

# Vendor Comparison: *in vitro* Transcription Systems



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The RNA polymerase from bacteriophage T7 is a well-characterized enzyme that is frequently used for *in vitro* transcription. Several vendors provide *in vitro* transcription systems, which are commonly of two types: for radioactive labeling or small-scale production of RNA, and for large-scale production of RNA. In this article, we compare Promega's T7 RNA Polymerase *in vitro* transcription systems to those from two other leading vendors.

## Introduction

T7 RNA polymerase is a DNA-dependent RNA polymerase that exhibits extremely high specificity for the T7 promoter sequence. *In vitro* transcription using T7 RNA polymerase takes advantage of this property to produce specific RNA transcripts from a DNA template which contains a T7 RNA polymerase promoter upstream of the region to be transcribed. This technique allows molecular biologists to generate well-defined transcripts for many different applications (1). To ensure the success of these applications it is often critical that the transcribed RNA meet certain requirements, such as high percent incorporation and high yield, or that the RNA is full-length and intact. In addition, it is important that the transcription systems provide consistent and reliable results.

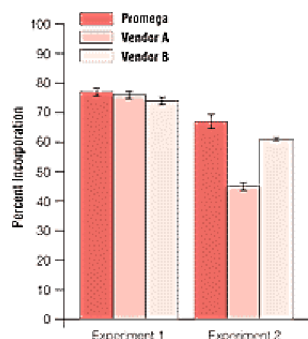
Promega provides two types of systems for *in vitro* transcription. The Riboprobe<sup>®</sup> Systems are designed for generating radioactively-labeled transcripts and small-scale production of unlabeled transcripts, while the RiboMAX<sup>™</sup> Systems are ideal for large-scale production of RNA transcripts. To investigate the quality of Promega's T7 RNA Polymerase *in vitro* transcription systems, we compared them with analogous systems provided by two other leading vendors. For the radioactive labeling systems, we based our analysis on percent incorporation, RNA integrity and T7 RNA polymerase activity. For the large-scale RNA production systems, we compared RNA yield and integrity.

## Systems for radioactive labeling

When producing radioactively labeled RNA by *in vitro* transcription, it is important to have a high percent incorporation of the radioactive nucleotide to ensure a high specific activity probe. It is also important that the RNA is the correct size and intact and that the *in vitro* transcription system performs consistently. We tested small-scale, radioactive labeling systems from Promega and two other vendors for percent incorporation. We also analyzed the transcripts by gel electrophoresis to confirm that they were intact and the correct size. In addition, we measured the T7 RNA polymerase activity before the first transcription experiment and after the second transcription experiment to determine whether the polymerase in the enzyme mix was stable.

The *in vitro* transcription reactions were performed following the directions provided by each vendor, using the same DNA template -- the pGEM<sup>®</sup>\* Express Positive Control Template DNA that is provided with Promega's Riboprobe<sup>®</sup> Systems. Transcription reactions were incubated at 37°C for 1 hour and percent incorporation was measured by TCA precipitation (2). The results of the percent incorporation assays for two experiments are provided in [Figure 1](#). For the first experiment, we obtained approximately 75% incorporation for systems from all three vendors, while in the second experiment, the percent incorporation was lower. For Promega's and Vendor B's systems the decrease was minor; however, for Vendor A's system we observed a decrease of 40%, indicating that this system may not give reproducible results.

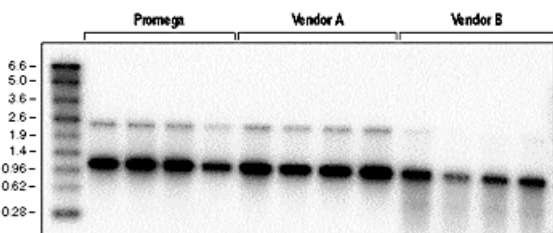
\*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.



**Figure 1. Percent incorporation of radioactively labeled transcripts from T7 *in vitro* transcription systems.** Transcription reactions were performed as recommended by each vendor, and percent incorporation was calculated

following TCA precipitation (2). For each experiment, data represent the average and standard deviation of five replicate transcriptions per vendor.

The transcripts from the first experiment described above were analyzed on a native 1% TAE agarose gel followed by autoradiography (Figure 2). Since the samples were denatured before loading on the gel, the transcript sizes could be compared. We observed that transcripts generated with Promega's and Vendor A's systems were the correct size and intact. In contrast, transcripts generated with Vendor B's system were the correct size, but all transcripts were not full-length, as indicated by the smearing in the lanes. The incomplete transcripts may be due to either RNase degradation (unlike the other two systems, this system did not contain an RNase inhibitor) or premature termination of transcription.



