TnT® Quick Coupled Transcription/Translation Systems

INSTRUCTIONS FOR USE OF PRODUCTS L1170, L1171, L2080 AND L2081.

Quick PROTOCOL

Transcription/Translation Procedure with Plasmid DNA

Before You Begin

Upon removal from storage at –70°C, rapidly thaw the TnT® Quick Master Mix 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.

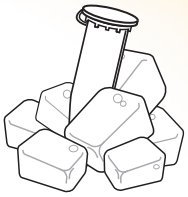
Translation Procedure

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

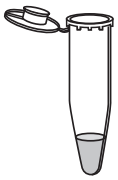
Components	Standard Reaction	Transcend™ Reaction	FluoroTect™ Reaction
TnT® T7 Quick Master Mix	40µl	40µl	40µl
Methionine, 1mM	–	1µl	1µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml)	2µl	–	–
Plasmid DNA Template (0.5µg)	2µl	2µl	2µl
Transcend™ Biotin-Lysyl-tRNA	–	1–2µl	–
FluoroTect™ Green$_{Lys}$ tRNA	–	–	1–2µl
Nuclease-Free Water to a final volume of	50µl	50µl	50µl

2. Incubate the reaction at 30°C for 60–90 minutes.
3. Analyze the results. Procedures for incorporation assays, gel analysis of translation products and an assay for luciferase production in the control reactions are provided in the *TnT® Quick Coupled Transcription/Translation Systems Technical Manual* #TM045.

*See additional protocol information in Technical Manual #TM045, available online at: **www.promega.com**

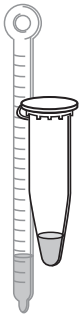


Keep all components on ice.

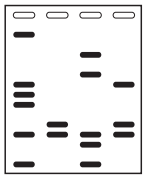


Assemble reaction components. Gently mix. Return unused components to –70°C.

Centrifuge briefly if necessary.



Incubate at 30°C for 60–90 minutes.



Analyze.

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TNT® Quick Coupled Transcription/Translation Systems

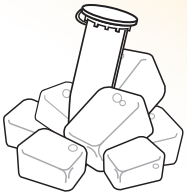
INSTRUCTIONS FOR USE OF PRODUCTS L1170, L1171, L2080 AND L2081.

Quick PROTOCOL

Transcription/Translation Procedure with PCR-Generated DNA

Before You Begin

Upon removal from storage at –70°C, rapidly thaw the TNT® Quick Master Mix by hand and place on ice. Thaw all other components at room temperature and store on ice.



Keep all components on ice.

Template Considerations

PCR products (5–7µl) can be used directly from the amplification reaction.

Note: For PCR-generated templates, include 1µl of theT7 TNT® PCR Enhancer in each 50µl reaction.

Translation Procedure

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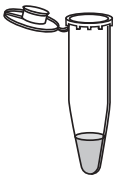
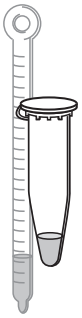


Assemble reaction components. Gently mix. Return unused components to –70°C.

Centrifuge briefly if necessary.

Components	Standard Reaction	Transcend™ Reaction	FluoroTect™ Reaction
TNT® T7 Quick Master Mix	40µl	40µl	40µl
Methionine, 1mM	–	1µl	1µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml)	2µl	–	–
PCR-Generated DNA Template (0.5µg)	2.4–5µl	2.5–5µl	2.5–5µl
T7 TNT® PCR Enhance	1µl	1µl	1µl
Transcend™ Biotin-Lysyl-tRNA	–	1–2µl	–
FluoroTect™ Green$_{Lys}$ tRNA	–	–	1–2µl
Nuclease-Free Water to a final volume of	50µl	50µl	50µl



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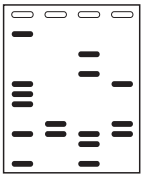

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