E. coli T7 S30 Extract System for Circular DNA

INSTRUCTIONS FOR USE OF PRODUCT L1130.

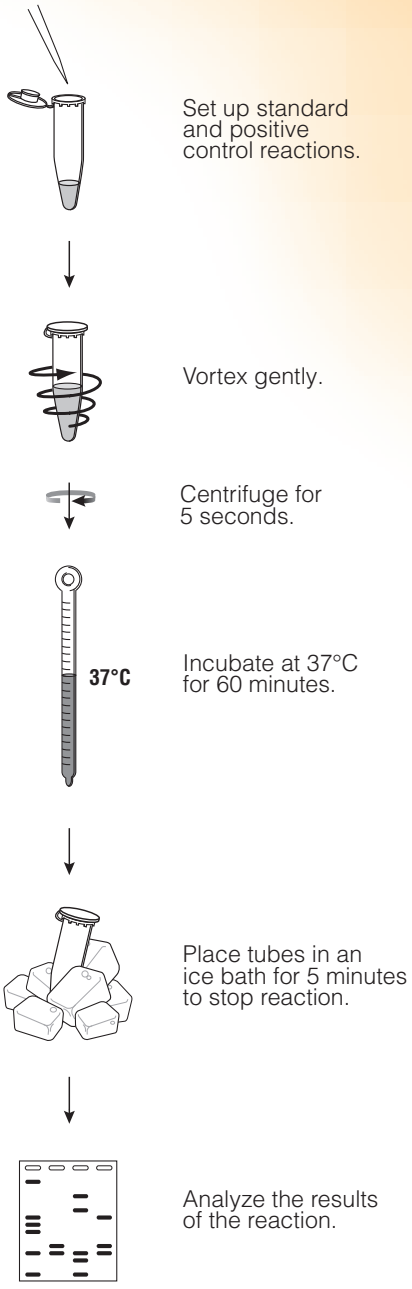
Quick PROTOCOL

Coupled Transcription/Translation Protocol

1. Set up the following reactions:

Component	Standard	Positive Control (see Note 4)
DNA template	≤4µg	4µl
Amino Acid Mixture Minus Methionine	5µl	5µl
S30 Premix Without Amino Acids	20µl	20µl
[^{35}S]methionine (1,200Ci/mmol at 15mCi/ml) (optional, see Notes 2 and 4)	1µl	1µl
T7 S30 Extract, Circular (mix gently prior to use)	15µl	15µl
Nuclease-Free Water (see Note 3) to a final volume of	50µl	50µl

2. Vortex gently, then centrifuge for 5 seconds.
3. Incubate at 37°C for 1–2 hours (see Note 5).
4. Place tubes in an ice bath for 5 minutes to stop reaction.
5. Analyze the results of the reaction. See Sections 5–9 of TB219 for incorporation assays and gel analysis of proteins.



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Notes

1. Optimize the amount of DNA added. In general, reactions should not contain more than 4µg of DNA.
2. For optimal results, store [^{35}S]methionine in aliquots at –70°C, and use once.
3. Template DNA and water purity are extremely important. If efficiencies are low, examine the quality of the template DNA and water.
4. When using the PinPoint™ Control Vector DNA template, the largest molecular weight band corresponds to the PinPoint™/CAT fusion protein (39kDa), and a prominent band corresponding to β-lactamase (28kDa) migrates below the PinPoint™/CAT fusion. Some full-length CAT protein is also observed, probably due to an internal translation initiation site. For a negative control, omit the DNA from the reaction. Use the negative control to determine background incorporation (see TB219).
5. The reaction may be incubated between 24–37°C. The fastest linear rate occurs at 37°C for approximately 2 hours, although the reaction will continue for several hours at a slower rate. Lower temperatures produce a slower rate of translation 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 #TB219, available online at: **www.promega.com/tbs**

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E. coli T7 S30 Extract System for Circular DNA

INSTRUCTIONS FOR USE OF PRODUCT L1130.

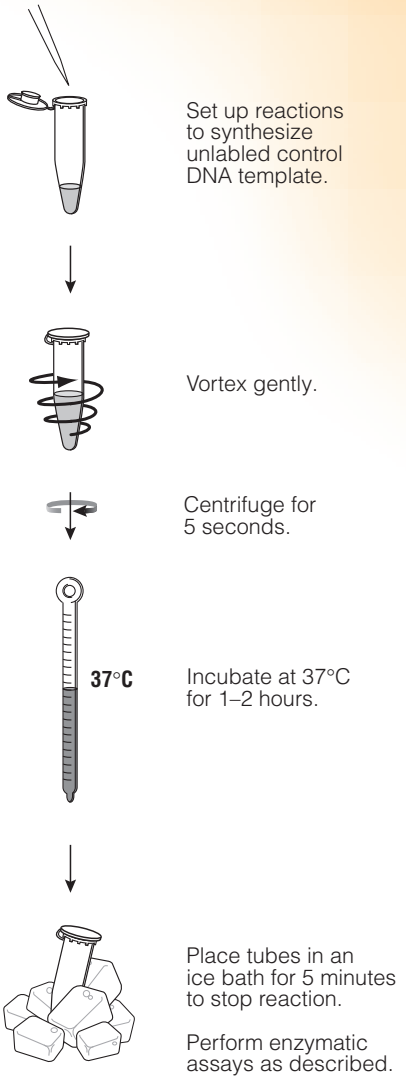
Quick PROTOCOL

Immunological and Enzymatic Assays of Control DNA Template

1. Synthesize unlabeled control DNA template using:

Component	Volume
PinPoint™ Control Vector DNA	4µl
Complete Amino Acid Mixture (mix gently prior to use)	5µl
S30 Premix Without Amino Acids (mix gently prior to use)	20µl
T7 S30 Extract, Circular (mix gently prior to use)	15µl
Nuclease-Free Water to a final volume of	50µl

2. Vortex gently, then centrifuge for 5 seconds.
3. Incubate at 37°C for 1–2 hours.
4. Place tubes in an ice bath for 5 minutes to stop reaction.
5. Use 5µl of reaction in immunoblotting assays. See Section 6 of TB219. Perform enzymatic assays as described in the *CAT Enzyme Assay System Technical Bulletin* #TB084.



Notes

1. Water purity is extremely important. If efficiencies are low, examine the quality of the water.
2. The reaction may be incubated within a temperature range of 24–37°C. The fastest linear rate occurs at 37°C for approximately 2 hours, although the reaction will continue for several hours at a slower rate. Lower temperatures produce a slower rate of catalysis 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.

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