High Capacity cDNA Reverse Transcription Kit

(formerly the High Capacity cDNA Archive Kit)

First Strand cDNA Synthesis Reverse Transcription Kit

The High Capacity cDNA Reverse Transcription Kit provides:

- Highest Level of Performance for Accurate and Precise RNA Quantification
- High Capacity for Maximum Dynamic Range
- High Reverse Transcription Efficiency
- Unsurpassed Value

Introduction

cDNA is widely used in a variety of applications, from determining the level of gene expression for a few genes, to large-scale screening of these levels in a thousand genes. Gene expression studies require access to large quantities of cDNA. The Applied Biosystems' High Capacity cDNA Reverse Transcription Kit (formerly the High Capacity cDNA Archive Kit) delivers uncompromised high reverse transcription efficiency, thereby maximizing the yield of cDNA.

RNA Quantification

One of the primary applications for real-time polymerase chain reaction (PCR) is RNA quantification.

In "two-step" real-time PCR chemistry, a reverse transcription (RT) step is performed first and the cDNA is then transferred for real-time PCR amplification. Therefore, accurate and precise quantification of RNA targets relies upon the performance of the reverse transcription step.

Quantification of gene targets using real-time PCR technology is based on threshold cycle (C_T) values. A C_T is the PCR cycle at which a geometric signal reaches a fixed fluorescent value.

C_T values are derived directly from cDNA, not RNA. The implication is that if the reverse transcription chemistry does not perform well, C_T values may no longer accurately reflect the original quantities of the RNA targets.

To investigate reverse transcription chemistry performance, RT kits from a variety of vendors were compared for their capacity, linearity and RT efficiency.

Researchers were asked to serially dilute RNA samples to create standard curves. Each RNA concentration was reverse transcribed using the same RT reaction volume. The resulting cDNA was then transferred into the real-time PCR Master Mix. Real-time PCR reactions were amplified using Applied Biosystems real-time instruments.

Figure 1 displays ideal RT chemistry performance for quantitation. RNA standard curve slopes should be approximately -3.3 and they should be parallel, reflecting 100%, and therefore equal, PCR efficiencies. The chemistry used in Figure 1 was the High Capacity Reverse Transcription Kit.

Capacity of a Reverse Transcription Reaction

Capacity is the maximum RNA mass that may be added to the reverse transcription reaction without loss of RT efficiency. Exceeding the capacity of reverse transcription chemistry not only makes inefficient use of RNA, but also runs the risk of disturbing the quantitative relationship between the gene target and the normalizing gene.

The performance of a reverse transcription reaction can be analyzed by looking at RNA standards, in which each RNA concentration is reverse transcribed using the same reverse transcription reaction volume. A common practice by users of the two-step RT-PCR chemistry is to run cDNA standards. However, serially diluting cDNA does not test the performance of the RT chemistry.

In Figure 2, the cDNA standards did not reveal the reverse transcription chemistry performance problems, but the RNA standards did. At high RNA concentrations, the reverse transcription efficiencies of the competitor's chemistry are reduced. In other words, this reverse transcription chemistry has exceeded its capacity.

Exceeding capacity of a reverse transcription reaction could lead to significant errors in the RNA quantification results (Figure 3). Figure 4 shows that the capacity of the reverse transcription chemistry from Competitor B is at 200 ng, compared to 10 µg for the Applied Biosystems High Capacity cDNA Reverse Transcription Kit.

