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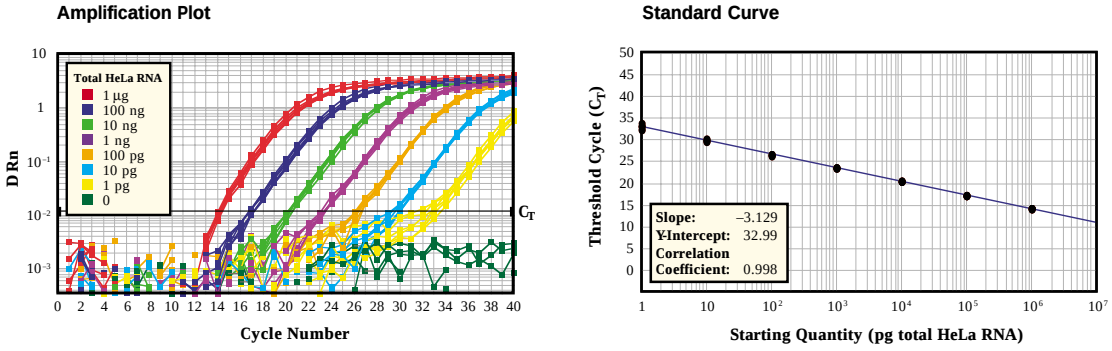
High-performance quantitative RT-PCR in one step

To achieve success in quantitative RT-PCR, you need the right RT and PCR technologies. With the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System, you get both with accurate real-time quantification of RNA. Obtain the high specificity, yield and sensitivity essential to your experiments, with one-step convenience.

One-step qRT-PCR protocol

The Platinum® Quantitative RT-PCR ThermoScript™ One-Step System is optimized to deliver accuracy and reliability. It combines two high-performance enzymes, ThermoScript™ reverse transcriptase and Platinum® *Taq* DNA polymerase, in a pre-mixed formulation. You use just one tube, where cDNA synthesis and amplification occur sequentially. Simply add total or poly(A)$^+$ RNA, gene-specific reverse transcription and amplification primers, and fluorogenic probes to your enzyme mixture and you're ready to go. This one-step protocol reduces reaction variability, lowers risk of contamination, and provides highly sensitive and efficient qRT-PCR. The convenient format makes it well suited for high throughput applications. As Figure 1 shows, the system provides exceptional sensitivity—you can achieve precise quantification from as low as 10 molecules of RNA template or 1 pg of total RNA.

Figure 1 - One-Step Quantitative RT-PCR



Quantitation of β-actin mRNA. Amplification of six replicates of various amounts of HeLa total RNA was performed with the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System and detected using a 6-FAM-labeled *Taq*Man® probe on an ABI PRISM® 7700. TAMRA was used as a passive reference for normalization of reporter signal (ΔRn).

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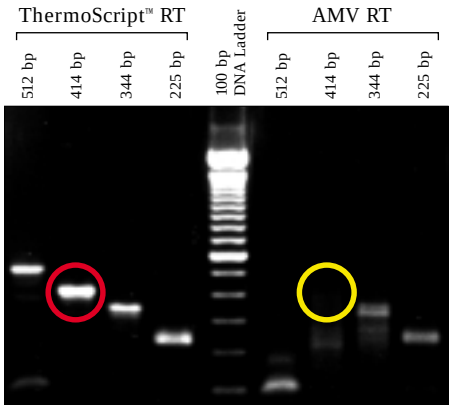
Thermostability for enhanced cDNA synthesis

ThermoScript™ RT, a cloned avian RNase H- reverse transcriptase, is engineered to provide highly specific cDNA synthesis at temperatures up to 70°C. It offers the same performance advantages of SuperScript™ II with added thermostability. With its reduced RNase H activity, ThermoScript™ improves the yields of full-length cDNA. With its high thermostability, you can overcome the challenges of high GC content and secondary structure to achieve highly efficient cDNA synthesis at elevated temperatures (Figure 2).

Figure 2 - High yield RT-PCR of GC-rich RNA with extensive secondary structure

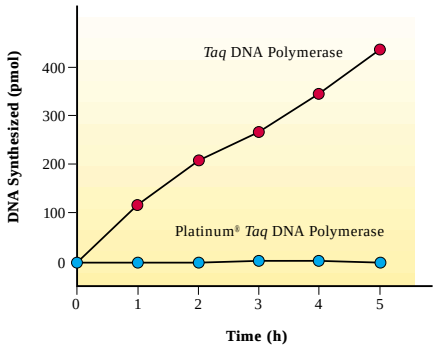


cDNA was synthesized from 10 ng total RNA using 15 units of ThermoScript™ RT or 15 units AMV RT according to manufacturers' recommendations. One tenth of each reaction was amplified with Platinum® *Taq* DNA Polymerase High Fidelity.

