

# RT Script Kit

**Designed for reverse transcription of RNA to yield template for PCR.**

**The kit accomplishes the RT step in RT-PCR. USB Taq PCR Master Mix, the Taq PCR Kit or other USB PCR reagents can be used for the PCR step.**

## This Kit is:

- Optimized for generation of cDNA specifically for use in PCR
- Useful for the analysis of expression of multiple genes or RNA splice variants in individual RNA samples

## cDNA Synthesis Optimized for RT-PCR

- Based on M-MLV Reverse Transcriptase provided at a concentration optimized for sensitivity in standard RT-PCR applications
- Includes an optimized RT reaction buffer, RNase inhibitor, Ultrapure dNTPs, supplemental magnesium chloride, and RNase-free water
- Allows simple set up of RT reactions to be followed by PCR

## RT Script Kit

Product Code	Pack Size	List Price
78360	50 reactions	\$151.00



## High Sensitivity, High Yield

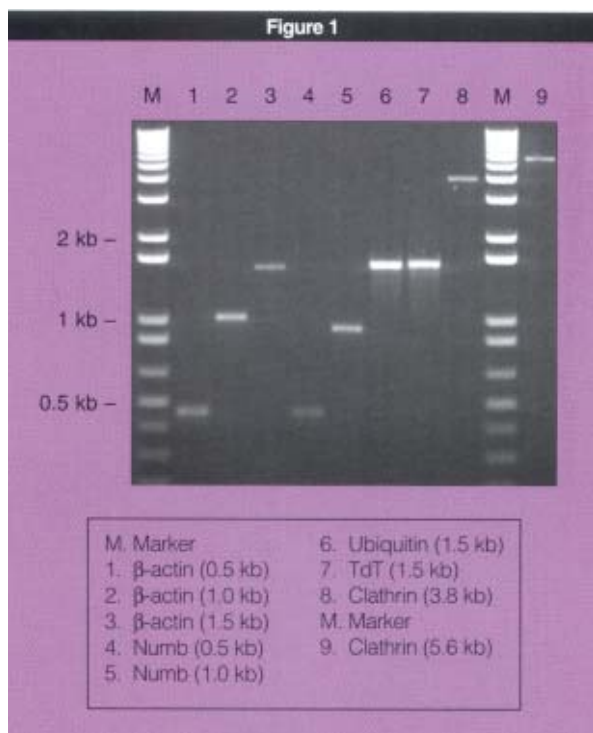
- Use for generating long cDNA (to at least 5.6 kb) from diverse RNA targets, based on use of either gene specific primers or oligo dT (*Fig. 1*)
- Targets can be reverse transcribed from 1 ng to 1 µg total RNA or 100 pg to 100 ng polyA RNA
- Highly expressed targets can be reverse transcribed from even lower amounts of RNA (*Fig. 2*)
- Resulting cDNA products can be detected readily by use of USB Taq PCR Master Mix (PN 71162), typically without need for optimization
- Yields from the RT step are typically sufficient to allow PCR detection of cDNA products from 10<sup>-1</sup> to 10<sup>-4</sup> dilutions of the RT reactions, depending on RNA target abundance (*Fig. 3*)

## Kit Components

- M-MLV Reverse Transcriptase
- RT Reaction Buffer, 5X [including MgCl<sub>2</sub>]
- PCR Nucleotide Mix, Ultrapure: 10mM each dATP, dCTP, dGTP, dTTP
- Ribonuclease Inhibitor, Recombinant (4 units/µl)
- Magnesium Chloride (25mM)
- RNase-Free (DEPC-treated) Water

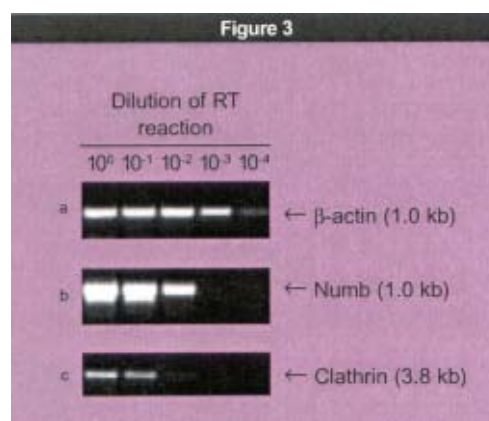
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**Figure 1:**  
Amplification of diverse RNA targets by two-step RT-PCR with the RT Script Kit and USB Taq PCR Master Mix.

Target (source):  $\beta$ -actin (human liver), Numb (human liver), Ubiquitin (Arabidopsis leaf), Terminal Deoxynucleotidyl Transferase (TdT) (calf thymus), and Clathrin (human liver). RT was carried out on 1  $\mu$ g total RNA by priming with 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.

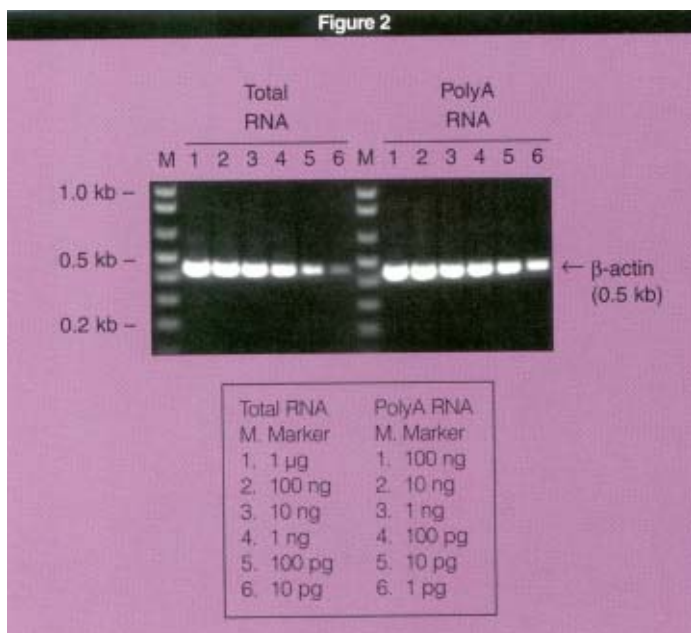


**Figure 3:**  
Detection of multiple RNA targets from a dilution series of a single RT reaction, by use of the RT Script Kit and USB Taq PCR Master Mix.

RT was carried out in a 25  $\mu$ l volume on 1  $\mu$ g total RNA from human liver with priming by oligo dT. A dilution series ( $10^{-1}$  to  $10^{-4}$ ) of the RT product was generated. PCR was conducted in a 25  $\mu$ l volume on 1  $\mu$ l aliquots from the RT reaction and each dilution. Gene specific primers were designed to generate products of particular sizes. (a) 1.0 kb  $\beta$ -actin. (b) 1.0 kb Numb. (c) 3.8 kb Clathrin.

### 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.



**Figure 2:**  
Highly sensitive detection of  $\beta$ -actin target from human liver total RNA and polyA RNA, by two-step RT-PCR with the RT Script Kit and USB Taq PCR Master Mix.

RT was carried out in a 25  $\mu$ l volume on indicated amounts of RNA by use of a gene specific primer. PCR was conducted in a 25  $\mu$ l volume on 1  $\mu$ l aliquot of RT reaction. For PCR, primers were used at 0.8  $\mu$ M, a relatively high concentration, in order to achieve high sensitivity.



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

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