Transcriptor One-Step RT-PCR Kit – High Sensitivity for Detecting RNA

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Introduction

One-step reverse-transcription (RT) PCR is a very sensitive technique for determining the presence or absence of RNA templates or quantifying expression levels through qualitative, semi-quantitative, or quantitative analysis of RNA transcription levels (*e.g.*, for virus-level quantification). This RT-PCR method also allows amplification of rare messages for cloning. The one-step format is convenient with a reduced reaction-to-reaction variability and prevents contamination by minimizing hands-on steps. With the Transcriptor One-Step RT-PCR Kit, Roche Applied Science now offers the excellent Transcriptor Reverse Transcriptase in a one-step format. The Transcriptor One-Step RT-PCR Kit is designed for fast, sensitive, and specific end-point RT-PCR analysis of RNA using gene-specific primers on standard block cyclers. Featuring an innovative reaction buffer (see below), the kit provides the improved performance of a hot-start system for high-fidelity amplification of a variety of templates, including GC-rich RNA. It combines the high sensitivity and yield of Transcriptor Reverse Transcriptase with the fidelity and yield of the Expand System. The kit can use either total RNA, mRNA, in vitro-transcribed RNA, or viral RNA as starting material.

The Enzyme Mix

The Transcriptor One-Step RT-PCR Enzyme Mix contains four different enzymes: Transcriptor Reverse Transcriptase ensures sensitive and robust reverse transcription with high

The Benefits of the Transcriptor One-Step RT-PCR Kit

- Obtain high sensitivity and yield in a wide amplification range: Detect as little as 1 fg of total RNA or 1 copy of viral RNA (in vitro transcript; Figure 1 and 2).
- Achieve high specificity and reduce primer-dimers: Count on excellent performance with the kit's proprietary hot-start buffer (patents pending, Figure 2).
- Produce long fragments: Generate transcripts up to 6.5 kb (Figure 3).
- Transcribe a variety of templates, even the most difficult (*e.g.*, GC-rich RNA): Overcome high secondary structure with thermostable Transcriptor Reverse Transcriptase in combination with an optimized buffer (Figure 1).
- Save time and improve results with a convenient kit: Minimize pipetting steps and prevent contamination by using a premixed enzyme blend and an optimized buffer that includes PCR-grade dNTPs and hot-start components.
- Protect your RNA sample: Safeguard your RNA from degradation by RNases with the supplied thermostable Protector RNase Inhibitor that is functional during one-step RT-PCR when other RNase inhibitors fail.
- Increase confidence in your results: Use the supplied control reactions to monitor performance.

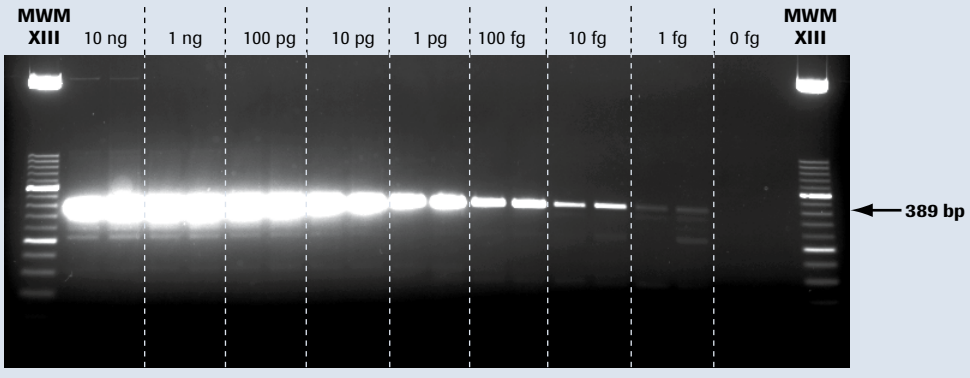

Figure 1: Transcribe difficult templates with high sensitivity. Various amounts (down to 1 fg) of total HeLa RNA were reverse transcribed with the Transcriptor One-Step RT-PCR Kit. A 389-bp fragment (GC content is 64%) was amplified with specific primers for human 28S ribosomal RNA according to the kit's standard RT-PCR protocol (reverse transcription at 50°C for 30 minutes). As shown by the band obtained after agarose gel electrophoresis and ethidium bromide staining, the kit transcribes even small amounts (1 fg) of template with high sensitivity (MWM, molecular weight marker).

