

# The TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System



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*The TNT<sup>®</sup>\* T7 Quick Coupled Transcription/Translation System offers researchers a convenient new alternative for eukaryotic in vitro translation -- a one tube, coupled transcription/translation reaction. This new addition to the TNT<sup>®</sup> Coupled Transcription/Translation Systems simplifies the process by including all of the reaction components in a single TNT<sup>®</sup> T7 Quick Master Mix. To use this system, 0.2-2.0µg of either circular or linear DNA is added to an aliquot of the Master Mix and incubated in a 50µl reaction volume for 60-90 minutes at 30°C. Synthesized proteins are then analyzed by SDS-PAGE and autoradiography or by functional activity.*

## Introduction

In 1992, Promega introduced the two original T7 and SP6 TNT<sup>®</sup> Coupled Reticulocyte Lysate Systems. These systems were the first to couple transcription from phage polymerases and promoters with translation in eukaryotic extracts (1,2). To offer a wider range of choices for eukaryotic coupled transcription/translation expression, Promega then introduced the TNT<sup>®</sup> T3 Coupled Reticulocyte Lysate System and the TNT<sup>®</sup> T7, SP6 and T3 Coupled Wheat Germ Extract Systems. All of the coupled transcription/translation systems greatly simplify *in vitro* translation reaction protocols by eliminating the laborious task of synthesizing large amounts of potentially labile mRNA (3,4) and allowing direct addition of DNA to the translation reaction. In addition to circular plasmid DNA, linear DNA templates such as those generated by PCR amplification can also be used in the TNT<sup>®</sup> Systems (5). In addition to saving work and time, the coupled eukaryotic systems almost always produce significantly (2- to 6-fold) more protein than standard mRNA programmed reactions (1,5).

## The TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System

The original TNT<sup>®</sup> Systems continue to be well received. However, we realized that further streamlining and optimization could make the reactions even faster, easier and more convenient. In the TNT<sup>®</sup> T7 Quick System, the coupled transcription/translation process has been further simplified by modifying and combining the separate standard TNT<sup>®</sup> Reticulocyte Lysate System components (T7 RNA Polymerase, Nucleotides, salt and RNasin<sup>®</sup> Ribonuclease Inhibitor) together with the Reticulocyte Lysate solution in a single Master Mix (Figure 1). The components of this Master Mix have been carefully adjusted to maximize both expression and fidelity for most gene constructs. The inclusion of RNasin<sup>®</sup> Ribonuclease Inhibitor directly in the Master Mix protects against potential disaster from the introduction of RNases carried over in the DNA solutions prepared using some miniprep protocols.

*\*U.S. Pat. Nos. 5,324,637 and 5,492,817 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.*

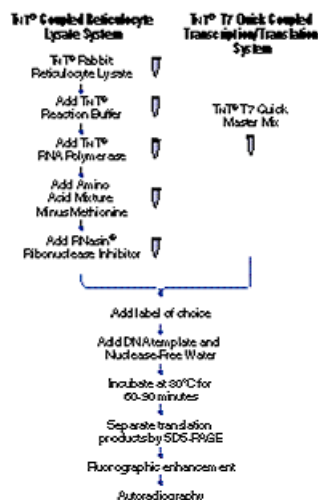


Figure 1. Comparison of the TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System and the TNT<sup>®</sup> Coupled

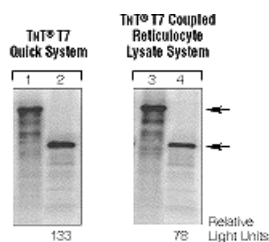
## Reticulocyte Lysate System.

# Applications of the TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System

During the past four years, the TNT<sup>®</sup> Systems have been utilized for a range of applications in addition to simple verification of the expected molecular weight of a particular gene product (6,7). The TNT<sup>®</sup> T7 Quick System is ideal for screening large numbers of genetic constructs for either naturally occurring or deliberately engineered mutations. This type of approach is currently used in a Protein Truncation Test (PTT) to rapidly identify truncation mutations (5). The protein products produced in TNT<sup>®</sup> T7 Quick reactions may also be tested for DNA/RNA binding or protein binding activity using gel shift assays or fusion polypeptides. Please contact Promega or your local Promega Branch Office or distributor and request the *TNT<sup>®</sup> Coupled Transcription/Translation Systems Bibliography* (#BL001) for further information regarding these and other applications of the TNT<sup>®</sup> Coupled Transcription/Translation Systems.

