Express More Functional Protein: TnT® Quick Coupled Transcription/Translation Systems

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Abstract

In this study the TnT® T7 and SP6 Quick Coupled Transcription/Translation Systems were compared with two other commercially available linked two-step transcription/translation systems. The TnT® Quick Systems synthesized more functional protein for each of two constructs examined.

The TnT® Quick Systems are robust systems for the expression of functional protein in vitro in a simple, one-step procedure.

Introduction

The TnT® T7 and SP6 Quick Coupled Systems[a–e] (Cat.# L1170, L2080) provide convenient single-tube coupled transcription/translation for eukaryotic protein expression. To use these systems, 0.2–2.0µg of circular plasmid DNA containing a T7 or SP6 DNA polymerase promoter is added to an aliquot of the TnT® Quick Master Mix and incubated in a 50µl reaction for 60–90 minutes at 30°C. The synthesized proteins can then be analyzed using a number of methods, including gel analysis or activity determination.

In addition to verifying the expected molecular weight of a gene construct, the TnT® Quick Systems are ideal for applications such as the protein truncation test (PTT; 1), in vitro expression cloning (IVEC), mutation and deletion analysis, protein-protein interactions, nucleic acid binding studies, post-translation modification analysis, and ligand binding assays (2). For many of these analyses, expression of functional protein is critical. In this study, the relative amount of functional protein synthesized using the TnT® Quick Coupled Transcription/Translation Systems was evaluated using firefly luciferase. The performance of the TnT® Quick Systems was compared with that of two other commercially available eukaryotic linked two-step transcription/translation systems.

Protein Expression and Activity Detection

The 61kDa firefly luciferase protein was expressed in three different eukaryotic transcription/translation systems, and the activity of the expressed protein was quantitated using the Bright-Glo™ Luciferase Assay System[a,f] (Cat.# E2610). The results shown in Figure 1 demonstrate that the TnT® Quick Systems synthesized more luciferase activity than the two other systems. This was seen for both SP6- and T7-driven templates. Similar results were obtained for expression of *Renilla* luciferase (data not shown).

To determine the amount of luciferase protein expressed in each system, an aliquot of each translation reaction was subjected to Western analysis with Anti-Luciferase pAb (Cat.# G7451). The results show that the TnT® Quick Systems synthesized more luciferase protein than the linked two-step systems (Figure 2). Duplicate blots incubated with secondary antibody alone showed no bands at the expected position of luciferase.

A comparison of the activity and protein amount results is presented in Figure 3. The relative luciferase activity and protein levels achieved using the TnT® Quick Systems were arbitrarily set to 100%. This figure illustrates that the difference in luciferase activity between the three systems is much greater than the difference in actual protein amount. Thus the TnT® Quick Systems produced more functional protein compared to the two-step linked systems.

Conclusions

In a direct comparison of the amount of functional luciferase produced, the TnT® Quick Systems outperformed two other linked transcription/translation systems tested. The level of performance achieved by the TnT® Quick Systems can make a critical difference, particularly for low-expressing proteins for which maximal functionality is desired.

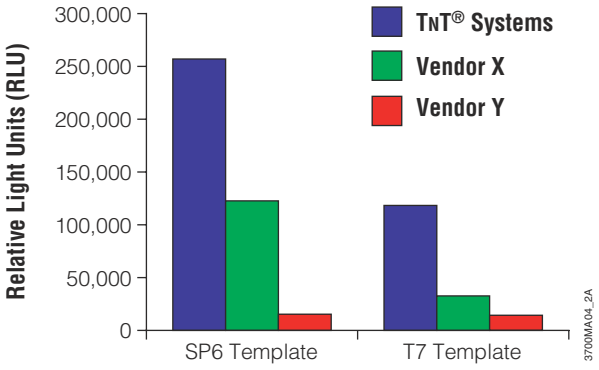

Figure 1. Firefly luciferase activity. Luciferase activity was detected from an SP6-luciferase DNA template (Luciferase SP6 Control DNA[d] [Cat.# L4741]) and a T7-luciferase DNA template (Luciferase T7 Control DNA[d] [Cat.# L4821]) expressed in the TnT® SP6 or T7 Coupled Transcription/Translation Systems and linked SP6/T7 transcription/translation systems from Vendors X and Y. Luciferase activity was measured using the Bright-Glo™ Luciferase Assay System (Cat.# E2610) as described in Technical Manual #TM052. An aliquot of each translation reaction was diluted 1:500 in PBS, and 5µl of the diluted reaction was assayed for luciferase activity. Luminescence was measured in a Berthold® EG&G plate-reading luminometer with a 1-second delay time and 3-second read time. Assays were performed in duplicate, and the averages are presented.