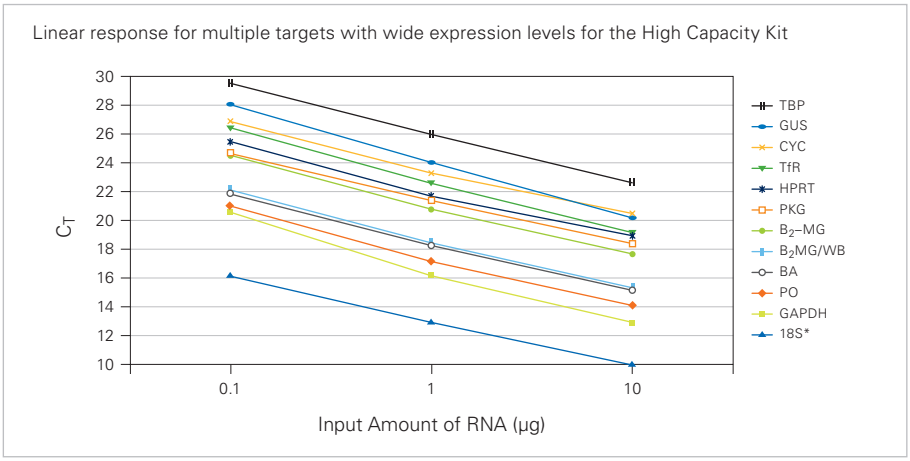

Figure 1. The expected ΔC_T value of -3.3 for each tenfold increase in input quantity is obtained for 11 different RNA transcripts converted to cDNA from different input quantities of total RNA.

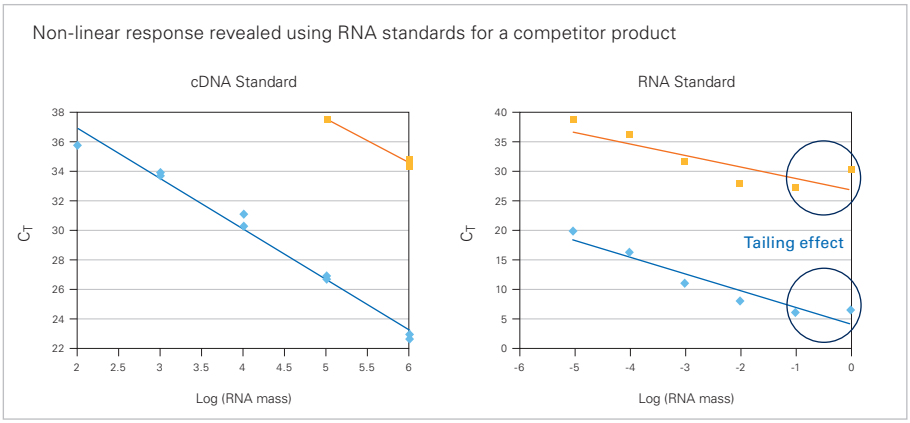

Figure 2. Comparison of cDNA standards to RNA standards using a competitor's reverse transcription kit. The blue line is the normalizing gene and the red line is the gene target. Note: RNA standards show reduced efficiency at higher RNA concentrations. Data provided by an Applied Biosystems customer.

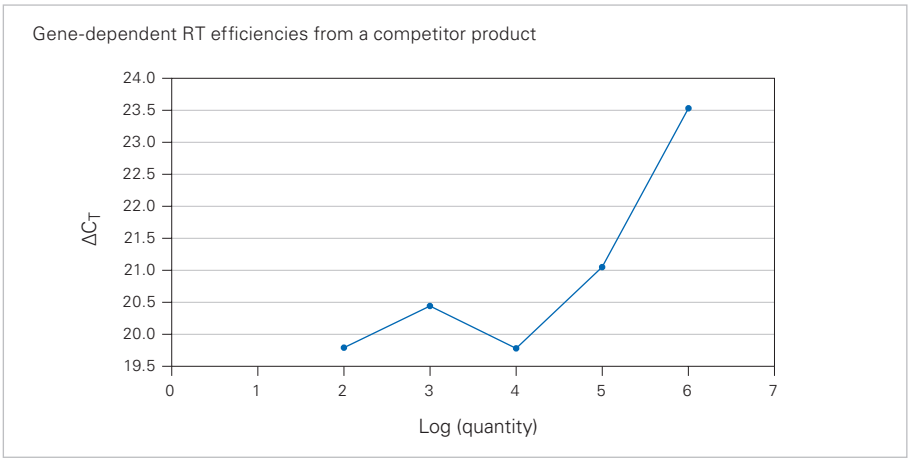

Figure 3. The RNA standards from Figure 2 were normalized to each other to determine whether the changes in reverse transcription efficiency between the gene target and the normalizing gene at high RNA concentrations could cancel out. The rise in ΔC_T values indicates that the reverse transcription efficiency changes are gene dependent. Data provided by an Applied Biosystems customer.

