Transcriptor High Fidelity cDNA Synthesis Kit – Accuracy Meets RT-PCR

*Renate Kolb, Waltraud Ankenbauer, and Brigitte Hloch**
Roche Applied Science, Penzberg, Germany
**Corresponding author: brigitte.hloch@roche.com*

New Enzyme Class

Retroviral reverse transcriptases commonly used for cDNA synthesis exhibit a higher error rate than other DNA polymerases used in nucleic acid analysis techniques. This lack of accuracy leads to a significant number of base exchanges or frameshifts which are further propagated in a subsequent PCR reaction. With the introduction of a new high-accuracy reverse transcriptase, Roche Applied Science now offers a new tool to increase the fidelity of cDNA synthesis. The Transcriptor High Fidelity cDNA Synthesis Kit not only reduces the amount of errors in RT-PCRs; it also allows the reverse transcription of full-length cDNAs with high yield. When using the kit, you will have the following benefits:

- Get 7-fold higher fidelity
- Generate full-length transcripts up to 14 kb with the anchored-oligo $(dT)_{18}$ primers included in the kit (Figure 1)
- Obtain excellent yields (Figure 1)
- Detect low-copy number templates; reverse transcription from as little as 1 ng template RNA is possible
- Simultaneously reverse transcribe rare and abundant RNA – without altering gene expression levels (Figure 2)
- Transcribe a variety of templates, even the most difficult (*e.g.*, GC-rich RNAs with high secondary structure) through reverse transcription at temperatures up to 55°C
- Get results faster. Reduce reverse transcription reaction time to as little as 10 minutes

The kit features Transcriptor High Fidelity Reverse Transcriptase, a blend of a recombinant reverse transcriptase and a proofreading mediating enzyme optimized for two-step RT-PCR, making this the product of choice for the following applications:

- Sequencing transcriptomes
- Quantitative RT-PCR applications
- RNA splicing analysis
- Cloning genes of interest
- Generating cDNA libraries with large and full-length inserts

As the kit is also tested with the LightCycler® Instruments and other real-time PCR instruments, the product is also ideal for quantitative RT-PCR applications that require high fidelity (Figure 2).

Increase Accuracy

High mutation rate is a hallmark of retrovirus replication. This originates in the mechanism of genome replication by the viral-encoded RT, which converts the genomic RNA of the virus to a dsDNA. During this process, RT produces frequent replication errors. One accepted explanation of this inaccuracy is the lack of RT 3´–5´ exonuclease activity. The naturally high error rate of reverse transcriptases is not optimal for a lot of different applications.

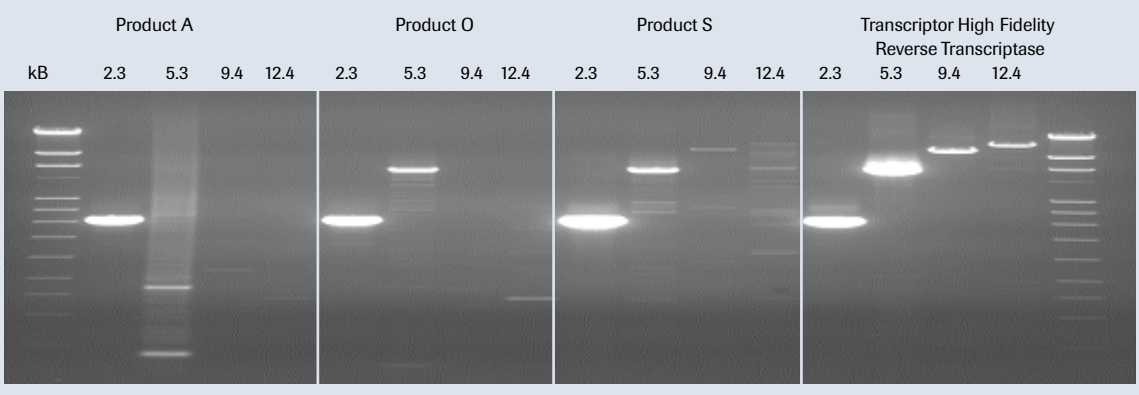

Figure 1: Comparison of several reverse transcriptases for the reverse transcription of total RNA for different fragment sizes. Total RNA samples (1 µg from human muscle total RNA for the 2.3-kb-fragment, 1 µg from HeLa total RNA for the 5.3-kb- and 9.4-kb-fragments, 2 µg of rat brain total RNA for the 12.4-kb-fragment) were reverse transcribed with different reverse transcriptases, according to the manufacturers' recommendations. A 5 µl aliquot of each cDNA reaction was subsequently amplified with the Expand Long Range dNTPack.