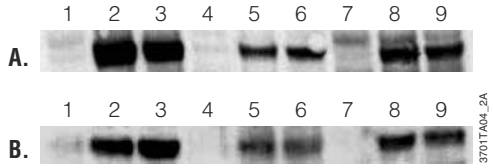

Figure 2. Western blot analysis of luciferase protein. Three microliters of duplicate translation reactions performed using either the TNT® Quick Systems (lanes 1–3), or two-step linked transcription/translation systems from Vendor Y (lanes 4–6), or Vendor X (lanes 7–9) were separated on a 4–12% Novex NuPAGE® Tris-Bis polyacrylamide gel (Invitrogen) and transferred to Hybond®-C-supported nitrocellulose (Amersham). The blots were blocked in 1X TBST containing 1% blot-qualified BSA (Cat.# W3841), then developed with 1:1,000 Anti-Luciferase pAb (Cat.# G7451), 1:10,000 Donkey Anti-Goat IgG HRP Conjugate (Cat.# V8051), and developed using the Transcend™ Chemiluminescent Non-Radioactive Translation Detection System (Cat.# L5080) followed by exposure to Kodak X-OMAT® film for 1–5 seconds. Band intensity was quantitated using an Alpha Innotech FluorChem® 800 scanner. After background subtraction, relative amounts of luciferase expressed were calculated. **Panel A:** SP6-driven reactions. **Panel B:** T7-driven reactions. Lanes 1, 4 and 7 contain no-DNA negative control reactions.

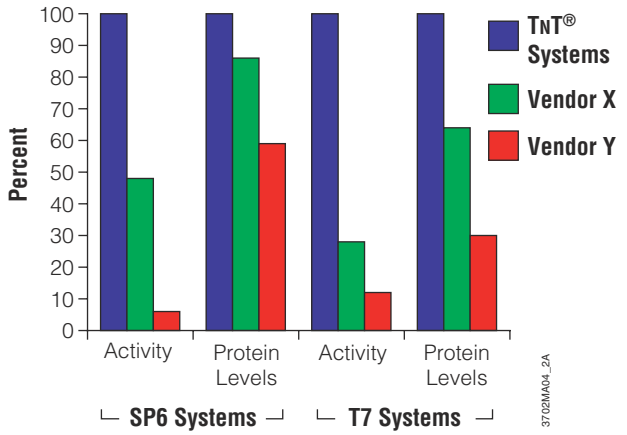

Figure 3. Relative luciferase activity and protein levels achieved using the TNT® Quick Systems. Relative luciferase activity and protein levels for the two-step transcription/translation systems from Vendors X and Y were compared to those achieved with the TNT® Quick Systems. After calculation of luciferase activity and protein quantitation as described in the legends to Figures 1 and 2, the relative luciferase activity and protein levels were set to 100% for the TNT® T7 and SP6 Quick Systems. Results for the two-step transcription/translation systems are depicted as a percentage of the activity and yield obtained with the TNT® Quick Systems.

Methods

In vitro protein expression: TNT® Quick Coupled Transcription/Translation reactions were assembled as described in Technical Manual #TM045. Other linked transcription/translation reactions were performed as described in the manufacturer's instructions. All translation reactions were performed in duplicate and incubated for 60 minutes at 30°C.

References

1. References for the Protein Truncation Test #BL002 (1998) Promega Corporation.
2. Bibliography of References Using the TNT® Coupled Transcription/Translation System #BL001 and #BL003 (1998) Promega Corporation.

Protocols

- *TNT® Quick Coupled Transcription/Translation System Technical Manual* #TM045, Promega Corporation. www.promega.com/tbs/tm045/tm045.html
- *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052, Promega Corporation. www.promega.com/tbs/tm052/tm052.html
- *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182, Promega Corporation. www.promega.com/tbs/tb182/tb182.html

Ordering Information

Product	Size	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System*	40 reactions	L1170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size*	5 reactions	L1171
TNT® SP6 Quick Coupled Transcription/Translation System*	40 reactions	L2080
TNT® SP6 Quick Coupled Transcription/Translation System, Trial Size*	5 reactions	L2081
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System*	30 reactions	L5080
Anti-Luciferase pAb	200µg	G7451

*For Laboratory Use.

[a] U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

[b] U.S. Pat. Nos. 5,324,637, 5,492,817, 5,665,563, Australian Pat. No. 660329 and other patents.

[c] U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

[d] The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

[e] U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

[f] Certain applications of this product may require licenses from others.

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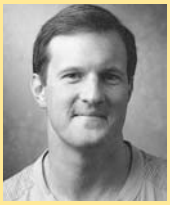

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