Promega

Technical Bulletin

T7 Sample System

INSTRUCTIONS FOR USE OF PRODUCT L5900.

www.promega.com

PRINTED IN USA.
Revised 5/09

Part# TB293

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T7 Sample System

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1. Description

This system contains samples of four unique in vitro translation systems: TNT® T7 Quick For PCR DNA[a–c], TNT® T7 Quick Coupled Transcription/Translation System[a–e], TNT® T7 Coupled Wheat Germ Extract System[a,d,e] and *E. coli* T7 S30 Extract System for Circular DNA[f,g]. Included are sufficient reagents to determine the system for the optimal expression of individual genes. A luciferase plasmid control is included to monitor successful incorporation of [^{35}S] methionine.

Table 1 provides a reference for what types of DNA work well with different in vitro translation systems. For more information on the in vitro translation systems included in this sample system, please refer to the online technical information listed at the end of Section 3.

Table 1. Comparison of DNA Templates for in vitro Translation Systems.

in vitro Translation System	Circular Plasmid	PCR-Generated DNA	Linear DNA
TNT® T7 Quick for PCR DNA System	+	+++	+++
TNT® T7 Quick Coupled Transcription/Translation System	+++	+	+
TNT® T7 Coupled Wheat Germ Extract	+	++	+++
E. coli T7 S30 Extract System for Circular DNA	+++	+	+

+++ Recommended
++ Satisfactory
+ Possible

2. Product Components and Storage Conditions

Product	Cat.#
T7 Sample System	L5900

Includes:

- 200µl TNT® T7 Quick for PCR Master Mix
- 200µl TNT® T7 Quick Master Mix
- 5µg Luciferase T7 Control DNA[(e)]
- 175µl Amino Acid Mixture Minus Methionine
- 175µl Amino Acid Mixture, Complete
- 50µl 1mM Methionine
- 90µl TNT® Reaction Buffer
- 200µl TNT® Wheat Germ Extract
- 30µl TNT® T7 Wheat Germ Polymerase
- 150µl T7 S30 Extract for Circular DNA
- 750µl S30 Premix without Amino Acids
- 1,250µl Nuclease-Free Water

Stability/Storage: Store all components at –70°C. Product is sensitive to CO_2 (avoid prolonged exposure) and multiple freeze-thaw cycles, which may have an adverse effect on activity/performance.

3. Protocols

Material to Be Supplied by the User

- radiolabeled amino acid (for radioactive detection) or Transcend™ tRNA (Cat.# L5061; for non-radioactive detection) and Transcend™ Colorimetric (Cat.# L5070) or Chemiluminescent (Cat.# L5080) Translation Detection System (for non-radioactive detection) or FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (Cat.# L5001)

Before You Begin

Thaw the lysate/extract on ice. Thaw all other components at room temperature, and store on ice.

Translation Procedures

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

TNT® T7 Quick for PCR DNA (standard reaction using [^{35}S] methionine, FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA)

Note: When using either FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA with certain in vitro translation systems, background bands are observed. Before ordering these systems, refer to the online technical information listed at the end of Section 3.

Component	Volume
TNT® T7 Quick for PCR Master Mix	40µl
[^{35}S] methionine (1,000Ci/mmol at 10mCi/ml; see Note 2)	1–4µl
PCR-generated DNA template (see Notes 1 and 4)	2.5–5µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60–90 minutes. Terminate reactions by placing on ice.

Component	Volume
TNT® T7 Quick for PCR Master Mix	40µl
1mM Methionine	1µl
PCR-generated DNA template (see Notes 1 and 4)	2.5–5µl
FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA	1–2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60 minutes. Terminate reactions by placing on ice.

TNT® T7 Quick Coupled Transcription/ Translation System (standard reaction using [^{35}S] methionine, FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA)

Component	Volume
TNT® T7 Quick Master Mix	40µl
[^{35}S] methionine (1,000Ci/mmol at 10mCi/ml; see Note 2)	2µl
plasmid DNA template (0.5µg/µl; see Notes 3 and 4)	2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60–90 minutes. Terminate reactions by placing on ice.

Component	Volume
TNT® T7 Quick Master Mix	40µl
1mM Methionine	2µl
plasmid DNA template (0.5µg/µl; see Notes 3 and 4)	2µl
FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA	1–2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60 minutes. Terminate reactions by placing on ice.

3. Protocols (continued)

TNT® Coupled Wheat Germ Extract System (standard reaction using [35S] methionine, FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA)

Note: Wheat Germ Extract contains five major endogenous biotinylated proteins, which migrate at 200kDa, 80kDa, 32kDa and a doublet at 17kDa.

Component	Volume
TNT® Wheat Germ Extract	25µl
Amino Acid Mix Minus Methionine	1µl
TNT® Reaction Buffer	2µl
TNT® T7 Wheat Germ Polymerase	1µl
[35S] methionine (1,000Ci/mmol at 10mCi/ml; see Note 2)	2µl
plasmid DNA template (0.5µg/µl; see Notes 3 and 4)	2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60–90 minutes. Terminate reactions by placing on ice.

