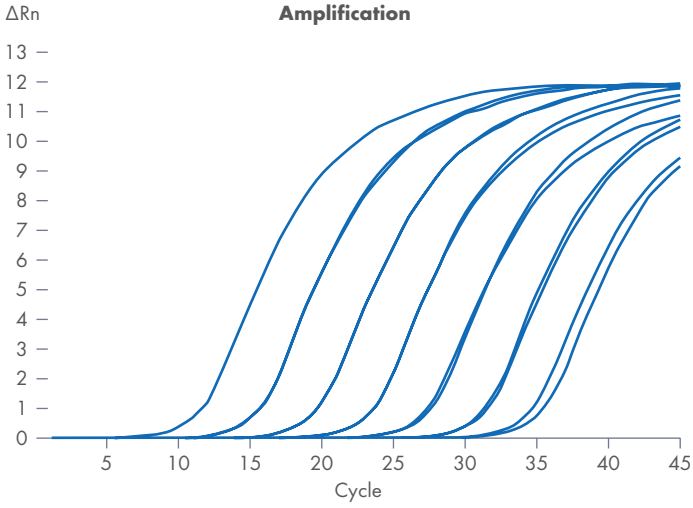

Figure 4
First-strand cDNA synthesis reaction set up with total human RNA (1 ng), decreasing copies of Kan mRNA (5 x 10^8 to 5 x 10 copies) primed with dT20 oligo, and run at 42°C. The cDNA product was added as template to qPCR using Fast SYBR® Green Master Mix (Applied Biosystems) and Kan mRNA specific primers.

Compatible with one-step RT-PCR

EnzScript is compatible with one-step RT-PCR. One-step RT-PCR has the advantage of faster analysis, fewer pipetting steps, a lower risk of errors and contamination and is suitable for high-throughput applications (Figure 3).

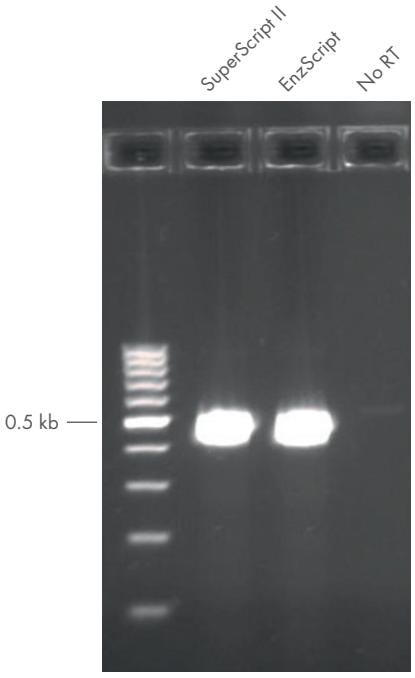

Figure 3
One step RT-PCR was set up in-house with the indicated reverse transcriptase (EnzScript, SuperScript II, or No RT) and Phoenix Hot Start Taq with 100 ng total human RNA input to detect 0.5 kb GAPDH target. PCR amplification was run following a 50°C, 30 min first-strand synthesis. PCR products visualized on 2% agarose gel.

Picogram level sensitivity in end-point RT-PCR

End-point RT-PCR can be used to measure changes in gene expression levels using three different methods: relative, competitive and comparative. The most common procedure for quantitating end-point RT-PCR results relies on detecting a fluorescent dye such as ethidium bromide. The results in Figure 5 show that EnzScript has sensitivity (down to 100 pg) comparable to Superscript II.

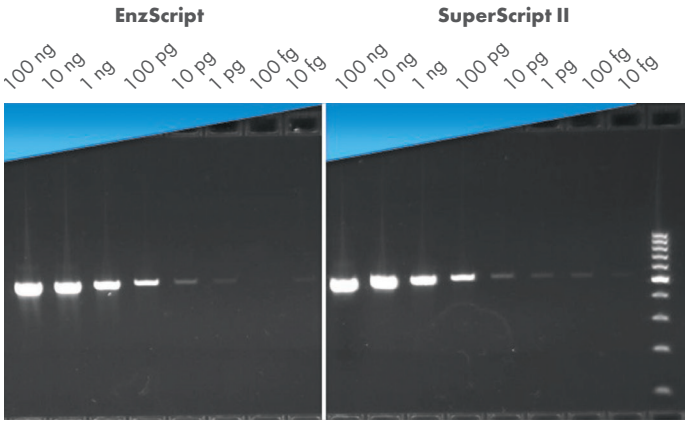

Figure 5
First-strand reactions were set up in-house with decreasing amounts of human total RNA (100 ng – 10 fg) primed with dT20 oligo. The cDNA added as template to PCR with Phoenix Hot Start Taq to amplify a 0.5 kb human target (GAPDH). PCR products were visualized on 2% agarose gel.

For full length transcripts

EnzScript generates high yields of full-length cDNAs. Yield of a 9.4 kb target is shown in Figure 6.

Quality and service you can count on

QIAGEN manufactures pure, highest quality enzymes and reagents for molecular biology and other applications. The company strives to resolve customers' challenges by providing high quality materials, an unbreakable supply chain and excellent service. With a manufacturing record unmatched in commercial enzyme production, QIAGEN designs analytical grade quality into all its products to meet the most rigorous specifications. EnzScript Reverse Transcriptase is evidence of our commitment to identifying, developing, and delivering the very best enzyme technologies. If your company requires products and a service partner that stand above the crowd, we'd love to hear from you.

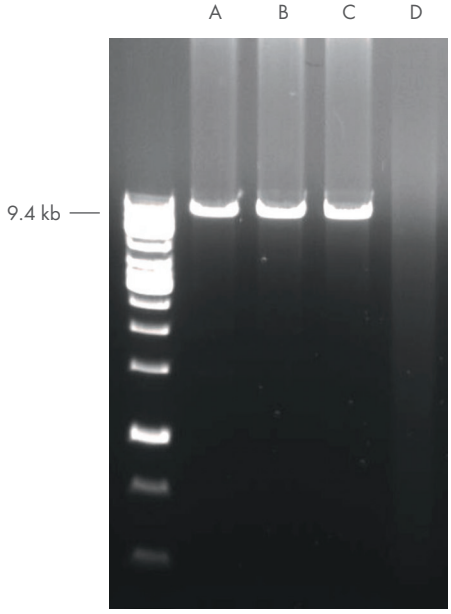

Figure 6
First-strand cDNA synthesis reactions in-house (1 µg total human RNA primed with oligo dT) using EnzScript (A and B) reveal high lot-to-lot consistency and comparable yield to Superscript II (C). Lane D is no RT control. High fidelity polymerase was used to amplify 9.4 kb target (Fibrillin 1). PCR products were visualized on 1% agarose gel.

Ordering Information

Product	Contents	Cat. no.
EnzScript Reverse Transcriptase	10,000 U of EnzScript Reverse Transcriptase (0.05 mL at 200,000 U/mL) and 5x M-MLV Reverse Transcriptase RNase H Minus Buffer (1 x 1.5 mL) and 100 mM DTT (1 x 1.5 mL)	P7600L
Phoenix Hot Start Taq DNA Polymerase	500 U at 5000 U/mL	P7590L

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