Affymetrix | USB® Products

PCR Tools

USB® First-Strand cDNA Synthesis Kit for Real-Time PCR

The First-Strand cDNA Synthesis Kit for Real-Time PCR is optimized for reverse transcription of RNAs and produces first-strand cDNA template suitable for real-time PCR.

- **Robust performance** – Optimized to reliably generate cDNA from full-length transcripts longer than 12 kb.
- **Sensitive** – Sensitive and reliable real-time RT-PCR analysis of multiple user-defined targets from as little as 10 pg of starting total RNA.
- **Consistent performance** – Optimized for generating first-strand cDNA to be used in real-time PCR. HeLa total RNA and primers for qPCR are included as a positive control.
- **Versatile** – Three different priming strategies are provided to meet experimental needs. Mixed priming strategy overcomes 5' and/or 3' end bias associated with typical oligo$(dT)_n$ or random hexamer priming strategies.

Conversion of RNA into single-stranded cDNA involves a complex, enzyme-catalyzed reaction known as reverse transcription (RT). RT followed by real-time or quantitative PCR (real-time RT-PCR or qPCR) amplification is the most sensitive technique for mRNA detection and quantification currently available[1].

This kit is specifically designed to convert RNA into first-strand cDNA that is suitable for real-time PCR applications.

First-strand cDNA generated by this kit is also suitable for end-point PCR. RT-PCR can be used in a variety of applications, such as qualitative and quantitative analyses of cellular RNAs, characterization of RNA splice variants, and the generation and cloning of cDNAs.

Mixed priming strategy eliminates end-bias

This kit includes an optimized Primer Mix which results in the generation of first-strand cDNAs from an entire transcript without the end-bias observed with typical oligo$(dT)_n$ or random hexamer primers (Fig. 2). This mixed primer strategy overcomes variability in real-time PCR gene expression analysis that can result from using different individual primers[2]. For convenience, anchored oligo$(dT)_{23}$VN and random hexamer primers are also included.

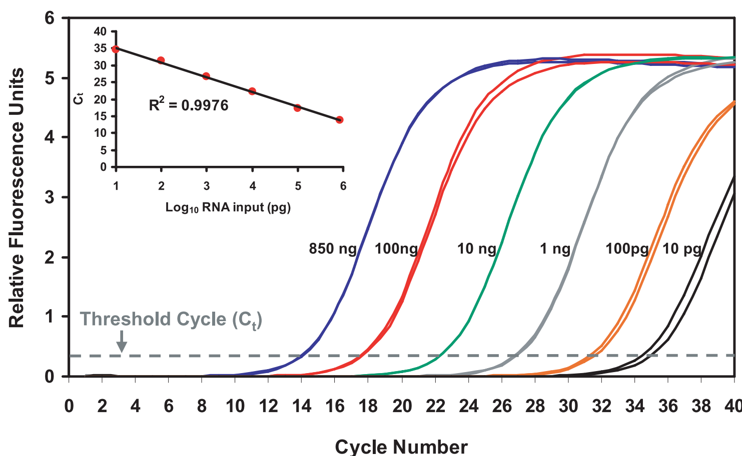
Robust and sensitive performance

This kit has been designed to routinely generate cDNAs from transcripts longer than 12 kb (Fig. 3). Consistent results are obtained using any of the three priming strategies provided with this kit. Reverse transcription can be performed with as little as 10 pg of total RNA, allowing sensitive downstream analysis (Fig. 1).

Kit components

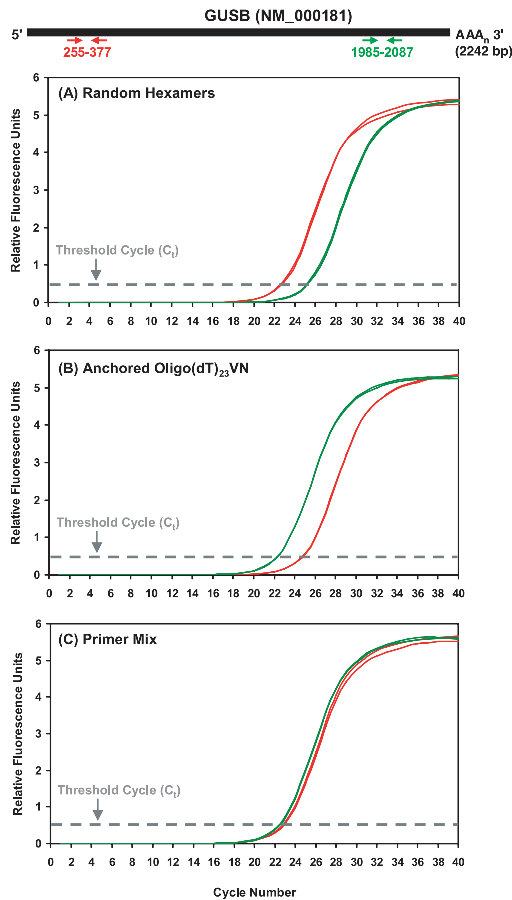
10X RT Buffer, 10 mM dNTPs, RNase Inhibitor (10 units/µl), RNase-Free, DEPC-Treated Water, M-MLV RT, Anchored Oligo$(dT)_{23}$VN (50 µM), Random Hexamers (75 µM), 10X Primer Mix, HeLa Total RNA (100 ng/µl), Control Primer Mix for qPCR.

