Roche Applied Science

Transcriptor First Strand cDNA Synthesis Kit

Bring Power and Sensitivity to Two-Step RT-PCR on Real-Time PCR Instruments

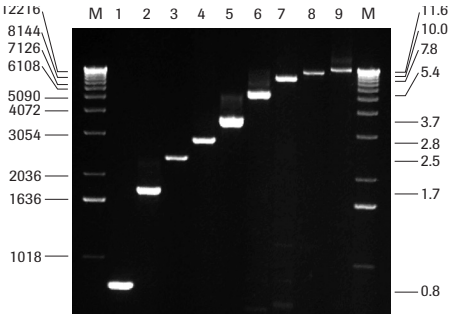

Figure 1: Produce full-length cDNA with the Transcriptor First Strand cDNA Synthesis Kit. In numbered lanes except Lane 3, reverse transcription was performed on 2 µg total RNA from human skeletal muscle research samples, using the kit's anchored oligo $(dT)_{18}$ primer. In a subsequent PCR, various fragments of the dystrophin gene were amplified using either the Expand High Fidelity* (Lanes 1, 2, 4) or the Expand Long Template (5 to 9) PCR Systems*. In lane 3, 1 µg of total RNA from human liver cells research samples was used as template for the reverse transcription, and Expand High Fidelity was used to amplify an ApoB gene fragment in the subsequent PCR.

Increase sensitivity of quantitative PCR

- Improve gene-expression studies by using this new kit to reverse transcribe RNA without distorting gene-expression levels; then amplify multiple targets on real-time PCR instruments (see reverse side).

Produce large quantities of full-length cDNA in two-step RT-PCR

- Employ a reverse transcriptase that generates very long cDNAs, up to 12 kb (Figure 1).
- Use the kit's unique anchored-oligo $(dT)_{18}$ primer, which binds at the beginning of the poly(A) tail, rather than mispriming at an internal site within the tail.

Overcome challenging secondary structures and fully protect your RNA with thermostable enzymes

- Transcribe GC-rich templates with high secondary structure at 55°C with the thermostable Transcriptor Reverse Transcripase. Simultaneously save your RNA from degradation with the included Protector RNase Inhibitor, a thermostable inhibitor that is fully active at this temperature.

Generate a representative pool of cDNA for subsequent quantitative PCR

Improve gene-expression studies by producing cDNA for both very rare and abundant RNAs in the same sample – without distorting gene expression levels.

Achieve accurate linear quantification over at least a 10^8-fold range of input RNA (*in vitro* transcripts)

Analyze genes with very low or extremely high expression levels.

Simplify results interpretation

Produce qPCR curves that are well shaped, exhibit a high fluorescence intensity, and maintain identical distances between RNA dilutions (Figures 2 and 3).

Insist on a kit that is designed and function tested for qPCR, and efficient with all qPCR instruments

Use the supplied control RNA and PBGD primers for the control reaction or as a housekeeping gene. In addition, we tested each lot on our LightCycler System.

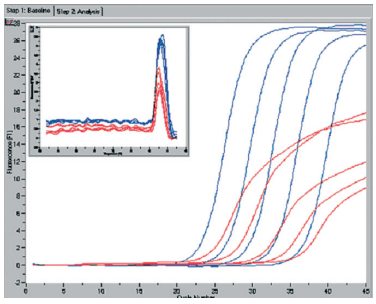

Figure 2: Sensitivity of two-step RT-PCR for the quantification of gene-expression levels on the LightCycler Instrument.
Either the Transcriptor First Strand cDNA Synthesis Kit (blue curves) or another suppliers's Reverse Transcriptase (red curves) was used to perform reverse transcription on 100 ng to 10 pg of total RNA from K-562 cells. LightCycler FastStart DNA Master^{PLUS} SYBR Green I‡,§ was then used with primers for G6PDH to quantify gene-expression levels.

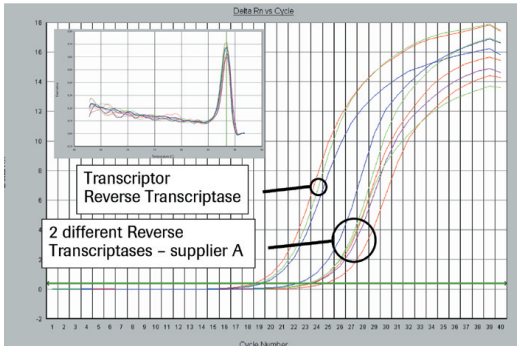

Figure 3: Sensitivity of two-step RT-PCR for the quantification of gene-expression levels on an ABI PRISM 7000 Sequence Detection System.
100 ng of total RNA from maize leaves was reverse transcribed with either Transcriptor or another supplier's Reverse Transcriptases (product A and B). A fragment from Phosphoenolpyruvate-Carboxylase with 50% GC content was then amplified **(data kindly provided by Dr. Peterhänsel, RWTH Aachen, Germany).**

Product	Cat. No.	Pack Size
Transcriptor First Strand cDNA Synthesis Kit†	04 379 012 001	1 kit (50 reactions)
Transcriptor Reverse Transcriptase† (single reagent)	03 531 317 001	250 U for 25 reactions
	03 531 295 001	500 U for 50 reactions
	03 531 287 001	2.000 U (4 x 500 U) for 200 reactions
Protector RNase Inhibitor (single reagent)	03 335 399 001	2.000 U
	03 335 402 001	10.000 U (5 x 2.000 U)

† This product is optimized for use in the Polymerase Chain Reaction ("PCR") process covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product.

‡ Purchase of this product includes a limited non-transferable end-user license to the purchaser under the SYBR Green I Technology owned by Idaho Technology under U.S. Patents 6.569.627 and foreign counterparts to use this product for any purpose.

* Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler.

§ Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process, including homogeneous PCR methods described in U.S. Patent Nos. 5.994.056 and 6.171.785 and their foreign counterparts, for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, i.e. an authorized thermal cycler. No rights for any other application, including any in vitro diagnostic application, are conveyed expressly, by implication or by estoppel under U.S. Patents 5.210.015, 5.487.972 and 5.804.375 or their foreign counterparts, or any other patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd claiming real-time amplification and detection methods.

The technology used for the LightCycler Instrument is licensed from Idaho Technology, Inc.

Roche Diagnostics

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