RT-PCR Master Mix (2X)

- **Flexible.** RT-PCR Master Mix (2X) with Taq DNA Polymerase is optimized for routine detection.
- **Sensitive.** Targets are easily detected down to 100 fg of total RNA.
- **Convenient.** Just add RNA template and primers in one easy step.
- **Stable.** RT-PCR Master Mix (2X) withstands repeated freeze-thaws with no loss in performance.

USB RT-PCR Master Mix (2X)

RT-PCR Master Mix (2X) provides maximum convenience and optimal performance for highly sensitive and specific one-step RT-PCR reactions. This unique formulation combines all the reagents necessary for successful RT-PCR with M-MLV Reverse Transcriptase and Taq DNA Polymerase. Simply add the RT-PCR Master Mix to RNA template, primers, and RNase-free water and the reactions are ready to begin.

During RT-PCR, reverse transcriptase converts RNA template to cDNA which is subsequently amplified by a thermostable DNA polymerase[1, 2]. One-step RT-PCR combines both the RT and PCR steps in a single tube and the reactions are performed sequentially[2]. This single-step, closed-tube approach simplifies the expression analyses of one or a few genes from multiple RNA samples and reduces the risk of contaminating samples. USB RT-PCR Master Mix further simplifies this technique by providing all the necessary reagents for one-step RT-PCR in a pre-mixed format.

Convenience

RT-PCR Master Mix saves time and reduces potential contamination errors by eliminating several pipetting steps. Since the mix is pre-formulated and thoroughly QC tested, experimental variability is significantly reduced. This translates into greater reproducibility in demanding, high-throughput experiments. For a 50 µl reaction, simply add 25 µl of RT-PCR Master Mix to primers, RNA template and RNase-free H_2O. Reactions can be performed in 10 µl, 25 µl, 50 µl or 100 µl volumes.

Figure 1

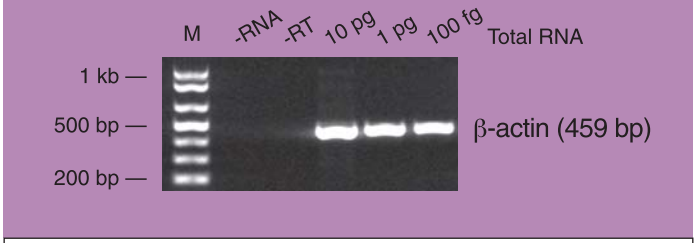

Fig. 1. Sensitivity of the RT-PCR Master Mix. A 459 bp fragment of the human β-actin gene was RT-PCR amplified from the indicated amounts of human placental total RNA. RT temperature was 50°C and primers were at 0.8µM. The -RNA control included no RNA in the sample and the -RT control included 100 ng of total RNA but used the Taq PCR Master Mix (PN 71162, without reverse transcriptase) to test for any contaminating genomic DNA in the RNA sample. "M" is the DNA marker lane. Since the typical human cell contains about 10 picograms of total RNA, this target's detection at 100 femtograms represents about 1/100th of the total RNA of a typical human cell.

Improve Specificity and Sensitivity

Amplify RT-PCR products from less template, with lower background, and with little or no optimization. Targets may be routinely detected from just 100 fg of total RNA. For example, human β-actin is detected from 100 femtograms of total RNA, which represents about 1/100th of the total RNA of a single human cell (Fig. 1).

USB Corporation
26111 Miles Road; Cleveland, OH 44128
800.321.9322 | www.usbweb.com

usb®
Fueling Innovation™

Rev 010407

Optimal Formulation

An enhanced buffer allows for RT reaction temperatures up to 50°C, which can improve detection of more difficult targets. This is because higher RT temperatures reduce non-specific priming and facilitate melting of RNA secondary structure[3]. In addition, the mix has the wild-type, RNase H-plus form of M-MLV Reverse Transcriptase which has been shown to improve the sensitivity for certain targets[4]. The RNase H activity degrades the RNA part of the cDNA/RNA hybrid following reverse transcription which may prevent its inhibition during subsequent PCR steps. RT-PCR Master Mix has been specifically designed to detect targets whose sizes are generally less than 1.0 kb. For targets greater than 1.0 kb and for high fidelity, use USB FideliTaq™ RT-PCR Master Mix [PN 71185].

Stable Performance

RT-PCR Master Mix withstands repeated freeze-thaw cycles with no observed decrease in performance (Fig. 2).