## The TNT<sup>®</sup> T7 Quick System compared to the original TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System

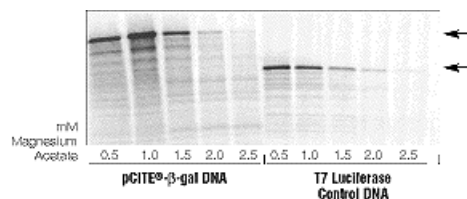
Comparisons of the TNT<sup>®</sup> T7 Quick System with the standard TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System consistently show either identical or higher expression for most genetic constructs in the TNT<sup>®</sup> T7 Quick System (Figure 2). The TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System is designed for optimal expression of most T7 promoter genetic constructs. This includes both prokaryotic and eukaryotic cloned genes. However, as is true for all *in vitro* translation expression systems, gene expression in the TNT<sup>®</sup> T7 Quick System is influenced by a variety of genetic elements. Important DNA sequences influencing expression include: 5' Untranslated Leader Region (UTR) sequence length, secondary structure and sequence (such as the 'Kozak' sequence surrounding the AUG start codon) (8,9), 3' UTR sequence length or sequence (such as polyadenylation sequences) and actual gene sequence (such as codon usage) (10).



**Figure 2. Comparison of expression in the TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System and the TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System.** 0.5µg of either pCITE<sup>®</sup>-beta-gal DNA (Lanes 1 and 3) or T7 Luciferase Control DNA (Lanes 2 and 4) were added to either TNT<sup>®</sup> T7 Quick reactions (Lanes 1 and 2) or standard TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System reactions (Lanes 3 and 4). After the transcription/translation reaction was complete, a 1µl aliquot of each 50µl TNT<sup>®</sup> T7 Quick reaction was separated on a 4-20% polyacrylamide gradient gel. The separated translation products were transferred onto a PVDF membrane by electroblotting for 1 hour at 100V. The membrane was then exposed to a PhosphorImager<sup>™</sup> screen for 16 hours. The positions of the beta-galactosidase and luciferase translation products are indicated by arrows. The level of functional luciferase expression was measured by mixing 2.5µl of the reaction mixture with 50µl of Luciferase Assay Reagent and detecting the light generated on a Turner Designs TD-20/20 Luminometer for 10 seconds after a 2 second delay and with a 256X filter. The expression of luciferase in the TNT<sup>®</sup> T7 Quick reaction was 169% of that in the standard TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System reaction.

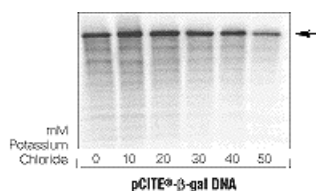
## Optimized expression

Each gene construct will exhibit particular optimal magnesium and potassium salt concentration requirements for maximal expression. Most gene constructs tested in the TNT<sup>®</sup> T7 Quick System exhibit similar optimal magnesium acetate and potassium chloride concentrations. The TNT<sup>®</sup> T7 Quick Master Mix contains optimized salt concentrations for most constructs, these represent the lower level of the effective concentration range for both magnesium and potassium. A few constructs, such as those containing encephalomyocarditis virus 5' UTR sequences, require higher concentrations of both magnesium acetate and, to a lesser extent, potassium chloride for optimal expression (2). Figure 3 shows the result of optimizing magnesium concentration for expression of one such construct (pCITE<sup>®</sup>-beta-gal) in the TNT<sup>®</sup> T7 Quick System. After optimization, expression of this viral construct in the TNT<sup>®</sup> T7 Quick System exceeds that obtained in the standard TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System.



**Figure 3. Optimization of expression of constructs containing translation enhancing sequences by adjustments to magnesium concentration.** 0.5µg of either pCITE<sup>®</sup>-beta-gal DNA or T7 Luciferase Control DNA were added to TNT<sup>®</sup> T7 Quick reactions containing varying amounts of added magnesium acetate as shown. After the transcription/translation reaction was complete, a 1µl aliquot of each 50µl TNT<sup>®</sup> T7 Quick reaction was processed as described in the legend to Figure 2. The positions of the beta-galactosidase and luciferase translation products are indicated by arrows. Maximal expression from the pCITE<sup>®</sup>-beta-gal DNA was obtained using an added magnesium acetate concentration of 1mM. No optimization is required for the T7 Luciferase Control DNA supplied with the TNT<sup>®</sup> T7 Quick System.

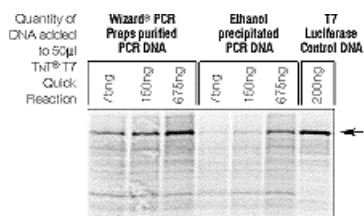
The addition of potassium chloride (not potassium acetate) at levels 10-20mM above those required for maximal expression of some constructs has been found to decrease the appearance of secondary low molecular weight polypeptide products in coupled transcription/translation reticulocyte lysate reactions (2). We have detected a similar slight improvement in fidelity with the addition of potassium chloride for certain gene constructs containing viral 5'-UTR sequences (e.g., pCITE<sup>®</sup>-beta-gal) in the TNT<sup>®</sup> T7 Quick System (Figure 4).



**Figure 4. Optimizing translation fidelity by alterations in Potassium Chloride concentration.** 0.5µg of pCITE<sup>®</sup>-beta-gal DNA was added to TNT<sup>®</sup> T7 Quick reactions containing varying amounts of added potassium chloride as shown. After the transcription/translation reaction was complete, a 1µl aliquot of each 50µl TNT<sup>®</sup> T7 Quick reaction was processed as described in the legend to Figure 3. The position of the beta-galactosidase translation product is indicated by an arrow. Maximal expression and fidelity of translation from the pCITE<sup>®</sup>-beta-gal DNA was obtained using an added potassium chloride concentration of 20-30mM.