Linearity—Maintain Reverse Transcription Efficiency with Increasing RNA Mass

Linearity is defined as the degree that the reverse transcription chemistry maintains a constant efficiency for all genes. All the assays in this study were assessed to have 100% geometric efficiency and therefore the standard slopes should have been equal to or approaching -3.3. RNA standards with slopes significantly less than |-3.3| (Figure 5) indicate a continuing decrease in reverse transcription efficiency with increasing RNA mass. The High Capacity cDNA Reverse Transcription Kit uses random priming, which significantly benefits linearity.

The shallow, non-parallel RNA standard slopes (Figure 6) using Competitor A's reverse transcription chemistry indicate that the chemistry is unable to reverse transcribe the RNA quantitatively, resulting in inaccurate data. This is an example of poor linearity. With poor linearity, reverse transcription efficiencies will be sample dependent, resulting in inaccurate data.

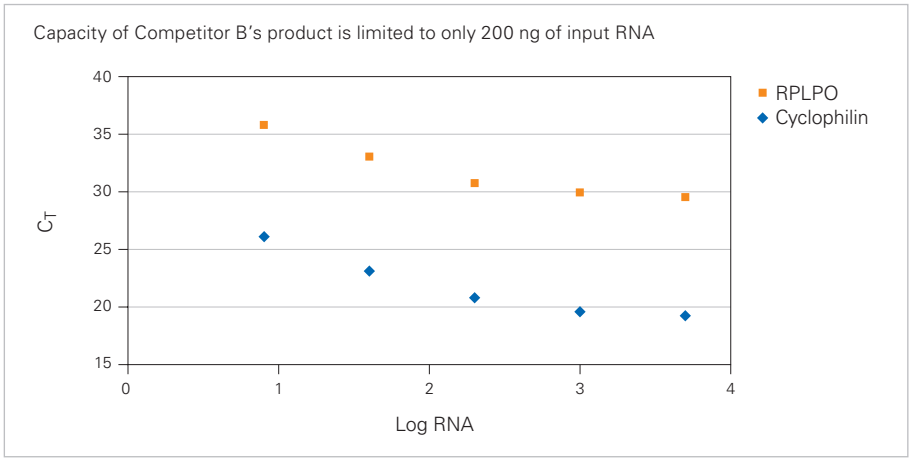

Figure 4. RNA standard curve using a 5′ nuclease assay. Slope RPLPO = -3.6, slope cyclophilin = -3.76, reverse transcription reaction volume = 100 µl. The reverse transcription chemistry was measured to be 200 ng. Data provided by an Applied Biosystems customer.

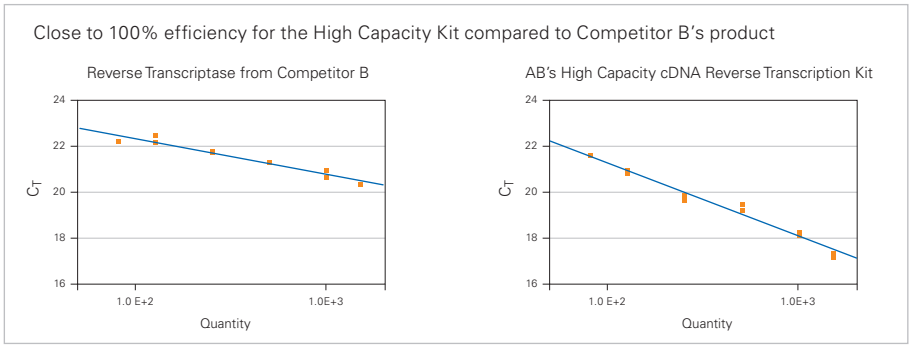

Figure 5. RNA standard curves comparing a reverse transcriptase from Competitor B (slope = -1.8) vs. the High Capacity Kit (slope = -3.2). Gene = GAPDH. Data provided by an Applied Biosystems customer.