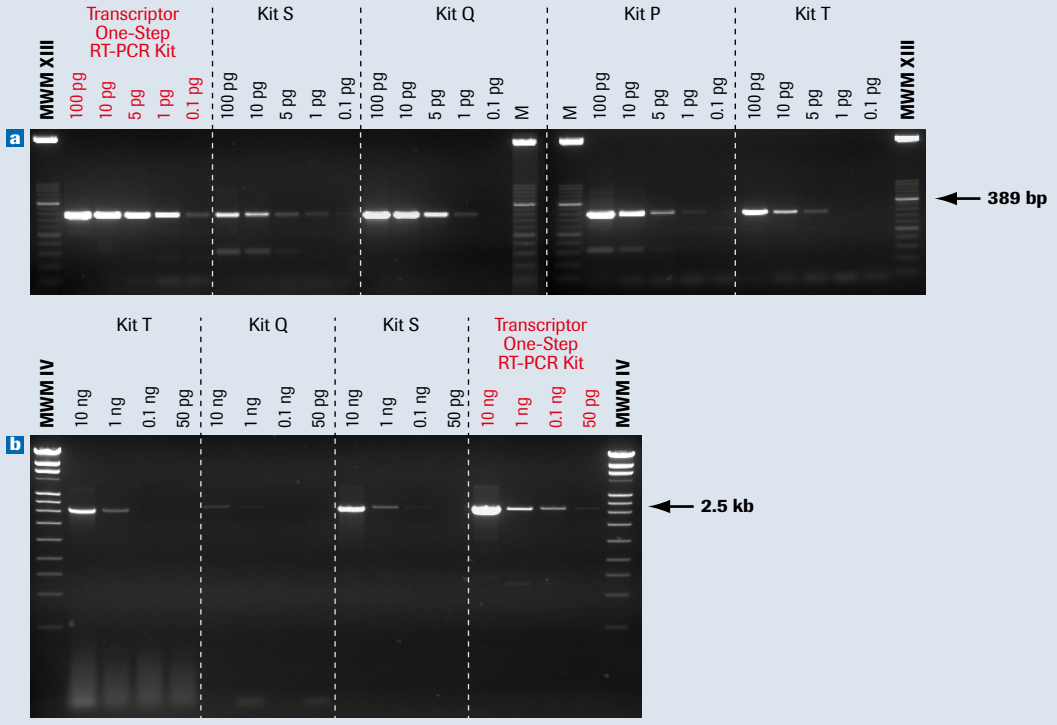

Figure 2: Achieve high sensitivity and specificity with the Transcriptor One-Step RT-PCR Kit. A 389-bp fragment of human 28S ribosomal **(a)** or a 2.5 kb-fragment of human ApoB **(b)** was amplified with Transcriptor One-Step RT-PCR Kit or one-step RT-PCR kits from other suppliers using manufacturers' recommended reaction conditions. Results show that the Transcriptor One-Step RT-PCR Kit efficiently amplifies a wide range of sample amounts with greater sensitivity and specificity compared with the other suppliers' kits (MWM, molecular weight marker).

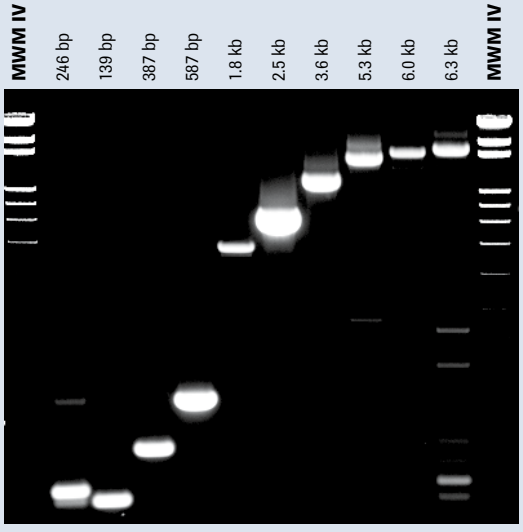

Figure 3: Generate fragments up to 6.5 kb. Various amounts of total or viral RNA were reverse transcribed with the Transcriptor One-Step RT-PCR Kit using the reaction conditions outlined in the pack insert. Samples: 10^6 copies of hepatitis A virus (HAV) RNA (246-bp fragment); 10 ng of total RNA of different tissue types or K562 cells (139-bp, 387-bp, 587-bp, and 1.8-kb fragments); 100 ng of total RNA of different tissue types or HeLa cells (2.5-kb, 3.6-kb, 5.3-kb, 6.0-kb, and 6.3-kb fragments). Results show that the Transcriptor One-Step RT-PCR Kit efficiently transcribes a broad range of fragment sizes (MWM, molecular weight marker).

yield; Protector RNase Inhibitor, fully active at elevated temperatures, provides maximum template protection during reverse transcription; and the Expand System, a blend consisting of Taq DNA Polymerase and a proofreading polymerase, minimizes the possibility of mutations, offering high yield and fidelity in PCR.

The Reaction Buffer

The optimized RT-PCR Reaction Buffer includes high-quality dNTPs and provides the overall improved performance of a hot-start system. The buffer's unique hot-start component binds and sequesters primers at lower temperatures to prevent the primers from binding to nonspecific sites. Another component binds magnesium in a temperature-dependent manner to prevent uncontrolled DNA synthesis. This new buffer formulation is effective during reverse transcription and PCR, resulting in increased specificity, sensitivity, and reduced primer dimers. ■

Free Sample

To test the new Transcriptor One-Step RT-PCR Kit request your free sample by contacting your local Roche representative..

Order INFO

Product	Pack Size	Cat. No.
NEW!		
Transcriptor One-Step RT-PCR Kit	1 kit (50 reactions, including 10 control reactions)	04 655 877 001
	1 kit (150 reactions)	04 655 885 001