E. coli S30 Extract System for Circular DNA

INSTRUCTIONS FOR USE OF PRODUCT L1020.

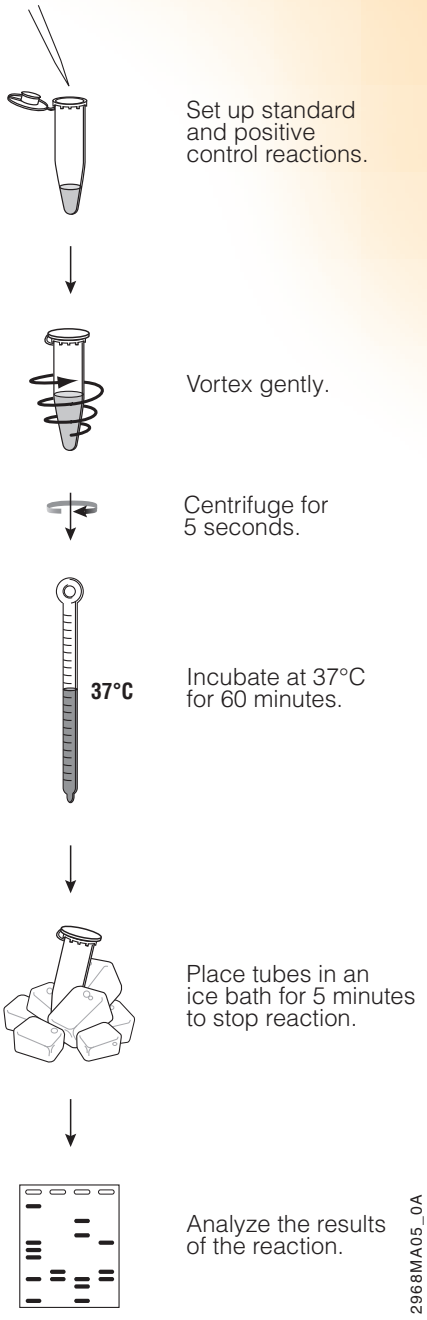
Quick PROTOCOL

Standard Coupled Transcription/Translation Protocol

1. Set up the following reactions:

Component	Standard	Positive Control (see Note 3)
DNA template (see Notes 1 and 2)	≤2µg	2µl
Amino Acid Mixture Minus Methionine	5µl	5µl
S30 Premix Without Amino Acids	20µl	20µl
[^{35}S]methionine (1,200Ci/mmol at 15mCi/ml) (PerkinElmer EasyTag™ L-[35S]methionine, Cat.# NEG709A) (optional, see Note 3)	1µl	1µl
S30 Extract, Circular	15µl	15µl
Nuclease-Free Water (see Note 2) to a final volume of	50µl	50µl

2. Vortex gently, then centrifuge for 5 seconds.
3. Incubate at 37°C for 60 minutes (see Note 4).
4. Place tubes in an ice bath for 5 minutes to stop reaction.
5. Analyze the results of the reaction. See Sections 6–10 of TB092 for incorporation assays and gel analysis of proteins.



Notes

1. Optimize the amount of DNA added. In general, reactions should not contain more than 2µg of DNA.
2. Template DNA and water purity are extremely important. If efficiencies are low, examine the quality of the template DNA and water.
3. Use pBEST*luc*™ DNA to synthesize luciferase. Luciferase migrates at 61kDa. An apparent internal translation start results in a second major gene product of 48kDa. Additionally, β-lactamase may appear as a faint band migrating at 31.5kDa.

   Unlabeled luciferase is used in a luminescence assay to monitor the efficiency of the S30 reaction. To generate unlabeled luciferase, see reverse side of this card.

   For a negative control, omit DNA from the reaction. Use the negative control to determine background radiolabel incorporation.
4. The reaction may be incubated within a temperature range of 24–37°C. The fastest linear rate of protein synthesis occurs at 37°C for approximately 1 hour, although the reaction will continue for several hours at a slower rate. Lower temperatures produce a slower rate of synthesis but often extend the time of the linear rate to several hours. If the standard reaction at 37°C for 1 hour does not produce the desired results, perform the reaction at a lower temperature for a longer time.

