

# Philisa<sup>®</sup> Thermal Cycler Technical Note

## One-tube Reverse Transcriptase PCR (RT-PCR) in Under One Hour Using the Streck Philisa Thermal Cycler

Joel R. TerMaat, Ph.D. and Sheila E. Norton, M.S.

### Overview:

One-step RT-PCR amplification is frequently used to save time and reduce hands-on processing. Standard heat-block thermal cyclers typically require 3 hours or more to complete the cycling protocol. The purpose of this study was to evaluate the feasibility of performing rapid one-tube RT-PCR with the Philisa Thermal Cycler using significantly reduced run times.

In this study, amplification of a human  $\beta$ -actin mRNA gene target was performed using a commercially available kit. Results showed the one-tube protocol could be reduced to 47 minutes when amplifying from 1 ng of RNA template and as little as 10 pg of total RNA was adequate to detect the specific RNA sequence in 60 minutes.

### Materials and Methods:

The Titan One Tube RT-PCR Kit from Roche Applied Science and its control primers (F) 5'-CCAAGGCCAACCGCGAGAAGATGAC-3' and (R) 5'-AGGGTACATGGTGGTGCCGCCAGAC-3' were used to amplify a 587 bp product from human  $\beta$ -actin mRNA. Each 25  $\mu$ l reaction mixture consisted of 5 mM MgCl<sub>2</sub>, 5 mM DTT, 2.5 U RNase Inhibitor, 0.5 mM each dNTP, 0.7  $\mu$ M each forward and reverse primers, 1x reaction buffer, and 0.75  $\mu$ l enzyme mix. Varying template amounts of K-562 human leukemia total RNA (Ambion) were used as described.

The thermal cycler was programmed as follows: RT at 50°C for 20 min; 45 second hot-start at 95°C; followed by 40 cycles of [95°C for 7 sec; 64°C for 6 sec; and 68°C for 20 sec], and a final extension at 68°C for 20 sec. In the sensitivity experiment, the RT step was increased to 30 min along with 45 PCR cycles. 15  $\mu$ l of each PCR product was electrophoresed on a 1% agarose gel and stained with ethidium bromide along with a 100 bp reference ladder.

### Results & Discussion:

Figure 1 shows PCR results for 4 replicate samples amplified using 1 ng total RNA template. High yields of product were observed in all 4 reactions. The Philisa Thermal Cycler was programmed to perform a 20 minute RT step and 40 PCR cycles with a final run time of 47 minutes. Faster run times can be achieved using higher amounts of RNA template.

To investigate the sensitivity of the Philisa Thermal Cycler when using a one-step RT-PCR kit, the instrument was programmed to perform a 30 minute RT step and 45 PCR cycles for a total run time of 60.5 minutes. PCR products generated from 1 ng, 100 pg and 10 pg starting template are shown in Figure 2. A high yield of product was obtained for the 1 ng and 100 pg samples and an amplified product is also visible for the 10 pg sample.



Figure 1 - 47 min RT-PCR time

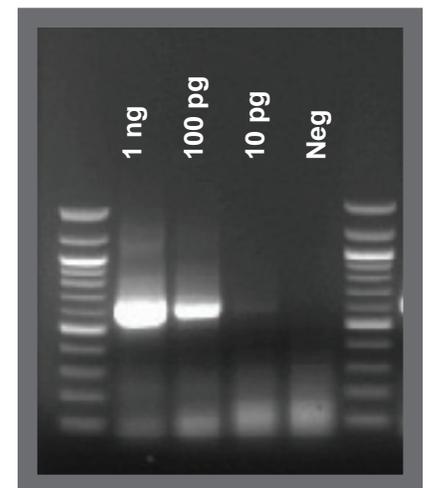


Figure 2 - 60.5 min RT-PCR time