**Figure 2. Gel analysis of radioactively labeled transcripts from T7 *in vitro* transcription systems.** Transcription reactions were performed as recommended by each vendor. Equivalent volumes (1µl of a 1:100 dilution) of each transcription reaction were mixed with 20µl of RNA sample buffer and 2µl of RNA loading buffer (4), incubated at 65°C for 10 minutes, and 10µl aliquots were analyzed by electrophoresis on a native 1% TAE agarose gel. The gel was fixed in 7.5% TCA for 30 minutes, compressed (3) and exposed to X-ray film. Transcript sizes are 2,346 and 1,065 bases.

We measured the unit activity of the T7 RNA polymerase in each vendor's enzyme mix, using Promega's activity assay (see preceding article in this issue). The results of the activity assays are provided in Table 1. The enzyme mixes from Promega and Vendor B had similar T7 RNA polymerase activity, but the measured activity of the mix from Vendor A was considerably lower. The same volume of enzyme mix was added to the transcription reaction for all three vendors; thus, with Vendor A's system, less T7 RNA polymerase is added to each reaction. The activity measurements before and after the transcription reactions were very similar. Based on these observations, it seems likely that the dramatic decrease in percent incorporation for Vendor A's system observed earlier was not due to a decrease in activity or stability of the T7 RNA polymerase in the enzyme mix.

Table 1. Activity of T7 RNA Polymerase in Enzyme Mixes.

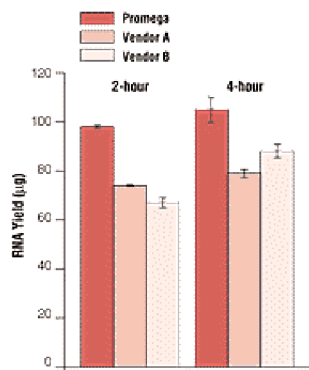
	Measured Activity (Units/µl)*		
	Promega	Vendor A	Vendor B
Before first experiment	15 ± 0.4	5.7 ± 0.1	13 ± 0.9
After second experiment	14 ± 0.5	5.2 ± 0.4	13 ± 0.9

\*Results represent the average and standard deviation of six assays.

## Large-scale *in vitro* transcription systems

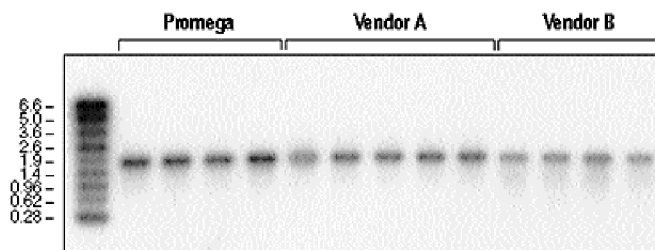
Large-scale *in vitro* transcription reactions are generally used to produce RNA for applications that require high yields and full-length, intact transcripts. In the next set of experiments, we tested large-scale *in vitro* transcription systems from Promega and two other vendors for these qualities. The *in vitro* transcription reactions were performed according to the directions provided by each vendor, using the same DNA template (Promega's T7 Linear Control DNA that is provided with the RiboMAX™ Systems). We added 5µCi of [alpha-<sup>32</sup>P]CTP to each reaction to monitor RNA yield. Reactions were incubated for 2 and 4 hours at 37°C. The RNA yield was determined by DE81 filter binding (3) and transcripts were analyzed on a native 1% agarose gel followed by autoradiography.

We initially performed two sets of reactions to compare Promega's RiboMAX™ System with Vendor A's large-scale *in vitro* transcription system; we subsequently performed two additional experiments to compare the RiboMAX™ System with Vendor B's system. The data from these experiments (not shown) were consistent with the results from our final comparison. In this final experiment, systems from all three vendors were compared directly and all systems had been subjected to multiple freeze/thaw cycles. The results (Figure 3) showed that Promega's system produced higher RNA yields than the systems from Vendors A and B. We observed no substantial difference between the 2-hour and 4-hour reactions, indicating that an extended reaction time does not produce correspondingly higher yields for all the systems tested. The data also demonstrate that all systems were stable to multiple freeze/thaw cycles, which is an important determinant of experimental reproducibility in everyday laboratory use.



