Cell-Free Protein Expression with the TNT® T7 Insect Cell Extract Protein Expression System

ABSTRACT The TNT® T7 Insect Cell Extract Protein Expression System is a new addition to the Promega line of products for cell-free expression of functional proteins. It is manufactured starting with extract from cultured insect cells. Specific Flexi® Vectors have been designed for, and are recommended for use with, this system to obtain optimal protein expression.

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Quick and easy: To begin protein synthesis with the TNT® T7 Insect Cell Extract Protein Expression System all you need to do is add your DNA template and water.

INTRODUCTION

Cell-free expression systems are powerful alternatives to cell-based protein expression for functional and structural proteomics. In comparison, they are especially useful for rapidly synthesizing many proteins in parallel and in high-throughput formats. Promega TNT® Systems (eukaryotic coupled transcription/translation systems) have the advantage of using DNA templates, eliminating the time-consuming processes of separately synthesizing and purifying RNA. These systems can be used to easily and rapidly produce active proteins.

The new TNT® T7 Insect Cell Extract Protein Expression System[a–e] is a convenient, quick, single-tube, coupled transcription/translation system for the cell-free expression of proteins (Figure 1). The extract is made from the commonly used *Spodoptera frugiperda* Sf21 cell line (1). However, the extract contains no endogenous glycosylation machinery. All necessary components are present in the TNT® T7 ICE Master Mix (ICE = insect cell extract). To begin protein synthesis, the only component you must provide is the DNA template. This format simplifies and reduces the time required to set up reactions.

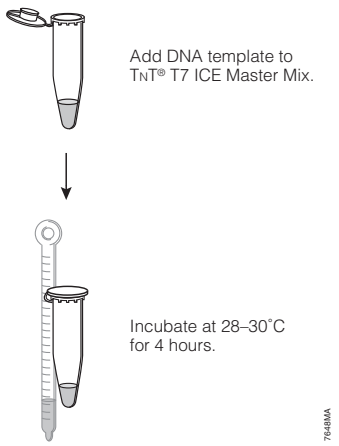

Figure 1. Schematic for the TNT® T7 Insect Cell Extract Protein Expression System.

Protein is expressed from genes cloned downstream of the T7 polymerase promoter. The recommended DNA templates for this system are vectors containing baculovirus polyhedrin gene 5′ and 3′ untranslated region (UTR) sequences to enhance translation efficiency (2). Such vectors have been designed for use with this system: the pF25A and pF25K ICE T7 Flexi® Vectors[a,d,e,f] (Cat.# L1061 and L1081, respectively). These companion vectors are used to achieve optimal protein yields with TNT® T7 Insect Cell Extract Protein Expression System. The vectors also are compatible with the Flexi® Cloning System (Cat.# C8640) for easy cloning and transfer of your sequence of interest to and from additional Flexi® Vectors, which are available for protein expression in other systems.

QUICK AND EASY PROTEIN PRODUCTION WITH DNA TEMPLATES

The TNT® systems allow cell-free protein expression to be initiated from a DNA template. For a standard 50 µl TNT® reaction, 4 µg of DNA template is recommended. The TNT® reaction volume can be scaled up or down without loss of activity by using proportionately less or more DNA (data not shown). The reactions should be incubated at 28–30 °C and are complete in 4 hours (Figure 2).

EXPRESSION LEVELS OF FUNCTIONAL PROTEIN

The yields of synthesized protein will vary depending on the specific protein being expressed. The level of luciferase produced in the experiment described in Figure 2 was approximately 40–45 µg/ml, as determined by measuring luciferase activity. Other functional proteins have been expressed in this system (Table 1). For this set of proteins the yields were determined by activity assays and ranged from 15–75 µg/ml. This range is higher than that expected from the rabbit reticulocyte lysate-based TNT® systems. All proteins were assayed directly in the TNT® reaction without further purification.

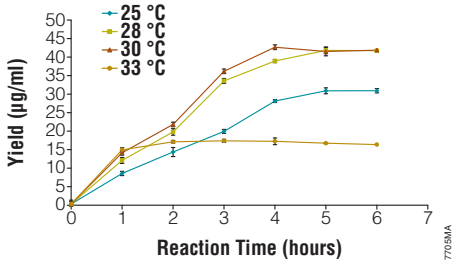

Figure 2. DNA time course and incubation conditions. The firefly luciferase gene was cloned into the pF25A vector. The resulting plasmid was purified using the PureYield™ Plasmid Midiprep System (Cat.# A2492) and ethanol precipitated. The DNA was resuspended in water and quantitated by absorbance at 260 nm. Four micrograms of plasmid DNA was added to each 50 µl reaction. The reactions were incubated at the specified temperatures for the indicated times. The luciferase activity was measured by adding 5 µl of each reaction to 100 µl of Steady-Glo® Luciferase Assay System (Cat.# E2510). Light output was measured using a microplate luminometer. Relative Light Units were converted to an estimate of protein concentration using a standard curve created from dilutions of QuantiLum® Recombinant Luciferase (Cat.# E1701). Data are the average +/– standard deviation of triplicate TNT® reactions.

