



TNT® T7 Quick Coupled Transcription/Translation System— Quick Screening of Protein Expression Using Common T7-Promoter-Containing Vectors

ABSTRACT Detectable amounts of protein can be expressed from many commonly used T7-promoter-containing protein expression vectors using the TNT® T7 Quick Coupled Transcription/Translation System and your choice of non-radioactive labels. In this article, we summarize data generated using sixteen different protein expression vectors to express seven different target proteins, highlighting the ability of the TNT® T7 Quick Coupled Transcription/Translation System to screen protein expression vectors quickly and easily.

Kate Qin Zhao, Promega Corporation

Save time and effort:
Use the convenient TNT® Quick Master Mix to assemble *in vitro* transcription/translation reactions when screening protein-coding regions.

QUICKLY AND EASILY SCREEN VECTORS TO CONFIRM A GENE PRODUCT

The TNT® Quick Coupled Transcription/Translation Systems are convenient single-tube, coupled transcription/translation systems for eukaryotic cell-free protein expression. The TNT® Quick Coupled Transcription/Translation Systems combine RNA polymerase, nucleotides, salts, amino acids and Recombinant RNasin® Ribonuclease Inhibitor with rabbit reticulocyte lysate to form the TNT® Quick Master Mix.

Screening vectors to confirm gene expression can be achieved easily and quickly using these systems, especially when combined with the FluoroTect™ Green_{lys} *in vitro* Translation Labeling System (Cat.# L5001), which allows fluorescent labeling and detection of proteins synthesized *in vitro*, eliminating the need for radioactive labeling.

At a minimum, a vector must contain a T7 promoter preceding the gene of interest for protein synthesis using the TNT® T7 Quick Coupled Transcription/Translation System^(a,b). Many widely used T7-promoter-containing vectors can produce detectable amounts of protein using this system (Figure 1), allowing you to quickly screen a protein-coding region in an existing T7-promoter-containing vector without recloning. Table 1 summarizes the vectors and protein targets that we tested in this study.

CHOOSE FROM A VARIETY OF T7-PROMOTER-CONTAINING VECTORS

In addition to the vectors tested in this study, we also have confirmed protein expression from other T7-promoter-containing vectors. It is possible to detect protein expression in the TNT® T7 Quick Coupled Transcription/Translation System with the following vectors:

1. Commonly used *E. coli* expression vectors, such as Flexi® Vectors [pF1A/K T7 Flexi® Vector, pFN6A/K (HQ) Flexi® Vector and pFN18A/K (HaloTag® 7) T7 Flexi® Vector] and pET vectors (pET3a, pET15b, pET32a and pET43.1a)
2. Vectors for S30 cell-free protein synthesis, such as pIVEX and pIX3.0
3. Vectors for wheat germ extract cell-free protein synthesis, such as pF3A/K WG (BYDV) Flexi® Vector, pFN19A/K (HaloTag® 7) T7 SP6 Flexi® Vector and pFC20A/K (HaloTag® 7) T7 SP6 Flexi® Vector
4. Vectors for insect extract cell-free protein synthesis, such as pIX4.0
5. Mammalian expression vectors, with similar elements such as pF4A CMV Flexi®, pFC14A/K (HaloTag® 7) CMV Flexi®, pFN21A/K (HaloTag® 7) CMV Flexi®, pRL-null, pRL-CMV, pRL-SV40, pRL-TK and pCI-neo Vectors