## PCR-generated DNA templates

The power of the TNT<sup>®</sup> coupled eukaryotic systems is centered around using DNA rather than RNA templates. As in the original TNT<sup>®</sup> Systems, plasmid DNA or linear DNA can be used to program the TNT<sup>®</sup> T7 Quick System. Plasmid DNA can be purified either using the Wizard<sup>®</sup> Plus Minipreps DNA Purification System (Cat.# A7100) or by standard alkaline lysis (11). PCR-generated DNA can either be used directly or after purification using the Wizard<sup>®</sup> PCR Preps DNA Purification System (Cat.# A7170) or a standard ethanol precipitation and wash (Figure 5). Higher yields of protein are achieved when PCR-generated template DNA is purified using the Wizard<sup>®</sup> PCR Preps DNA Purification System rather than by ethanol precipitation. When designing an amplification strategy it is recommended that the resulting PCR-generated DNA template contain at least 20bp upstream of the T7 RNA Polymerase promoter for efficient promoter binding. The TNT<sup>®</sup> T7 Quick System can be utilized to express gene segments as well as full length gene products. This can be useful for analyzing the gene segment product for specific activity (i.e., binding) or for mapping an epitope of a monoclonal antibody (5). It is also important to add a stop codon (usually UAA) to a designed truncated gene segment to prevent ribosomes from stalling at the ends of mRNA generated from linear DNA (5).



**Figure 5. Gel analysis of TNT<sup>®</sup> T7 Quick reactions using PCR-generated DNA or linearized plasmid DNA.** Equimolar amounts of T7 Luciferase Control plasmid DNA and luciferase encoding PCR DNA purified using either the

Wizard<sup>®</sup> PCR Preps DNA Purification System or by standard ethanol precipitation were added to the TNT<sup>®</sup> T7 Quick Master Mix in standard 50µl reactions. After the transcription/translation reaction was complete, a 1µl aliquot of each 50µl TNT<sup>®</sup> T7 Quick reaction was processed as described in the legend to Figure 3, except that the PVDF membrane was exposed to a PhosphorImager<sup>™</sup> for 2 hours. The position of the luciferase translation product is indicated by an arrow. PCR-generated DNA fragments gave best results when cleaned up using the Wizard<sup>®</sup> PCR Preps DNA Purification System. Increasing the amount of PCR-generated DNA added to each reaction increased the amount of luciferase protein produced. Increasing the amount of T7 Luciferase Control plasmid DNA above 200ng did not result in any further increase in expression.

## Summary

The TNT<sup>®</sup> T7 Quick Coupled Transcription/Translation System is optimized for expression of most standard T7 promoter driven gene constructs. Compared to the standard TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System, the TNT<sup>®</sup> T7 Quick System is faster, easier and more convenient. The TNT<sup>®</sup> T7 Quick System often produces increased levels of protein synthesis compared with the original TNT<sup>®</sup> T7 Coupled Reticulocyte Lysate System, and the one tube Master Mix formulation ensures no left over unused reagents. The inclusion of the RNasin<sup>®</sup> Ribonuclease Inhibitor in the Master Mix maximizes successful expression from DNA templates purified using a variety of different protocols, some of which can be contaminated with carry-over RNase

A. In addition, the TNT<sup>®</sup> T7 Quick System allows greater flexibility for additional optimization of expression for some genetic constructs by the addition of magnesium acetate or potassium chloride. The TNT<sup>®</sup> T7 Quick System is available in one configuration (minus methionine) and is supplied with a firefly luciferase Control DNA and Luciferase Assay Reagent to allow fast, convenient, non-isotopic verification of proper system performance.

## References

1. Beckler, G.S. (1992) *Promega Notes* **35**, 1.
2. Craig, D. *et al.* (1992) *Nucl. Acids Res.* **20**, 4987.
3. Pelham, H.R.B. and Jackson, R.J. (1976) *Eur. J. Biochem.* **67**, 247.
4. Krieg, P. and Melton, D. (1984) *Nucl. Acids Res.* **12**, 7057.
5. *Bibliography of References using the TNT<sup>®</sup> Coupled Transcription/Translation Systems*, #BL001, Promega Corporation.
6. Beckler, G.S. (1994) *Promega Notes* **47**, 20.
7. Beckler, G.S. (1995) *Promega Notes* **53**, 30.
8. Francis, V., Morle, F. and Godet, J. (1992) *Biochim. Biophys. Acta* **1130**, 29.
9. Kozak, M. (1989) *Mol. Cell. Biol.* **9**, 5073.
10. Jackson, R.J and Standart, N. (1990) *Cell* **62**, 15.
11. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

## Ordering Information

Product	Cat.#
TNT <sup>®</sup> T7 Quick Coupled Transcription/Translation System	L1170
TNT <sup>®</sup> T7 Quick Coupled Transcription/Translation System, Trial Size	L1171

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