Platinum® automatic hot-start for high specificity

Along with ThermoScript™ Plus RT, you get the power of Platinum® technology, which confers a high level of specificity in PCR. Platinum® *Taq* DNA polymerase provides antibody-mediated automatic hot-start capability. With significantly reduced mispriming and non-specific amplification, you get higher yields, vastly improved specificity, and greater sensitivity than with other DNA polymerases (Figure 3).

Figure 3 - Complete inhibition of DNA polymerase activity at room temperature

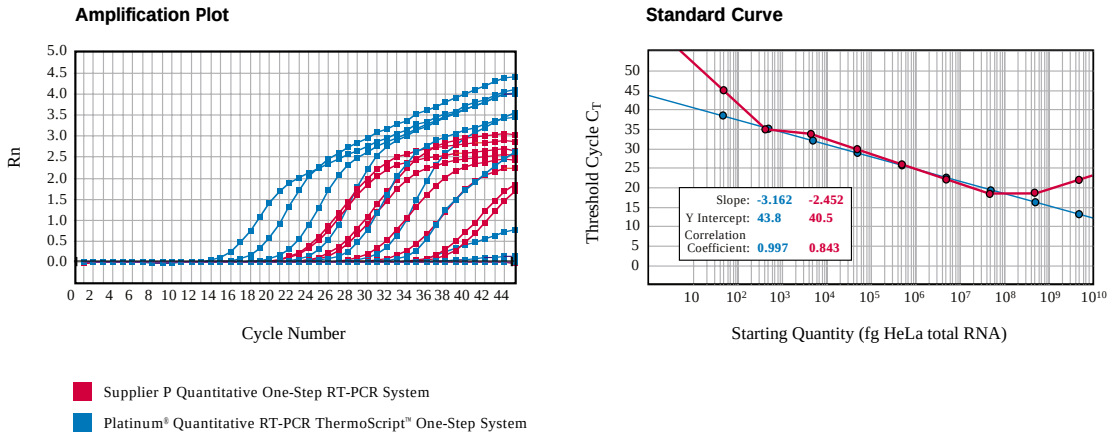


Activity assays were run at 37°C for 5 hours with *Taq* DNA polymerase and Platinum® *Taq* DNA Polymerase.

The right combination to surpass the competition

Together, the combination of RT and PCR technologies gives you the elements you need—yield, specificity, and sensitivity—for high performance qRT-PCR. Figure 4 compares the results achieved with the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System to the leading competitor's. The Platinum® kit demonstrates lower Ct (threshold cycler) and gives higher yield, making it more sensitive to detect low copies of mRNA transcripts.

Figure 4 - High yield with Platinum® Quantitative RT-PCR ThermoScript™ One-Step System



qRT-PCR of a 145 bp β-actin fragment was performed with the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System (blue plot) or Supplier P Quantitative One-Step RT-PCR System (red plot) according to manufacturers' recommendations. Starting RNA varied from 5 µg to 50 fg in 10-fold serial dilutions. Normalized relative fluorescence (ΔRn) was collected using TAMRA as the passive reference. **Panel A:** Linear scale amplification plots. **Panel B:** Standard curve plots.

More options for detection and analysis

Choose from TaqMan® probes, Molecular Beacons, or whatever fluorescent detection system you desire. Complete your analysis on any number of instruments, such as the ABI PRISM® 7700/7000/7900, Bio-Rad's iCycler, Stratagene MX4000™, Cepheid SmartCycler®, etc. With the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System, you don't need to limit yourself to one particular detection method or one real-time PCR machine.

One step to accurate qRT-PCR

The Platinum® Quantitative RT-PCR ThermoScript™ One-Step System provides the leading RT and PCR technologies in real-time quantification. Order today, and you'll get accurate quantitative RT-PCR, one-step convenience, and the flexibility to use the fluorescent detection method and instrument of your choice.

Ordering information

Product	Quantity	Cat. no.
Platinum® Quantitative RT-PCR ThermoScript™ One-Step System	100 rxns	11731-015
	500 rxns	11731-023
Rox Reference Dye*	500 rxns	12223-012

* There is no additional charge for Rox reference dye when ordered in conjunction with the Platinum® Quantitative RT-PCR ThermoScript™ One-Step System.

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Corporate headquarters:
1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com
European headquarters:
Invitrogen Ltd, 3 Fountain Drive • Inchinnan Business Park • Paisley PA4 9RF, UK • Tel: +44 (0) 41 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com

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