Table 1. Proteins Tested for Functionality and Yield. Proteins were expressed and tested for activity. Protein concentration was estimated from standard curves using purified protein standards of known specific activity. Reagents used for each protein include: firefly luciferase, Steady-Glo® Luciferase Assay System (Cat.# E2510); *Renilla* luciferase, *Renilla* Luciferase Assay System (Cat.# E2810); procaspase-3, Caspase-Glo® 3/7 Assay (Cat.# G8091) after activation by recombinant caspase-8; β-galactosidase, Beta-Glo® Assay System (Cat.# E4720). HaloTag® protein activity was determined by covalent binding to its TMR Ligand (Cat.# G8251) followed by gel imaging.

Protein	Molecular Weight (kDa)	Yield (µg/ml)
Firefly luciferase	61	45
Renilla luciferase	36	15
Procaspase-3	34	75
HaloTag® Protein	33	20
β-galactosidase	118	N.D.

N.D. The β-galactosidase enzyme was functional, but the amount produced was not quantitated.

DETECT PROTEIN USING INCORPORATED LABELS

The TNT® T7 Insect Cell Extract Protein Expression System is compatible with the FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (Cat.# L5001). This is a rapid method of fluorescently labeling and detecting synthesized proteins. Labeled proteins can be detected by gel electrophoresis immediately after the reaction incubation period. Eleven human kinase genes were cloned into the pF25A or pF25K ICE T7 Flexi® Vectors, expressed using the TNT® T7 Insect Cell Extract Protein Expression System and detected with the FluoroTect™ System (Figure 3). All 11 human kinase proteins were synthesized, and the TNT® reactions had no endogenous fluorescence that interfered with detection.

SUMMARY

The TNT® T7 Insect Cell Extract Protein Expression System is a cell-free system for the rapid expression of functional proteins. It is formulated to allow quick and easy reaction setup, requiring the addition of only the DNA template and water to begin the reaction. The Flexi® Vectors are designed for optimal protein expression in this system at levels higher than those expected from rabbit reticulocyte lysate systems. The versatile open format of the TNT® reaction allows the addition of labels that can be incorporated into the synthesized proteins for easy detection or for downstream analysis.

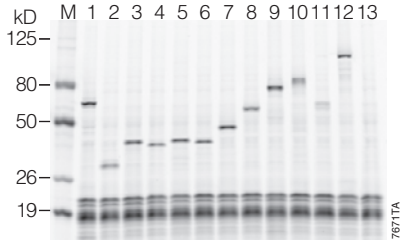

Figure 3. Expression of human kinase proteins. Eleven human kinase genes were cloned into the pF25A or pF25K T7 ICE Flexi® Vector. Four micrograms of purified plasmid was used for expression in 50 µl TNT® reactions. FluoroTect™ $Green_{Lys}$ tRNA (Cat.# L5001) (1 µl) was included in each reaction to facilitate rapid protein detection. Reactions were incubated for 4 hours, and 1 µl of each reaction in reducing sample buffer was loaded onto a 4–20% Tris-Glycine gel. Lanes: M, molecular weight markers (Cambrex); lane 1, pF25A-luciferase positive control; lanes 2 through 12: kinases AURKC, MAPK1, AURKB, PKA, MAPK14, AURKA, SRC, SYK, PKCgamma, AKT, PI3K, respectively; lane 13, no-DNA negative control; lane 14, no-DNA and no-FluoroTect™ $Green_{Lys}$ tRNA negative control. Fluorescent imaging was performed using a Typhoon® 9410 Imager (GE Healthcare) at the settings recommended for detecting fluorescein. Unincorporated fluorescent tRNA is visible at the bottom of the gel.

REFERENCES

1. Ezure, T. *et al.* (2006) *Biotechnol. Prog.* **22**, 1570–7.
2. Suzuki, T. *et al.* (2006) *J. Biosci. Bioeng.* **102**, 69–71.

ORDERING INFORMATION

Product	Size	Cat.#
TNT® T7 Insect Cell Extract Protein Expression System	40 × 50 µl reactions	L1102
	10 × 50 µl reactions	L1101
pF25A ICE T7 Flexi® Vectors	20 µg	L1061
pF25K ICE T7 Flexi® Vectors	20 µg	L1081

[a]Patent Pending.

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

[c]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.

[d]Use of this vector for applications outside of the TNT® T7 Insect Cell Extract Protein Expression System may require a license from Shimadzu Corporation. For more information contact t-direct@shimadzu-biotech.jp

[e]Ezure, T., Suzuki, T., Higashide, S., Shintani, E., Endo, K., Kobayashi, S., Shikata, M., Ito, M., Tanimizu, K., Nishimura, O. (2006) Cell-free protein synthesis system prepared from insect cells by freeze-thawing. *Biotechnol. Prog.* **22**, 1570–7.

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