One-Step RT-PCR Kit

Designed for simplicity and convenience in carrying out RT-PCR in a one-tube format.

This Kit is Ideal for:

- Qualitative analysis of expression of one or a few genes in multiple RNA samples
- Analysis of specific RNA splice variants
- Streamlining the optimization of RT and PCR steps simultaneously
- A starting point for analysis of new RNA targets, given that many targets can be amplified successfully without need for optimization

Convenient One-Tube Format

- Quick and simple reaction set-up
- Eliminates the need to set up RT and PCR independently
- Saves time and eliminates potential contamination

Highly Sensitive, Highly Specific

- Detects diverse RNA targets based on generation of short (~0.2 to 1.5 kb) PCR products *(Fig. 1)*
- Targets may be reliably detected in 1 ng to 1 µg total RNA or 100 pg to 100 ng polyA RNA
- Sensitivity and specificity may be optimized easily *(Figs. 2 & 3)*

Kit Components

- RT-PCR Enzyme Mix
- RT-PCR Reaction Buffer, 5X [including $MgCl_2$]
- Ultrapure PCR Nucleotide Mix: 10mM each dATP, dCTP, dGTP, dTTP
- Ribonuclease Inhibitor, Recombinant (4 units/ul)
- Magnesium Chloride, 25mM
- RNase-Free (DEPC treated) Water

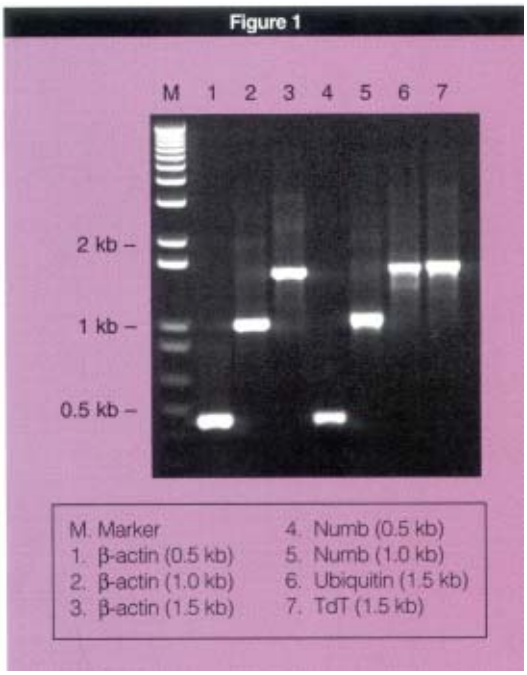

Figure 1:
Amplification of diverse RNA targets by one-step RT-PCR. Target (source): β-actin (100 ng total RNA, human liver), Numb (100 ng total RNA, human liver), Ubiquitin (1 µg total RNA, Arabidopsis leaf), and Terminal Deoxynucleotidyl Transferase (TdT) (100 ng polyA RNA, calf thymus). Gene specific primers were designed to generate products of particular sizes.

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Product Code	Pack Size	List Price
78350	50 reactions	$193.00

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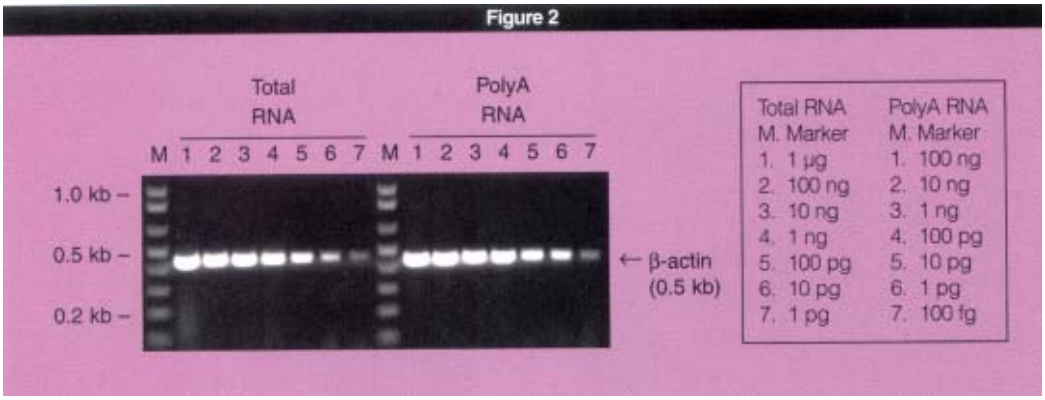

Figure 2:
Highly sensitive detection of β-actin target from human liver total RNA and polyA RNA, by one-step RT-PCR.
Primers were used at 0.8µM, a relatively high concentration, in order to achieve high sensitivity.

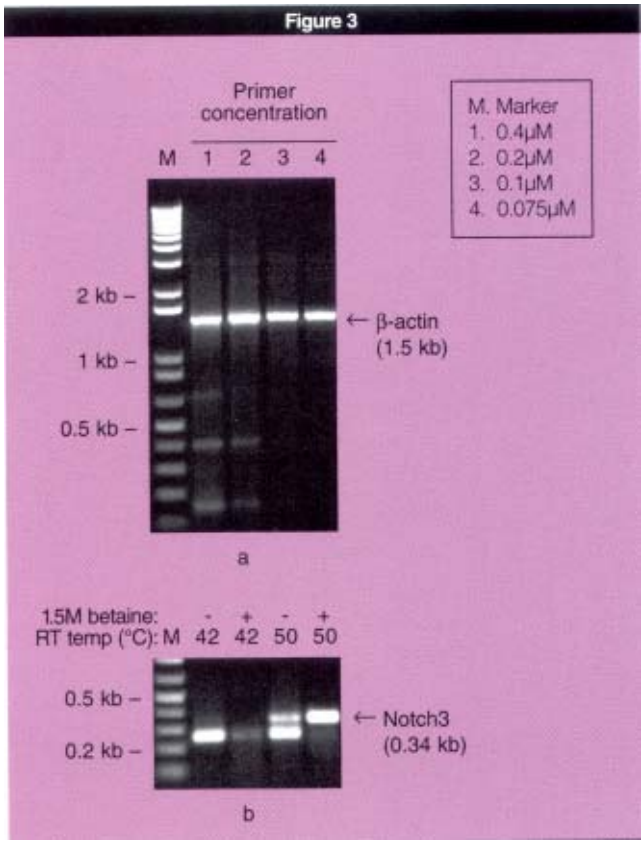

Figure 3:
Highly specific detection of β-actin and Notch3 from human liver total RNA (100 ng), by one-step RT-PCR.
(a) For many targets, such as 1.5 kb β-actin, specificity may be improved by decreasing the primer concentration. Compare results for 0.4µM versus 0.075µM primer. (b) For targets with high G+C contents, such as 0.34 kb Notch3 (G+C: 77%), adding supplements and/or increasing the temperature of the reverse transcription step, may improve specificity. Compare results for standard reaction conditions versus reaction with supplement and elevated temperature.

References:

1. SAMBROOK, J. AND RUSSEL, 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. ROTH, M. J., TANESE, N., AND GOFF, S. P. (1985) *J. Biol. Chem.* **260**, 9326-9335.

4. SAIKI, R. K., GELFAND, D. H., STOFFEL, S., SCHARF, S. J., HIGUCHI, R., HORN, G. T., MULLIS, K. B., AND ERLICH, H. A. (1988) *Science* **239**, 487-491.

*The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F. Hoffmann-La Roche Ltd.

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