Two-Step RT-PCR Kit

Designed for flexibility and versatility in carrying out RT-PCR in one or two-tube formats.

This Kit is Ideal for:

- Qualitative analysis of gene expression
- Highly detailed analysis of RNA splice variants
- Optimization of PCR independent of RT
- Analysis of the expression of multiple genes in individual RNA samples with oligo dT primers

Complete RT-PCR Kit

- Complete and ready to use for diverse RT-PCR applications
- Based on M-MLV Reverse Transcriptase and Taq DNA Polymerase provided in individual tubes at concentrations optimized to balance sensitivity and specificity in two-step RT-PCR
- Allows flexibility in setting up RT and PCR individually
- A simple enzyme dilution step also allows use of the kit for one-step RT-PCR

Highly Sensititve, Highly Specific

- Detects diverse RNA targets based on generation of long cDNAs (to at least 5.6 kb) followed by amplification 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

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

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.

Two-Step RT-PCR Kit

Product Code	Pack Size	List Price
78355	50 RT reactions & 100 PCR reactions	$216.00

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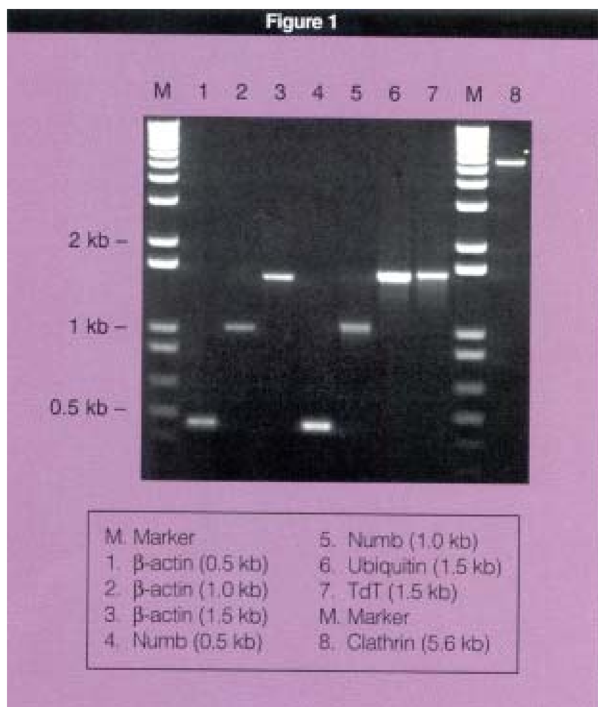

Figure 1:
Amplification of diverse RNA targets by two-step RT-PCR.
Target (source): β-actin (human liver), Numb (human liver), Ubiquitin (Arabidopsis leaf), and Terminal Deoxynucleotidyl Transferase (TdT) (calf thymus). RT was carried out on 1 µg total RNA with priming by oligo dT, and PCR was conducted on 10^{-1} dilution of RT reaction (except for TdT, for which non-diluted RT reaction was used). Gene specific primers were designed to generate products of particular sizes. Amplification of 5.6 kb Clathrin target (human liver) by use of a long-PCR method implies that RT reaction can generate cDNA of at least 5.6 kb.

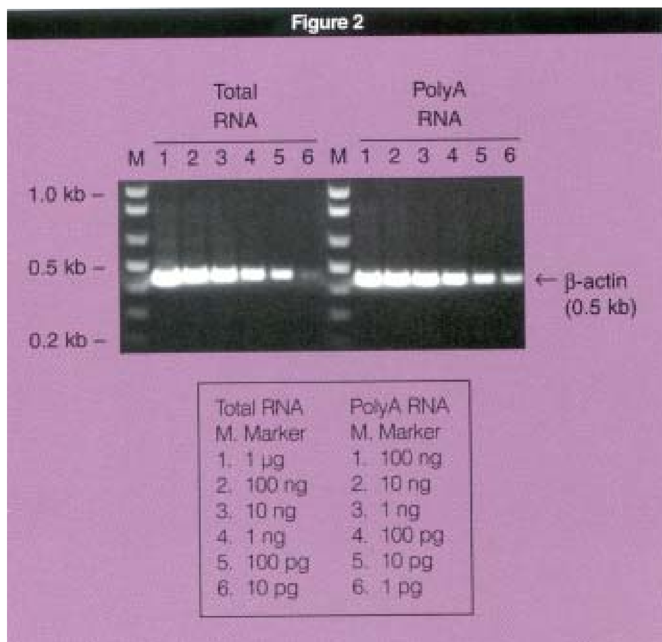

Figure 2:
Highly sensitive detection of β-actin target from human liver total RNA and polyA RNA, by two-step RT-PCR.
RT was carried out in 25 µl volume on indicated amounts of RNA by use of a gene specific primer. PCR was conducted in 50 µl volume on 2 µl aliquot of RT reaction. For PCR, primers were used at 0.8µM, a relatively high concentration, in order to achieve high sensitivity.

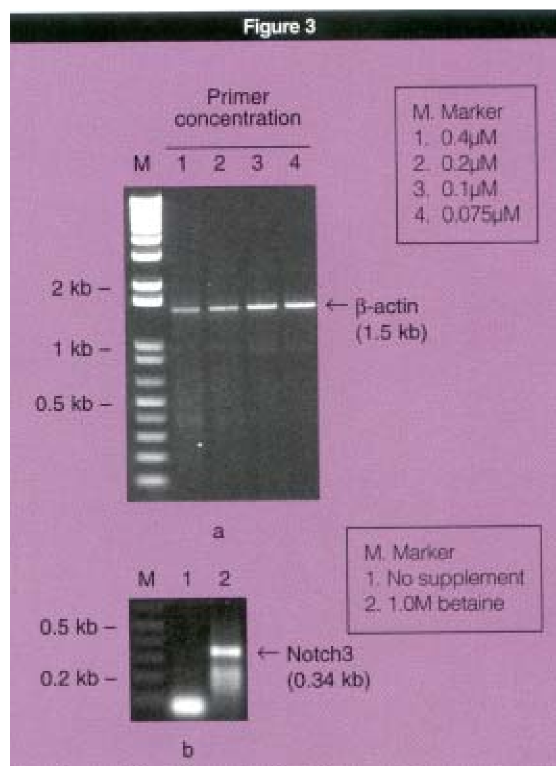

Figure 3:
Specific detection of β-actin and Notch3 from human liver total RNA (100 ng), by two-step RT-PCR.
(a) For many targets, such as 1.5 kb β-actin, specificity may be improved by decreasing the primer concentration in the PCR step. Compare results for 0.4µM versus 0.075µM primer.
(b) For targets with high G+C contents, such as 0.34 kb Notch3 (G+C: 77%), increasing the temperature of the reverse transcription step and/or adding supplements for the RT and/or PCR steps, may improve specificity. RT reaction was carried out at 50°C without supplements, and PCR was conducted with no supplements or with 1.0M betaine. The presence of betaine results in generation of desired product.

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

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