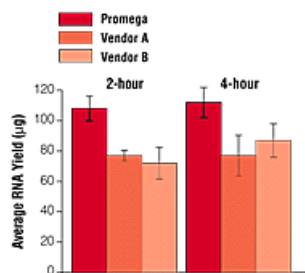
**Figure 3. RNA yield from large-scale T7 *in vitro* transcription systems.** Transcription reactions were performed as recommended by each vendor, after the system had been subjected to multiple freeze/thaw cycles (5 times for Promega's system; 3 times for other vendors' systems). RNA yields were determined by DE81 filter-binding assays (3). Data for each incubation (2- and 4-hour) represent the average and standard deviation of a single experiment in which five replicate transcription reactions were performed for each vendor's system.

Next, we compared the integrity of the transcripts obtained from the experiment described in Figure 3. Aliquots from the 2-hour transcription reactions were denatured and analyzed on a native agarose gel (Figure 4); similar results were obtained for the 4-hour transcription reactions (data not shown). Since equal volumes of the transcription reactions were loaded on the gel, the differences in RNA yield are also evident in this experiment. We observed that transcripts from all systems were the correct size. The transcripts generated by Promega's RiboMAX™ System and by Vendor A's system were intact. However, when we compared the intensity of the full-length transcript with the intensity of the smear of incomplete transcripts below the full-length transcripts (Figure 4), we observed that there were significantly more incomplete transcripts for Vendor B's system. Since all three vendors provide RNase inhibitors in their systems, it is likely that the incomplete transcripts produced by Vendor B's system are caused by either premature termination of transcription or contamination by an RNase that is resistant to the RNase inhibitor provided in Vendor B's system.



**Figure 4. Gel analysis of transcripts from large-scale T7 *in vitro* transcription systems.** Transcription reactions were performed as recommended by each vendor. Equivalent volumes (1µl of a 1:10 dilution) of each transcription reaction were mixed with 20µl of RNA sample buffer and 2µl of RNA loading buffer (4), incubated at 65°C for 10 minutes and 10µl aliquots were analyzed by electrophoresis on a native 1% TAE agarose gel. The gel was fixed in 7.5% TCA for 30 minutes, compressed (3) and exposed to X-ray film. The transcript size is 1,800 bases.

To obtain a summary of the data from all experiments performed with the large-scale transcription systems, we calculated the average RNA yield obtained from each vendor's system (Figure 5). We found that the yields obtained from Promega's T7 RiboMAX™ System were significantly higher than those from other vendors' systems. The average yield from Promega's system was 44% higher than that for Vendor A and 36% higher than that for Vendor B.



**Figure 5. Average RNA yield from large-scale *in vitro* transcription systems.** Transcription reactions were performed as recommended by each vendor and yields were determined as described in Figure 3. Data for each incubation (2- and 4-hour) represent the average and standard deviation of five separate experiments per vendor.

## Summary

Our comparison of Promega's *in vitro* transcription systems to those from other vendors demonstrates the quality and consistency of Promega's T7 RNA Polymerase Riboprobe<sup>®</sup> and RiboMAX<sup>™</sup> Systems. The Riboprobe<sup>®</sup> System provides consistent yields, high percent incorporation and full length, intact transcripts. This ensures that RNA probes generated with the Riboprobe<sup>®</sup> Systems can be used in detection applications that require high sensitivity and specificity. The RiboMAX<sup>™</sup> System provides high yield and full-length, accurately transcribed RNA, ensuring consistent results in downstream applications.

## References

1. Schenborn, E.T. (1995) *Methods in Molecular Biology*, **37**, 1.
2. *Riboprobe<sup>®</sup> in vitro Transcription Systems Technical Manual #TM016*, Promega Corporation.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Laboratory, Cold Spring Harbor, NY.
4. *Protocols and Applications Guide, Third Edition* (1996) Promega Corporation.

## Ordering Information

Product	Cat.#
Riboprobe <sup>®</sup> System - SP6	P1420
Riboprobe <sup>®</sup> System - T3	P1430
Riboprobe <sup>®</sup> System - T7	P1440
Riboprobe <sup>®</sup> System - T3/T7	P1450
Riboprobe <sup>®</sup> System - SP6/T7	P1460
RiboMAX <sup>™</sup> Large Scale RNA Production System, SP6	P1280
RiboMAX <sup>™</sup> Large Scale RNA Production System, T3	P1290
RiboMAX <sup>™</sup> Large Scale RNA Production System, T7	P1300

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