The MasterAmp™ Real-Time RT-PCR Kit Provides Superior Sensitivity and Consistent Quantitation

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Introduction

The MasterAmp™ Real-Time RT-PCR Kit provides all the necessary components to perform high sensitivity one-step quantitative RT-PCR. The kit includes a ready-to-use reaction mix containing SYBR® Green I dye, buffer, dNTPs, $MgCl_2$, and MasterAmp™ PCR Enhancer (with betaine)*. In addition, the kit uses RetroAmp™ RT DNA Polymerase, which is both a thermostable DNA polymerase and has reverse transcription activity. RetroAmp™ Polymerase allows for higher RT temperatures (up to 70ºC), significantly increasing the specificity and sensitivity of the RT reactions while decreasing secondary structure of RNA templates.

Another factor that improves the specificity and sensitivity of RT-PCR is the use of the MasterAmp™ PCR Enhancer, which is incorporated in the MasterAmp™ Real-Time RT-PCR Kit. The Enhancer reduces DNA and RNA secondary structure, eliminates sequence composition dependence of nucleic acid melting, and reduces pauses during DNA synthesis, thus improving the yield and specificity of many template amplifications.[1-3]

Here we demonstrate consistent real-time RT-PCR amplification of gene sequences from as little as 1-5 pg of total cellular RNA from *E. coli* or human cells.

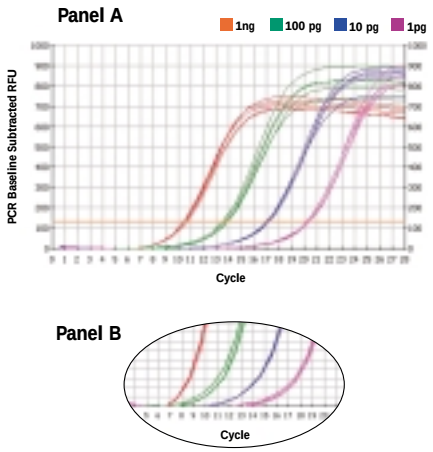

Figure 1. High sensitivity and C_T uniformity of RT-PCR using the MasterAmp™ Real-Time RT-PCR Kit. Panel A, Real-time RT-PCR using 16S rRNA consensus primers and 1 ng, 100 pg, 10 pg, or 1 pg of total *E. coli* RNA. **Panel B**, Expanded view showing cycle threshold uniformity.

Methods

Real-time RT-PCR was performed using total *E. coli* RNA and consensus primers to the *E. coli* 16S rRNA gene or total cellular RNA from HeLa human cells and gene-specific primers to the human β-actin gene. Total cellular RNA was purified using the MasterPure™ RNA Purification Kit as described in the product protocol. Real-time RT-PCR was carried out with 1 pg to 50 ng of the respective total cellular RNA using the MasterAmp Real-Time RT-PCR Kit according to the one-step protocol supplied with the kit. Real-time RT-PCR reactions were monitored using Bio-Rad's iCycler iQ™. Amplification products were also analyzed by agarose gel electrophoresis.

Results

Figure 1 presents the results obtained using the MasterAmp Real-Time RT-PCR Kit to amplify from 1 pg to 1 ng of *E. coli* cellular RNA using 16S rRNA consensus primers. The data show high sensitivity and cycle threshold (C_T) uniformity over the complete range of template.

We next examined RT-PCR amplification of the β-actin gene sequence using total RNA from human HeLa cells. Under standard RT-PCR conditions, little or no RT-PCR product was detected using the β-actin primers (Figure 2). However, 1X or 2X MasterAmp PCR Enhancer in the reaction resulted in an abundant specific amplification of the β-actin gene sequence from total HeLa cell RNA.

One-step real-time RT-PCR of the β-actin gene sequence was then performed using 5 pg to 50 ng of total HeLa cell RNA and either the MasterAmp™ Kit or a leading competitor's kit according to the manufacturer's protocol. As seen in Figure 3, amplification using the MasterAmp Real-Time RT-PCR Kit resulted in consistently higher sensitivity based on lower C_T values compared to results obtained with the competitor's kit.

Conclusions

The MasterAmp Real-Time RT-PCR Kit provides superior sensitivity and C_T uniformity in a ready-to-use pre-mix format. The patented MasterAmp PCR Enhancer significantly increases the specificity and consistency of real-time RT-PCR and eases the burden of primer design for both standard and difficult templates.

References

1. Schanke, J.T. and Grunenwald, H.L. (1997) *EPICENTRE Forum* **4** (1), 2.
2. Mytelka, D.S. and Chamberlin, M.J. (1996) *Nucl. Acids Res.* **24**, 2774.
3. Weissensteiner, T. and Lanchbury, J.S. (1996) *BioTechniques* **21**, 1102.

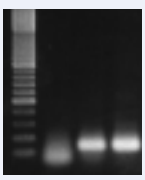

Figure 2. Improved RT-PCR due to the incorporated MasterAmp™ PCR Enhancer. A 132-bp region of β-actin was amplified using the MasterAmp™ Real-Time RT-PCR Kit from 100 ng of human placental cellular RNA. Lane M, 100-bp ladder; Lane 1, no added Enhancer; Lane 2, 1X Enhancer; Lane 3, 2X Enhancer.

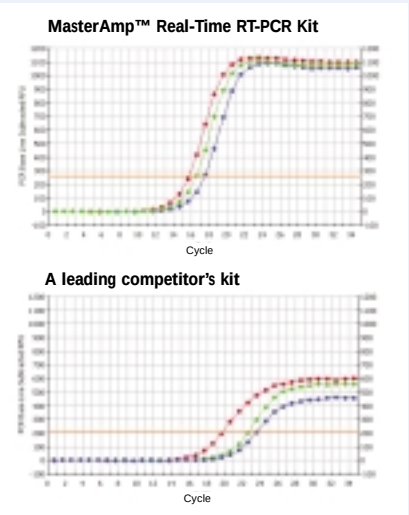

Figure 3. Increased sensitivity using the MasterAmp™ Real-Time RT-PCR Kit. Real-time amplification of β-actin RNA was performed using 50 ng (red), 500 pg (green) and 5 pg (blue) of total cellular RNA comparing the MasterAmp Real-Time RT-PCR Kit to a leading competitor's kit.

www.epicentre.com/realtimertpcr.asp

MasterAmp™ Real-Time RT-PCR Kit

MAR03100 100 Reactions

Contents:

RetroAmp™ RT DNA Polymerase, 2X Green RT-PCR PreMix, MasterAmp™ 10X PCR Enhancer, 25 mM $MgCl_2$, 25 mM $MnSO_4$, and Sterile Water.

* Covered by issued and pending patents as described on page 3.

See license and trademark information on page 3.