Flexi® Rabbit Reticulocyte Lysate System

INSTRUCTIONS FOR USE OF PRODUCT L4540.

Quick PROTOCOL

In Vitro Translation Protocol

Before You Begin

Remove the reagents from –70°C storage. Rapidly thaw the lysate by hand-warming, and immediately store on ice. Thaw other components at 37°C, and store on ice.

Preparation of Template RNA

Denature the template mRNA at 65°C for 3 minutes, and immediately cool on ice. The template should be free of ethanol, calcium, RNases and salts.

Translation Procedure

1. Assemble the reaction components, appropriate for the label being used, in a 0.5ml polypropylene microcentrifuge tube. After all components are added, gently mix the lysate by pipetting and stirring the reaction with the pipette tip. If necessary, centrifuge briefly to return the sample to the bottom of the tube.

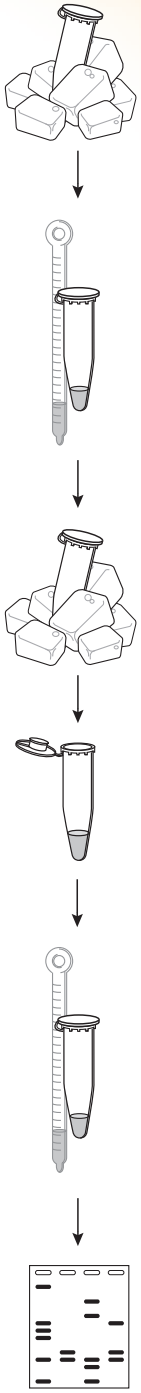
Component	Reaction Using [^{35}S]methionine	Reaction Using Transcend™ tRNA
Flexi® Rabbit Reticulocyte Lysate	33µl	33µl
Amino Acid Mixture, Minus Leucine, 1mM	—	0.5µl
Amino Acid Mixture, Minus Methionine, 1mM	1µl	0.5µl
[^{35}S]methionine (1,200Ci/mmol at 10mCi/ml)	2µl	—
Magnesium Acetate, 25mM	0–4µl	0–4µl
Potassium Chloride, 2.5M	1.4µl	1.4µl
DTT, 100mM	0–1µl	0–1µl
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl	1µl
RNA substrate	1–12µl	1–12µl
Transcend™ tRNA	—	1–2µl
Nuclease-Free Water to a final volume of	50µl	50µl

2. Incubate the translation reaction at 30°C for 90 minutes.
3. Analyze the results of translation. See Technical Bulletin #TB127 for procedures for incorporation assays, gel analysis of translation products and an assay for luciferase production in the control reactions.

Control Translation Reactions

1. We recommend including a control reaction containing no added mRNA. This allows measurement of any background incorporation of labeled amino acids.
2. The protocol for a positive control reaction using the Luciferase Control RNA is on the opposite side of this card.

See additional protocol information in Technical Bulletin #TB127, available online at: **www.promega.com**



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Flexi® Rabbit Reticulocyte Lysate System

INSTRUCTIONS FOR USE OF PRODUCT L4540.

Quick PROTOCOL

Luciferase Positive Control Translation Reaction

Before You Begin

Remove the reagents from –70°C storage. Rapidly thaw the lysate by hand-warming, and immediately store on ice. Thaw other components at 37°C, and store on ice.

Non-Radioactive Luciferase Control Reaction

1. Assemble the following reaction:

Component	Volume
Flexi® Rabbit Reticulocyte Lysate	35µl
Amino Acid Mixture, Minus Leucine, 1mM	0.5µl
Amino Acid Mixture, Minus Methionine, 1mM	0.5µl
Potassium Chloride, 2.5M	1.4µl
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl
Luciferase Control RNA, 1mg/ml	1µl
Nuclease-Free Water	10.6µl
final volume	50µl

2. Incubate the translation reaction at 30°C for 60–90 minutes.

   The luciferase control reactions can be stored at –20°C for up to 2 months or at –70°C for up to 6 months with little loss of luciferase activity.

3. Detect the synthesis of functional luciferase using the standard luciferase assay (see Section 7 of #TB127).

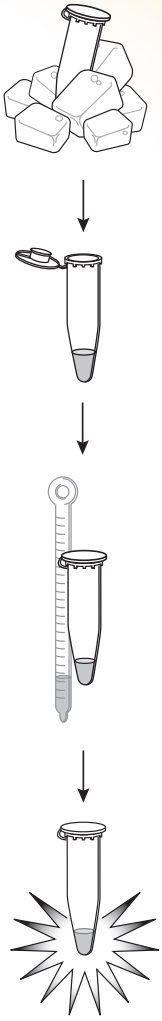
Radioactive Luciferase Control Reaction

1. Assemble the following reaction:

Component	Volume
Flexi® Rabbit Reticulocyte Lysate	35µl
Amino Acid Mixture, Minus Methionine, 1mM	1µl
[^{35}S]methionine (1,200Ci/mmol) at 10mCi/ml	2µl
Potassium Chloride, 2.5M	1.4µl
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl
Luciferase Control RNA, 1mg/ml	1µl
Nuclease-Free Water to a final volume of	50µl

2. Follow Steps 2–3 for the non-radioactive luciferase control reaction.

See additional protocol information in Technical Bulletin #TB127, available online at: **www.promega.com**



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