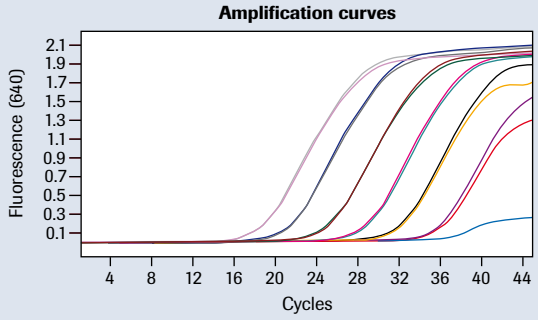

Figure 2: Highly efficient transcription over a broad range of template concentrations. 1 µg to 10 pg of K562 total RNA was reverse transcribed with anchored-oligo(dT)$_{18}$ primer followed by amplification of β-actin with Universal ProbeLibrary probes using the LightCycler® 2.0 Instrument.

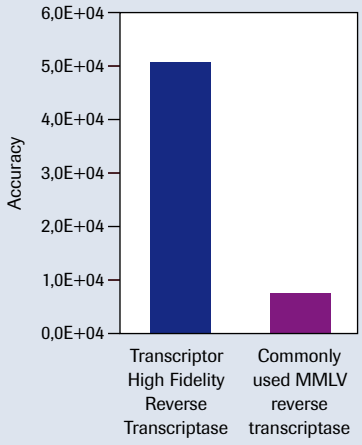

Figure 3: Accuracy of the Transcriptor High Fidelity Reverse Transcriptase and a commonly used MMLV reverse transcriptase. Error rate was determined by sequencing using the Genome Sequencer 20 System. RNA was reverse transcribed with the Transcriptor High Fidelity Reverse Transcriptase and a commonly used MMLV reserve transcriptase. After purification of the cDNA and amplification with a proofreading polymerase, the error rate of the reverse transcriptases was calculated by subtracting the error rate of the PCR control performed with plasmid DNA carrying the same sequence. The error rate of the Transcriptor High Fidelity Reverse Transcriptase is a mean value of 4 independent experiments in which at least 3.1 x 10^6 bases were sequenced. For the MMLV reverse transcriptase, 4.5 x 10^6 bases were sequenced. The accuracy is represented as error rate^{-1}.

With Transcriptor High Fidelity Reverse Transcriptase you can benefit from an optimized enzyme blend with a 7-fold higher fidelity compared with other commonly used reverse transcriptases (Figure 3). The data are true-fidelity data, as determined by the sequencing of several million bases with the Genome Sequencer 20 System.

Impressive Gene Expression Levels

Simultaneously reverse transcribe rare and abundant RNA without altering gene expression levels (Figure 2). The buffer is optimized also for use on LightCycler® Instruments, resulting in high fluorescence intensities, well-shaped curves, and expected C_T distances. Achieve additional safety in your qRT-PCR by using this proofreading reverse transcriptase.

Obtain Accurate Full-Length Results from Templates with Medium or High GC-Content

The Transcriptor High Fidelity Reverse Transcriptase enzyme blend efficiently reverse transcribes templates up to 14 kb. Compared with RTs of other suppliers, Transcriptor High Fidelity Reverse Transcriptase enzyme blend offers higher yield and robust cDNA synthesis over the whole fragment range (Figure 1). Owing to the high thermostability of both enzyme components and the specially optimized buffer system, reverse transcription is possible at temperatures up to 55°C. This allows the reverse transcription of GC-rich templates with high secondary structure, without the need to include additives that may negatively influence the accuracy of the reverse transcription reaction. In addition, you can profit from a very fast RT. With Transcriptor High Fidelity Reverse Transcriptase enzyme blend results were generated in 10 minutes.

The kit contains all components required for synthesizing cDNA suitable for direct use in qualitative RT-PCR with conventional thermal cyclers or quantitative RT-PCR on real-time PCR instruments. The 50-reaction pack size also includes 10 control reactions (control RNA and control primer mix). For priming, three different primer systems can be used. Two cDNA synthesis primers are provided with the kit: random hexamer primers and an anchored-oligo(dT)$_{18}$ primer. The latter is designed to bind at the beginning of the poly(A) tail in order to generate full-length cDNAs and to prevent priming from internal sites of the poly(A) tail. The 5´-ends of long mRNAs are often under-represented; therefore, this priming method is preferred for most applications. The use of random hexamer primers enables priming throughout the length of RNA for uniform representation of all RNA sequences and allows reverse transcription of RNAs that do not carry a poly(A) tail. The thermostable Protector RNase Inhibitor, that is also included in the kit, protects RNA from degradation at high reaction temperatures.

Free Sample

Obtain more information and request your free sample at www.transcriptor-highfidelity.com. ■

Order INFO

Product	Pack Size	Cat. No.
Transcriptor High Fidelity cDNA Synthesis Kit	kit for 50 reactions, including 10 control reactions	05 081 955 001
	kit for 100 reactions	05 091 284 001
	kit for 200 reactions	05 081 963 001

Please note that in the 2008 catalog, the catalog numbers for Transcriptor High Fidelity cDNA Synthesis Kit are incorrect. The correct catalog numbers are given here.