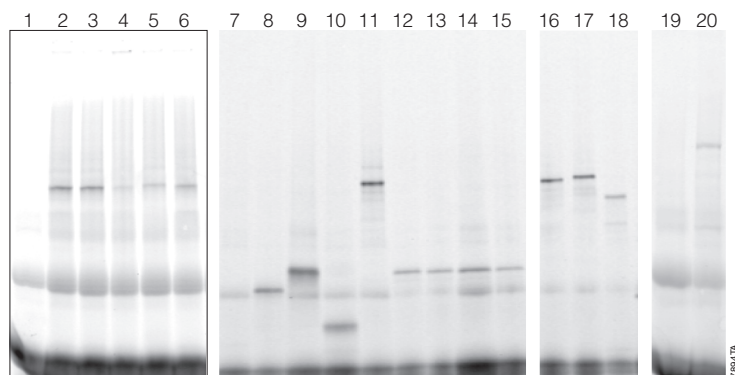


Figure 1. T7-promoter-containing vectors tested using the TNT® T7 Quick Coupled Transcription/Translation System. One microgram of each DNA template was used in a 50 μ l reaction in the presence of FluoroTect™ Green_{lys} tRNA. Several of the DNA templates were used directly from tubes supplied by the vendor; these included the pRL-null (Cat.# E2271), pRL-SV40 (Cat.# E2231), pRL-CMV (Cat.# E2261) and pRL-TK Vectors (Cat.# E2241); Luciferase T7 Control DNA, which expresses firefly luciferase, from the TNT® T7 Quick Coupled Transcription/Translation System; and pET32a and pET43.1a vectors. All other DNAs were prepared using the PureYield™ Plasmid Miniprep System (Cat.# A1221). The equivalent of 1 μ l of each reaction was resolved by SDS-PAGE (4–20% Tris glycine gel), and fluorescence was visualized on a Typhoon® scanner. Lane 1. No-DNA control. Lane 2. pF1A-flLuc. Lane 3. pF4A-flLuc. Lane 4. pIX4.0-luc. Lane 5. pET15b-flLuc. Lane 6. pF3K-HQ-flLuc. Lane 7. No-DNA control. Lane 8. pIVEX-GFP. Lane 9. pIX3.0-EF-Tu. Lane 10. pET32a. Lane 11. pET43.1a. Lane 12. pRL-null. Lane 13. pRL-CMV. Lane 14. pRL-SV40. Lane 15. pRL-TK. Lane 16. pFN19K-flLuc. Lane 17. pFC20K-flLuc. Lane 18. Luciferase T7 Control DNA. Lane 19. No-DNA control. Lane 20. pCI-neo p65-HaloTag® Vector.

Table 1. Elements and Vectors Tested Using the TNT® T7 Quick Coupled Transcription/Translation System.

Expression Vector Name	Vector Backbone	Original Use	Protein Tested	Expected Protein Size (kDa)	Protein Expressed
pFIA-ffLuc	pFIA T7 Flexi® Vector	<i>E. coli</i>	firefly luciferase	61	Yes
pET15b-ffLuc	pET15b	<i>E. coli</i>	His-Tag-firefly luciferase	62	Yes
pET32a	pET32a	<i>E. coli</i>	thioredoxin-HisTag-S-tag-HisTag	21	Yes
pET43.1a	pET43.1a	<i>E. coli</i>	NusA-HisTag-S-Tag	66	Yes
pIVEX-GFP	pIVEX	S30 cell-free systems	green fluorescent protein (GFP)	25	Yes
pIX3.0-EF Ts	pIX3.0	S30 cell-free systems	Elongation factor Ts (EF Ts)	37	Yes
pIX4.0-ffLuc	pIX4.0	Insect cell-free extracts	firefly luciferase	61	Yes
pF3K-HQ-ffLuc	pF3K WG (BYDV) Flexi® Vector	Wheat germ extracts	HQ-Tag-firefly luciferase	62	Yes
pFN19K-ffLuc	pFN19K (HaloTag® 7) T7 SP6 Flexi® Vector	Cell-free extracts	HaloTag® 7-firefly luciferase	96	Yes
pFC20K-ffLuc	pFC20K (HaloTag® 7) T7 SP6 Flexi® Vector	Cell-free extracts	firefly luciferase-HaloTag® 7	96	Yes
pF4A-ffLuc	pF4A CMV Flexi® Vector	Mammalian systems	firefly luciferase	61	Yes
pRL-null	pRL	Mammalian systems	<i>Renilla</i> luciferase	36	Yes
pRL-CMV	pRL	Mammalian systems	<i>Renilla</i> luciferase	36	Yes
pRL-SV40	pRL	Mammalian systems	<i>Renilla</i> luciferase	36	Yes
pRL-TK	pRL	Mammalian systems	<i>Renilla</i> luciferase	36	Yes
pCl-neo p65-HaloTag®	pCl-neo	Mammalian systems	p65-HaloTag® fusion protein	100	Yes

CONCLUSION

The TNT® T7 Quick Coupled Transcription/Translation System is a versatile tool to detect protein expression and validate constructs from many T7-promoter-containing vectors without recloning.

ACKNOWLEDGMENTS

We would like to thank the following Promega scientists for providing the clones or bacterial cultures that made this study possible: Donna Leippe, Pete Stecha, Natasha Karassina, Jami English, Robin Hurst and Denise Garvin.

ORDERING INFORMATION

Product	Size	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	40 reactions	LI170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size	5 reactions	LI171

For Laboratory Use.

©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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

©For Laboratory Use. Any use of the product for diagnostics requiring clearance or approval by the FDA may require a license under Mayo Clinic U.S. Pat. Nos. 6,027,913 and 6,361,949.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

Flexi, HaloTag RNasin and TNT are registered trademarks of Promega Corporation. FluoroTect and PureYield are trademarks of Promega Corporation.

Typhoon is a registered trademark of GE Healthcare Bio-sciences.