TnT® T7 Quick for PCR DNA

INSTRUCTIONS FOR USE OF PRODUCT L5540.

Quick PROTOCOL

Transcription/Translation Procedure

Before You Begin

Rapidly thaw the TnT® T7 PCR Quick Master Mix by hand and place on ice. Thaw all other components at room temperature and store on ice.

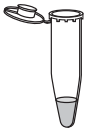
Transcription/Translation Procedure

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® T7 PCR Quick Master Mix	40µl	40µl
Methionine, 1mM	–	1µl
[35S]methionine (1,000Ci/mmol at 10mCi/ml)*	1–4µl	–
PCR-generated DNA template*	2.5–5µl	2.5–5µl
Transcend™ Biotin-Lysyl-tRNA*	–	1–2µl
Nuclease-Free Water to a final volume of	50µl	50µl

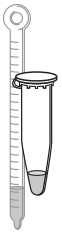
2. Incubate the reaction at 30°C for 60–90 minutes.
3. Analyze the results. For procedures for incorporation assays and gel analysis of translation products, please refer to the *TnT® T7 Quick for PCR DNA Technical Manual* #TM235.

Keep all components on ice.

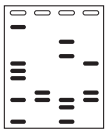


Assemble reaction components. Gently mix. Return unused components to –70°C.

Centrifuge briefly if necessary.



Incubate at 30°C for 60–90 minutes.



Analyze.

2927MA04_0A

*See notes 1–3 on back.

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Notes

1. We recommend using a grade of [^{35}S]methionine, such as PerkinElmer EasyTag™ L-[^{35}S]methionine (PerkinElmer Cat.# NEG709A), which does not cause the background labeling of the rabbit reticulocyte lysate 42kDa protein. Background labeling of the 42kDa protein 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 dispensing into aliquots. 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 DTT. Between 10–40µCi (1–4µl) of [^{35}S]methionine can be added to the TnT® Quick reactions, depending upon the balance between labeling efficiency and cost. For gene constructs that express well and contain several methionines, the 10µCi level (1µl) is sufficient for adequate detection.
2. PCR-generated templates can be used directly from the amplification reaction. We recommend using 2.5–5µl from the amplification reaction, but up to 7µl can be used in a 50µl reaction.
3. The level of added Transcend™ tRNA can be increased (up to 4µl) to allow more sensitive detection of proteins that contain few lysines or are poorly expressed.

See additional protocol information in Technical Manual #TM235, available online at: **www.promega.com**

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