Component	Volume
TNT® Wheat Germ Extract	25µl
TNT® Reaction Buffer	2µl
TNT® T7 Wheat Germ Polymerase	1µl
Amino Acid Mixture, Complete	1µl
plasmid DNA template (0.5µg/µl; see Notes 3 and 4)	2µl
FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA	1–2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 30°C for 60 minutes. Terminate reactions by placing on ice.

***E. coli* T7 S30 Extract System for Circular DNA (standard reaction using [35S] methionine, FluoroTect™ Green$_{Lys}$ or Transcend™ tRNA)**

Note: The provided T7 Luciferase Control DNA does not contain a ribosomal binding site. However, it does produce proteins in the *E. coli* T7 S30 Extract. For optimal expression with the *E. coli* T7 S30 Extract, we recommend that the insert or vector contains a ribosomal binding site.

Component	Volume
Amino Acid Mixture Minus Methionine	5µl
S30 Premix without Amino Acids	20µl
[35S] methionine (1,000Ci/mmol at 10mCi/ml; see Note 2)	1µl
T7 S30 Extract for Circular DNA	15µl
plasmid DNA template(0.5µg/µl; see Notes 3 and 4)	2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 37°C for 60–90 minutes. Terminate reactions by placing on ice.

Amino Acid Mix, Complete	5µl
S30 Premix without Amino Acids	20µl
T7 S30 Extract for Circular DNA	15µl
plasmid DNA template (0.5µg/µl; see Notes 3 and 4)	2µl
FluoroTect™ $Green_{Lys}$ or Transcend™ tRNA	1–2µl
Nuclease-Free Water to a final volume of	50µl

Incubate at 37°C for 60 minutes. Terminate reactions by placing on ice.

Notes:

1. PCR-generated templates can be used directly from the amplification reaction.
2. We recommend using a translational grade [^{35}S] methionine such as PerkinElmer EasyTag™ L-[^{35}S]methionine (PerkinElmer Cat.# NEG709A).
3. Plasmid template should be free of ethanol, calcium, RNase and salt.
4. We recommend using 1µg of control DNA to monitor incorporation of labeled amino acids. For the reactions using TNT® T7 Quick for PCR, we recommend using 18–20ng of control template per reaction.

Analysis

For incorporation assays and gel analysis, please refer to the appropriate Technical Manual/Technical Bulletin available online:

TNT® T7 Quick for PCR DNA Technical Manual **#TM235**
(www.promega.com/tbs/tm235/tm235.html)

TNT® Quick Coupled Transcription/Translation Systems Technical Manual **#TM045**
(www.promega.com/tbs/tm045/tm045.html)

TNT® Coupled Wheat Germ Extract Systems Technical Bulletin **#TB165**
(www.promega.com/tbs/tb165/tb165.html)

E. coli *T7 S30 Extract System for Circular DNA Technical Bulletin* **#TB219**
(www.promega.com/tbs/tb219/tb219.html)

FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System Technical Bulletin **#TB285**
(www.promega.com/tbs/tb285/tb285.html)

Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin **#TB182**
(www.promega.com/tbs/tb182/tb182.html)

4. Related Products

Product	Size	Cat.#
TNT® T7 Quick for PCR DNA*	40 reactions	L5540
TNT® T7 Quick Coupled Transcription/ Translation System*	40 reactions	L1170
	5 reactions	L1171
TNT® T7 Coupled Wheat Germ Extract System*	40 reactions	L4140
E. coli T7 S30 Extract System for Circular DNA	30 reactions	L1130
FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System*	20–40 reactions	L5001
Transcend™ Colorimetric Non-Radioactive Translation Detection System*	30 reactions	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System*	30 reactions	L5080
Transcend™ Biotinylated tRNA*	30µl	L5061

*For Laboratory Use.

Product	Size	Cat.#
pFN19A (HaloTag® 7) T7 SP6 Flexi® Vector	20µg	G1891
pFN19K (HaloTag® 7) T7 SP6 Flexi® Vector	20µg	G1841
pFN20A (HaloTag® 7) T7 SP6 Flexi® Vector	20µg	G1681
pFN20K (HaloTag® 7) T7 SP6 Flexi® Vector	20µg	G1691

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[a]U.S. Pat. Nos. 5,324,637 and 5,492,817, Australian Pat. No. 660329, Japanese Pat. No. 2904583 and other patents pending.

[b]U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596.

[c]For Laboratory Use. Any use of the product for diagnostics requiring clearance or approval by the FDA may require a license under Mayo Clinic U.S. Pat. Nos. 6,027,913 and 6,361,949.

[d]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595.

[e]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. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

[f]For research purposes only. Not for diagnostic or therapeutic use. For nonresearch uses of the portion of the vector encoding the biotinylation sequence, please contact Promega Corporation for licensing information.

[g]Licensed under U.S. Pat. No. 5,252,466 and Australian Pat. No. 647025.

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