Fig. 1. Assay sensitivity and dynamic range



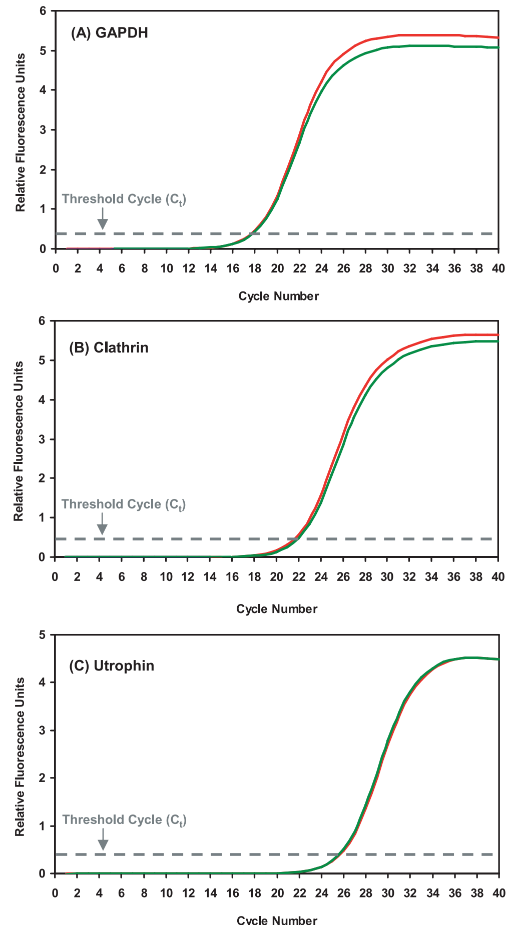
Various amounts of HeLa cell total RNA, ranging from 850 ng down to 10 pg, were reverse transcribed using anchored oligo$(dT)_{23}$VN as the primer. 1 µl of each reverse transcription reaction was then used in 20 µl real-time PCR reactions (in duplicate) to amplify a 122 bp GAPDH amplicon (on an ABI 7500 Fast instrument), using HotStart-IT® SYBR® Green qPCR Master Mix (PN 75762). GAPDH amplification and linear correlation curve above show the wide dynamic range and sensitivity of the First-Strand cDNA Synthesis Kit for Real-Time PCR.

Fig. 2. The primer mix solution



The Primer Mix solution greatly reduces bias for sequences near the 5' and/or 3' ends of cDNAs produced. The β-glucuronidase (GUSB) mRNA was reverse transcribed from 100 ng of HeLa cell total RNA using (A) Random Hexamers, (B) Anchored Oligo$(dT)_{23}$VN, or (C) the Primer Mix. Amplicons located near the 5' end (orange) or 3' end (green) of the GUSB transcript were amplified by real-time PCR (on an ABI 7500 Fast instrument) using 1 µl of each reverse transcription reaction in 20 µl real-time PCR reactions (in duplicate) and HotStart-IT SYBR Green qPCR Master Mix (PN 75762).

Fig. 3. Assay flexibility



The First-Strand cDNA Synthesis Kit for Real-Time PCR has been optimized for assay flexibility in carrying out reverse transcription using various RT-priming strategies and diverse RNA templates (GAPDH, ~1.5 kb transcript; Clathrin, ~6.7 kb transcript; Utrophin, ~12.5 kb transcript). Reverse transcription was performed using HeLa cell total RNA (100 ng) and two different priming strategies: anchored oligo$(dT)_{23}$VN primers (red) or random hexamers (green). 1 µl of each reverse transcription reaction was then used in 20 µl real-time PCR reactions (on an ABI 7500 Fast instrument) using HotStart-IT SYBR Green qPCR Master Mix (PN 75762).
(A) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), (B) Clathrin, and (C) Utrophin amplicons were amplified using gene specific primers designed for real-time PCR.

Product code	Pack size
75780	50 reactions (20 µl)

Shipping and storage:

Shipped on dry ice. Store at -20°C. Mix all components well prior to use. Light sensitive components should be protected from excessive light exposure.

References:

1. Bustin, S., Benes, V., Nolan, T., and Pfaffl, M. (2005) *J. Mol. Endocrinology* **34**, 597-601.
2. Ståhlberg, A., Håkansson, J., Xian, X., Semb, H., and Kubista, M. (2004) *Clinical Chem.* **50**(3), 509-515.

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