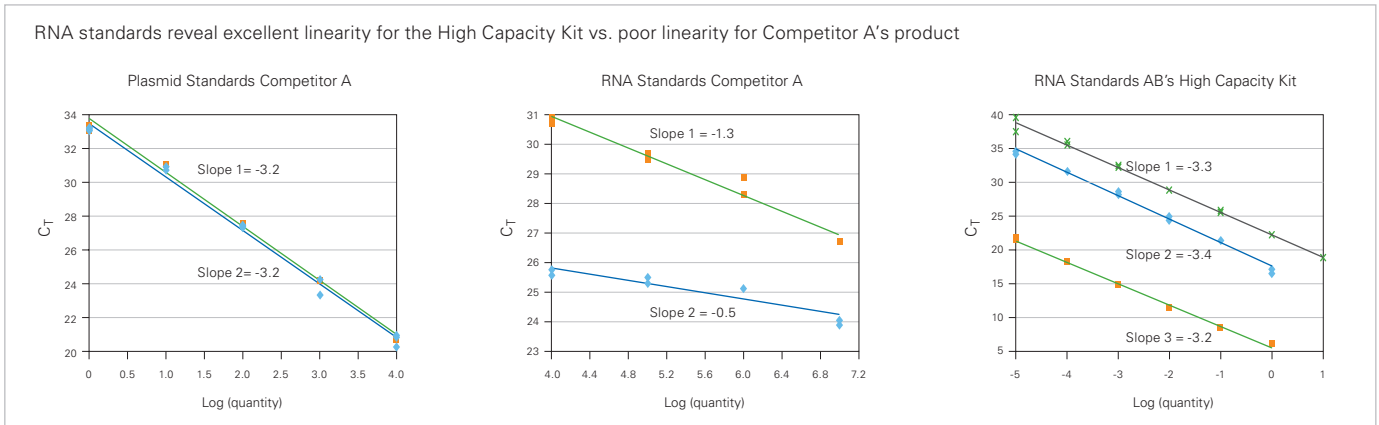

Figure 6. Comparison of plasmid standards and RNA standards using Competitor A's reverse transcription chemistry and the High Capacity Kit. The real-time PCR chemistry was the 5′ nuclease assay amplified on an Applied Biosystems 7300 Real-Time PCR System. Samples were bacterial RNA. Data provided by an Applied Biosystems customer.

High Reverse Transcription Efficiency—Minimizes RNA Template Input

High reverse transcription efficiency is desirable to minimize RNA usage and allow measurements of low expressed transcripts. In Figure 7, a customer compared the reverse transcription efficiencies of a competitor RT kit and the High Capacity cDNA Reverse Transcription Kit. The High Capacity Kit had a 32-fold higher efficiency for the target gene than Competitor B's reverse transcription chemistry.

The Applied Biosystems' High Capacity cDNA Reverse Transcription Kit offers superior capacity, efficiency, and linearity and has the performance necessary for accurate quantitation of RNA targets.

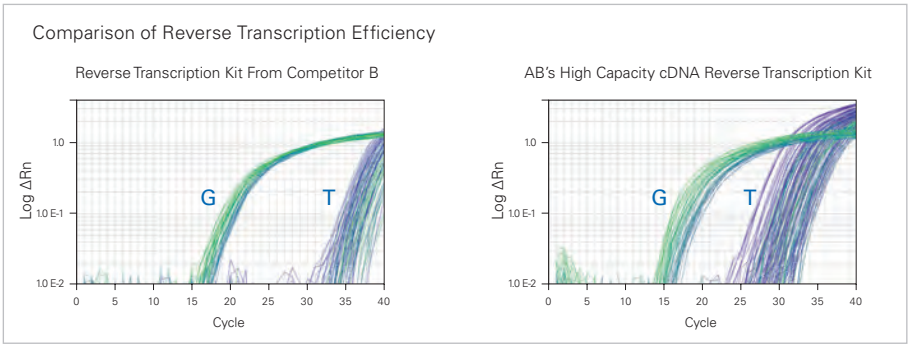

Figure 7. Comparison of Competitor B's reverse transcription chemistry vs. the High Capacity Kit. The High Capacity Kit produced an efficiency which was 32-fold higher for the gene target (T) and approximately 4-fold higher for GAPDH (G). Data provided by an Applied Biosystems customer.

ORDERING INFORMATION

Product Description	Quantity	Part Number
High Capacity cDNA Reverse Transcription Kit	200 Reactions	4368814
	1,000 Reactions	4368813
High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	200 Reactions	4374966
	1,000 Reactions	4374967
High Capacity cDNA Reverse Transcription Kit Protocol	1 Protocol	4375575

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