See additional protocol information in Technical Bulletin #TB092, available online at: **www.promega.com**

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2968MA05_0A

E. coli S30 Extract System for Circular DNA

INSTRUCTIONS FOR USE OF PRODUCT L1020.

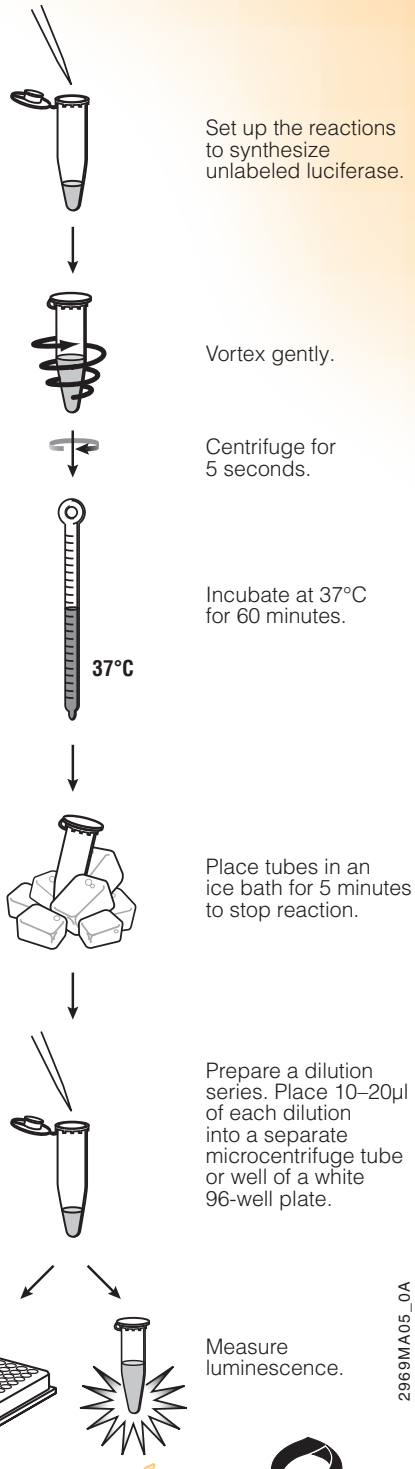
Quick PROTOCOL

Synthesis and Assays of Luciferase Control

1. Synthesize unlabeled luciferase using:

Component	Volume
pBEST*luc*™ DNA (1µg/µl)	2µl (2µg)
Complete Amino Acid Mixture	5µl
S30 Premix Without Amino Acids	20µl
S30 Extract, Circular	15µl
Nuclease-Free Water (see Note 1) to a final volume of	50µl

2. Vortex gently, then centrifuge for 5 seconds.
3. Incubate at 37°C for 60 minutes (see Note 2).
4. Place tubes in an ice bath for 5 minutes to stop reaction.
5. Prepare a dilution series:
   a. At room temperature, add 50µl of Luciferase Dilution Reagent to each of four microcentrifuge tubes (see Note 3).
   b. Add 50µl of the luciferase S30 control reaction to the first tube, mix and pipet 50µl from first tube to second tube. Mix, and continue the series of twofold dilutions in the remaining two tubes.
6. Place 10–20µl of each dilution into a microcentrifuge tube or the well of a white 96-well plate.
7. Measure luminescence by luminometry, scintillation counting, photography or visual detection (see Sections 5.B, 5.C and 5.D of TB092).



Notes

1. Water purity is extremely important. If translation efficiencies are low, examine the water quality.
2. The reaction may be incubated within a temperature range of 24–37°C. The fastest linear rate of protein synthesis occurs at 37°C for approximately 1 hour, although the reaction will continue for several hours at a slower rate. Lower temperatures produce a slower rate of synthesis but often extend the time of the linear rate to several hours. If the standard reaction at 37°C for 1 hour does not produce the desired results, perform the reaction at a lower temperature for a longer time.
3. If the samples are to be quantitated in a scintillation counter, further dilutions (five- to tenfold) using Luciferase Dilution Reagent may be needed at Step 5, as these instruments experience signal saturation at high light intensities.

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