TnT® Coupled Wheat Germ Extract Systems

INSTRUCTIONS FOR USE OF PRODUCTS L4120, L4130, L4140, L5030 AND L5040.

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® Wheat Germ Extract by hand and place on ice. Thaw all other components at room temperature and store on ice.

Preparation of Template

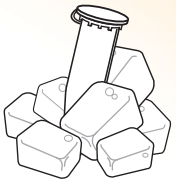
The template should be free of ethanol, calcium, RNase and salt. DNA from the Wizard® *Plus* Minipreps DNA Purification System, the Wizard® PCR Preps System or the standard alkaline lysate method (Sambrook *et al.*) will work with TnT® reactions.

Translation Procedure

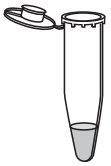
1. Assemble the reaction components, appropriate for the label being used, in a 0.5ml microcentrifuge tube. Gently mix by pipetting or stirring with pipette tip and, if necessary, centrifuge briefly.

Component	Standard Reaction Using [^{35}S]methionine
TnT® Wheat Germ Extract	25µl
TnT® Reaction Buffer	2µl
TnT® RNA Polymerase (SP6, T3 or T7)	1µl
Amino Acid Mixture, Minus Methionine, 1mM	1µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml)*	2–4µl
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl
DNA Template (0.5µg/µl)	2µl
Nuclease-Free Water to a final volume of	50µl

2. Incubate the translation reaction at 30°C for 60–120 minutes.
3. Analyze the results of translation. 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® Wheat Germ Extract System Technical Bulletin* #TB165.

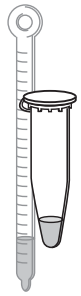


Keep all components on ice.

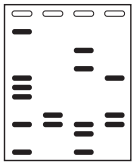


Assemble reaction components. Gently mix. Return unused components to –70°C.

Centrifuge briefly if necessary.



Incubate at 30°C for 60–120 minutes.



Analyze.

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*See additional protocol information in Technical Bulletin #TB165, available online at: **www.promega.com**

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Notes

1. We recommend using PerkinElmer EasyTag™ L-[^{35}S]methionine (PerkinElmer 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. In addition, a stabilizer has been added to this product to increase the stability over conventional radiolabeled 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.

For additional protocol information see Technical Bulletin #TB165, available online at **www.promega.com**

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