Tested User Friendly™ Functional Test

Tested by amplifying a 459 bp β-actin target from 10 pg of human placental total RNA.

RT-PCR Master Mix Formulation (2X)

RT-PCR Master Mix is a unique, proprietary formulation that includes M-MLV Reverse Transcriptase, Taq DNA Polymerase, recombinant Ribonuclease Inhibitor, nucleotides, and magnesium in a novel RT-PCR buffer. Magnesium concentration is 3mM in the 2X RT-PCR Master Mix.

Components

RT-PCR Master Mix is supplied in kit form with the following components sufficient for 100 reactions in a 50 µl reaction volume:

4 x 625 µl RT-PCR Master Mix (2X)
3 x 1 ml RNase-Free Water [PN 70783]
1 x 1 ml 25mM $MgCl_2$ Solution [PN 71167]

Shipping and Storage:

Shipped on dry ice. Store at -20°C. Mix well prior to use.

Figure 2

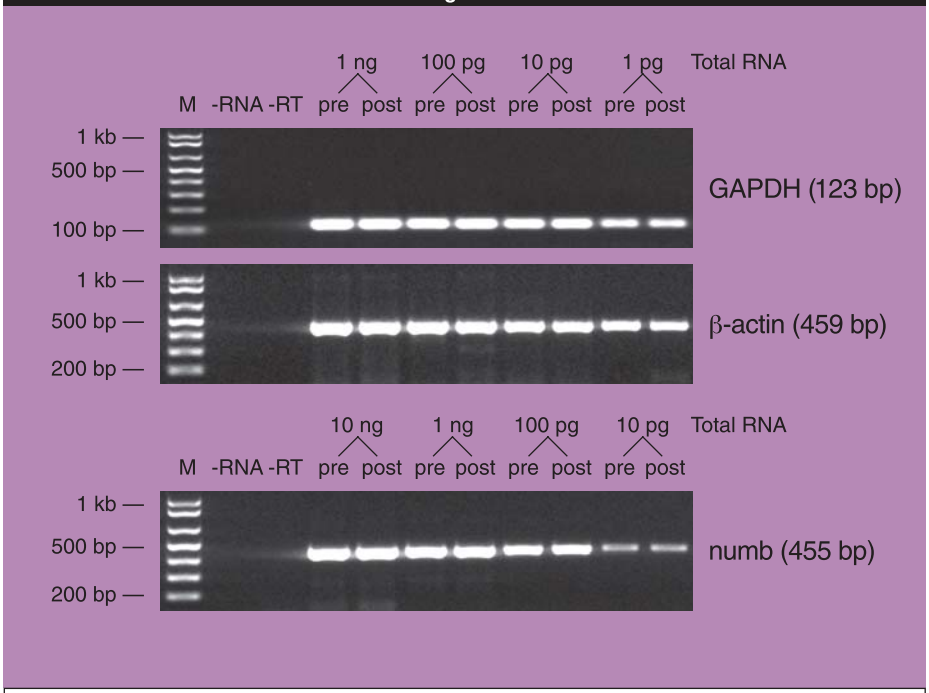

Fig. 2. Stability of the RT-PCR Master Mix with a variety of targets. Three targets were RT-PCR amplified from the indicated amounts of human placental total RNA. RT temperature was 50°C and primers were at 0.8µM. The -RNA control included no RNA in the sample and the -RT control included 100 ng of total RNA but used the Taq PCR Master Mix (PN 71162, without reverse transcriptase) to test for any contaminating genomic DNA in the RNA sample. "Pre" indicates lanes in which the Master Mix was not freeze-thawed and "Post" indicates lanes in which the Master Mix was freeze-thawed 15 times, alternating between dry ice and a room temperature water bath. "M" is the DNA marker lane.

References:

1. SAMBROOK, J. AND RUSSELL, D. W. (2001) "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, 8.46-8.53.
2. SELLNER, L. N., COELEN, R. J., AND MACKENZIE, J. S. (1992) *Nucleic Acids Res.* **20**, 1487-1490.
3. MALBOEUF, C. M., ISAACS, S. J., TRAN, N. H., AND KIM, B. (2001) *BioTechniques* **30**, 1074-1084.
4. POLUMURI, S. K., RUKNUDIN, A., AND SCHULZE, D. H. (2002) *BioTechniques* **32**, 1224-1225.

RT-PCR Master Mix (2X)

Product Code	Pack Size	List Price
78370	100 reactions	$288.00

usb®
800.321.9322 | www.usbweb.com