TnT® Coupled Reticulocyte Lysate Systems

INSTRUCTIONS FOR USE OF PRODUCTS L4600, L4610, L4950, L5010, L5020, L4601 AND L4611.

Quick PROTOCOL

Translation Procedure

Before You Begin

Upon removal from storage at –70°C, immediately place TnT® RNA Polymerase on ice. Rapidly thaw the TnT® Reticulocyte Lysate by hand and place on ice. Thaw all other components at room temperature and store on ice.

Preparation of Template

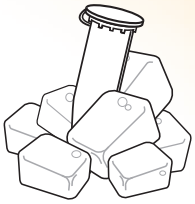
The template should be free of ethanol, calcium, RNase and salt. DNA from the Wizard® *Plus* Minipreps DNA Purification System, the Wizard® PCR Preps System or the standard alkaline lysate method (Sambrook *et al.*) will work with TnT® reactions.

Translation Procedure

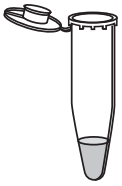
1. Assemble the reaction components, appropriate for the label being used, in a 0.5ml microcentrifuge tube. Gently mix by pipetting or stirring with pipette tip and, if necessary, centrifuge briefly.

Components	Standard Reaction Using [35S]methionine	Standard Reaction Using Transcend™ tRNA
TnT® Rabbit Reticulocyte Lysate*	25µl	25µl
TnT® Reaction Buffer*	2µl	2µl
TnT® RNA Polymerase (SP6, T3 or T7)	1µl	1µl
Amino Acid Mixture, Minus Leucine, 1mM	–	0.5µl
Amino Acid Mixture, Minus Methionine, 1mM	1µl	0.5µl
[35S]methionine (1,000Ci/mmol at 10mCi/ml)*	2µl	–
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl	1µl
DNA Template(s) (0.5µg/µl)*	2µl	2µl
Transcend™ Biotin-Lysyl-tRNA*	–	1µl
Nuclease-Free Water to a final volume of	50µl	50µl

2. Incubate the reaction at 30°C for 90 minutes.
3. Analyze the results. For procedures for incorporation assays, gel analysis of translation products and an assay for luciferase production in the control reactions, please refer to the *TnT® Coupled Reticulocyte Lysate Systems Technical Bulletin* #TB126.

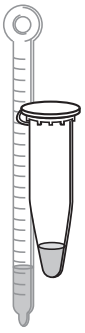


Keep all components on ice.

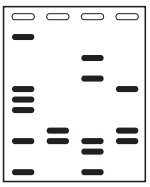


Assemble reaction components. Gently mix. Return unused components to –70C.

Centrifuge briefly if necessary.



Incubate at 30C for 90 minutes.



Analyze.

2934MA04_0A

*See notes on back.

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Printed in USA Revised 1/13
Part# 9FB027

TNT® Coupled Reticulocyte Lysate Systems

INSTRUCTIONS FOR USE OF PRODUCTS L4600, L4610, L4950, L5010, L5020, L4601 AND L4611.

Quick PROTOCOL

Translation Procedure

Notes

1. We have found that 50% lysate concentration is optimal for most TNT® Lysate reactions. In some cases, a lysate concentration of 55% (27.5µl) will ehnance translation.
2. The TNT® Reaction Buffer may contain a precipitate after thawing and sitting on ice. Redissolve the precipitate by vortexing at room temperature for 30 seconds.
3. We recommend using EasyTag™ L-[^{35}S]-Methionine (Perkin Elmer Cat.# NEG709A). This grade of [^{35}S]methionine does not cause the background labeling of the rabbit reticulocyte lysate 42kDa protein that can occur using other grades of label (7). In addition, a stabilizer has been added to this product to increase the stability of this product over conventional radio-labeled amino acids, so that the release of volatile gases is reduced substantially. This [^{35}S]methionine may be stored at 4°C without aliquoting. Other types of ^{35}S-labeled amino acids may be oxidized easily to translation-inhibiting sulfoxides and should be stored in aliquots at –70°C in buffer containing 0.1% DTT.
4. **New 1/13:** For optimal protein expression using the TNT® SP6 RNA polymerase, we recommend titrating magnesium acetate in 0.1mM increments between 0.1mM and 0.5mM. In some instances the addition of 0.2mM magnesium acetate has been shown to increase protein expression by 40%. Magnesium acetate is supplied only with Cat.# L4600 and L4601.

See additional protocol information in Technical Bulletin #TB126, available online at: **www.promega.com**

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

Printed in USA Revised 1/13.
Part# 9FB027