

EPICENTRE offers two different kits for RT-PCR. The MasterAmp RT-PCR Kit for High Fidelity, which utilizes an enzyme blend with proofreading activity, should be used for generating cDNA for cloning and expression. On the other hand, the MasterAmp RT-PCR Kit for High Sensitivity should be used for detection of small quantities of RNA.

The MasterAmp™ RT-PCR Kit for High Sensitivity Outperforms Other Kits for Sensitive Detection of RNA

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Introduction

Standard RT-PCR protocols recommend the use of up to one microgram of RNA template per reaction. In reality, researchers often have only a few nanograms or picograms of RNA with which to work. The amplification of these RNAs can be further complicated by the secondary structure of the RNA sequence of interest.

Thus, in order to achieve the highest sensitivity, both reverse transcription and PCR reactions must be optimized to strike the best balance between maximizing denaturation of RNA secondary structure, primer binding and primer extension, and minimizing high temperature RNA template degradation and enzyme inactivation.^{1,2} These variables are optimized for the MasterAmp RT-PCR Kit for High Sensitivity by incorporating several advances that permit greater sensitivity in a single RT and PCR reaction mixture. This report compares the MasterAmp Kit for High Sensitivity with RT-PCR kits from six other suppliers for its ability to detect a small amount of a specific mRNA in 25-100 picograms of total cellular RNA.

Methods

The MasterAmp RT-PCR Kit for High Sensitivity uses a single reaction mix for both reverse transcription and PCR. Each MasterAmp reaction contained: 1X RT-PCR Buffer, 3mM MgCl₂, 0.5 mM MnSO₄, 400 μM each dNTP, 12.5 pmoles of each primer, 25-100 pg placental RNA and 2.5 U of RetroAmp™ RT DNA Polymerase. RNA was reverse transcribed at 60°C for 30 minutes, and then immediately PCR amplified using the following parameters: denature at 94°C for 45 seconds, anneal to primers at 55°C for 45 seconds, and primer extend at 72°C for 60 seconds for 40 cycles.

RT-PCR was performed using dilutions of total cellular RNA from human placenta. Primers were designed to amplify a 343 base RNA region of the human glycoprotein hormone alpha subunit. The sequences of the primers were: 5'GTCAACGCGCT-GAACACATCCTGC and 5'GACACTCCCCATTAC-CATGACCCTG.

Reverse transcription and RT-PCR were performed with kits from other vendors using the protocols recommended by the respective manufacturers. Reverse transcription temperatures ranged from

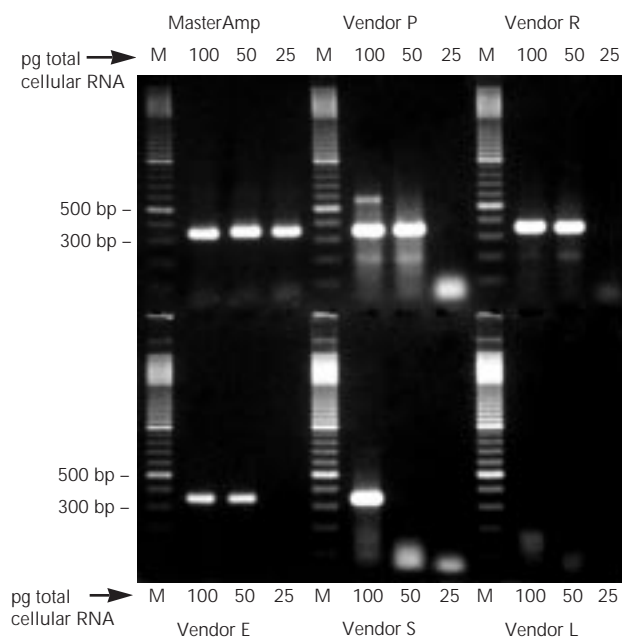


Figure 1. The MasterAmp RT-PCR Kit for High Sensitivity detects lower levels of RNA than RT-PCR kits from other vendors. RT-PCR was performed as described in Methods using 25pg of total human placental RNA and primers to the human glycoprotein hormone alpha subunit. M, DNA ladder.

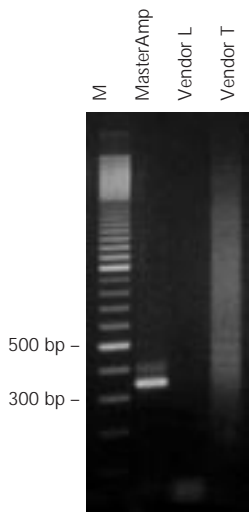


Figure 2. The MasterAmp RT-PCR Kit for High Sensitivity results in a more specific, high temperature RNA amplification. RT-PCR was performed as described in Methods using 25-100 pg of total human placental RNA and primers to the human glycoprotein hormone alpha subunit. M, DNA ladder.

37°C to 60°C. PCR cycling conditions were identical to those described above for the MasterAmp Kit with the exception of two kits, which recommended significantly longer extension times. Those recommendations were followed.

Results

The MasterAmp RT-PCR Kit was more sensitive than any other RNA amplification systems (Figure 1). Prominent, specific RT-PCR products were produced from 25-100 picograms of total cellular RNA from human placenta using the MasterAmp RT-PCR Kit for High Sensitivity, whereas none of the other kits or systems tested resulted in a detectable product from 25 picograms of RNA. The higher sensitivity of the MasterAmp Kit held true, both for systems that used a single reaction mix for reverse transcription and PCR (Vendors P and R) and for more time-consuming systems that used two different reaction mixtures and separate steps for reverse transcription and PCR (Vendors E, T, S and L).

The MasterAmp RT-PCR Kit was also compared to two vendors' high temperature RNA amplification kits (Figure 2). Reverse transcription was performed at 50°C to 60°C for 30 minutes followed by PCR amplification with

identical cycling profiles. As recommended by the supplier, PCR was performed as a second separate reaction with Vendor L's kit. As found previously, the MasterAmp RT-PCR Kit for High Sensitivity detected lower levels of RNA and produced a single, prominent RT-PCR product. Amplifications using the other kits resulted in a non-specific amplification (i.e., a smear) or no amplification when only 25 picograms of RNA was used as template.

Summary

The MasterAmp RT-PCR Kit for High Sensitivity is the easiest and most sensitive method for RNA detection. The kit uses a single reaction mix for both reverse transcription and PCR. Since reverse transcription is performed at the highest temperature, there is no need for a separate template denaturation step, and problems due to RNA secondary structure are eliminated.

References

1. Brooks, E.M. *et al.* (1995) *BioTechniques* **19**, 806.
2. Freeman, W.M. *et al.* (1996) *BioTechniques* **20**, 782.

MasterAmp™ RT-PCR Kit for High Sensitivity

RT71225-F73	25 Reactions	_____	_____
RT712100-F73	100 Reactions	_____	_____

MasterAmp™ High Fidelity RT-PCR Kit

RF91025-F73	25 Reactions	_____	_____
RF910100-F73	100 Reactions	_____	_____

*Special offer 25% off, ends September 15, 2000.

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