Technical Bulletin

E. coli T7 S30 Extract System for Circular DNA

INSTRUCTIONS FOR USE OF PRODUCT L1130.

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Promega

E. coli T7 S30 Extract System for Circular DNA

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1. Description

Systems that use bacteriophage T7 RNA polymerase and T7 promoters are popular for high-level expression of proteins in vivo (1). These expression systems require the presence of T7 RNA polymerase, which is typically supplied by induction of a recombinant T7 polymerase gene contained on a lambda lysogen in the host strain (2). Coupled in vitro transcription/translation of these sequences also requires the presence of T7 RNA polymerase in the extract.

The *E. coli* T7 S30 Extract System for Circular DNA[a,b] simplifies the transcription/translation of DNA sequences cloned in plasmid or lambda vectors containing a T7 promoter by providing an extract that contains T7 RNA polymerase for transcription and all necessary components for translation (Figure 1). The investigator supplies only cloned DNA containing a T7 promoter and a ribosome binding site. The T7 S30 Extract for Circular DNA Templates is prepared by modifications of the method described by Zubay (3–5) from an *E. coli* strain B deficient in OmpT endoproteinase and lon protease activity, which results in greater stability of expressed proteins that would otherwise be degraded by proteases if expressed in vivo (2,6).

The T7 S30 System contains an S30 Premix Without Amino Acids that is optimized for each lot of S30 Extract and contains all other required components, including rNTPs, tRNAs, an ATP-regenerating system, IPTG and appropriate salts. Amino acid mixtures lacking cysteine, methionine or leucine are provided to facilitate radiolabeling of translation products. This system also includes a control DNA template, the PinPoint™ Control Vector (see Section 11.B). This vector contains the chloramphenicol acetyltransferase gene fused to the PinPoint™ peptide sequence positioned downstream from the T7 and *tac* promoters and a ribosome binding site. Expression of this CAT fusion in the T7 S30 System for Circular DNA can be assayed by ^{35}S incorporation or by non-radioactive immunological or enzymatic methods.

The most common application of S30 Extracts is the synthesis of small amounts of radiolabeled protein. The synthesis of a protein of the correct size is a useful way to verify the gene product of a particular DNA sequence. Proteins expressed in the S30 Extract also may be used for a variety of functional transcription and translation studies (7). Additional applications of the *E. coli* T7 S30 Extract System for Circular DNA include synthesis of small amounts of radiolabeled protein for use as a tracer in protein purification (7), incorporation of unnatural amino acids into proteins for structural studies (8) and screening of compounds that affect translation.

For *E. coli* T7 S30 Extract System for Circular DNA citations visit:
www.promega.com/citations

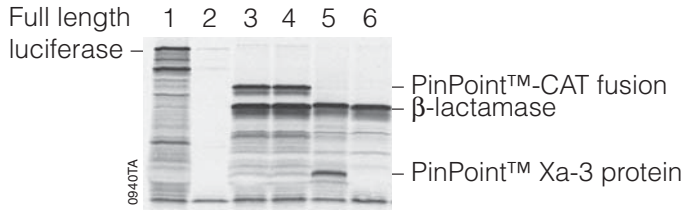

Figure 1. Coupled in vitro transcription/translation of circular DNA templates using the *E. coli* T7 S30 Extract System for Circular DNA. Several DNA constructs were expressed in the *E. coli* T7 S30 Extract System. The proteins were expressed as described in Section 4 using [^{35}S]methionine. Where indicated, the reactions also contained 50ng of rifampicin. The proteins were resolved by SDS-PAGE (4–20% acrylamide), transferred to nitrocellulose and visualized by autoradiography. Templates: lane 1, pBEST*luc*™ Vector; lane 2, pBEST*luc*™ Vector (+ rifampicin); lane 3, PinPoint™ Control Vector; lane 4, PinPoint™ Control Vector (+ rifampicin); lane 5, PinPoint™ Xa-3 Vector; lane 6, pGEM®-9Zf(–) Vector.

2. Product Components and Storage Conditions

Product	Cat.#
E. coli T7 S30 Extract System for Circular DNA	L1130

Each system contains sufficient reagents for 30 × 50µl reactions. Includes:

- 175µl Amino Acid Mixture Minus Cysteine, 1mM
- 175µl Amino Acid Mixture Minus Methionine, 1mM
- 175µl Amino Acid Mixture Minus Leucine, 1mM
- 450µl T7 S30 Extract, Circular (3 × 150µl)
- 750µl S30 Premix Without Amino Acids
- 5µg PinPoint™ Control Vector DNA, Circular (1µg/µl)

Storage Conditions: Store all components at –70°C. Avoid prolonged exposure of this product to CO_2 and multiple freeze-thaw cycles, which may have an adverse affect on activity and performance of this product.

Note: Please see Related Products (Section 11.C) for information on Amino Acid Mixture, Complete (Cat.# L4461), and Amino Acid Mixture Minus Methionine and Cysteine (Cat.# L5511). Bulk quantities (over 50ml) of the Extract and Premix are available from Promega.

3. General Considerations

3.A. Template Considerations

Expression of cloned DNA fragments in the *E. coli* T7 S30 Extract System for Circular DNA requires that a gene be under the control of either a T7 promoter or a good *E. coli* promoter. Examples of good *E. coli* promoters include lambda P_R, lambda P_L, *tac*, *trc* and *lac*UV5. Expression from T7 promoters is typically higher than from *E. coli* promoters using this extract system. Expression from *E. coli* promoters can be inhibited by the addition of rifampicin to the extract (compare lanes 1 and 2, Figure 1); transcription by T7 RNA polymerase is resistant to rifampicin (compare lanes 3 and 4, Figure 1).

It is important to emphasize that large differences in gene expression result from the context in which the gene resides. Changes in the position of the gene relative to the ribosomal binding site (RBS) will affect expression levels (9). The RBS is generally located approximately 7 bases upstream of the AUG start codon. In addition, many eukaryotic genes contain sequences within the coding region that can function as ribosomal binding sites when they precede a methionine codon. The presence of such internal sequences can result in internal translation initiation and the synthesis of potentially undesired truncated proteins in the prokaryotic system. An example of this can be seen in the expression of the firefly luciferase gene in the *E. coli* S30 Extract System.

The firefly luciferase gene contains 14 methionine codons, several of which are preceded by potential RBS sequences and produce truncated translation products (see Figure 1, lane 1).

Use only highly purified DNA templates (e.g., CsCl- or gel-purified), and avoid adding high concentrations of salts or glycerol with the DNA template. The activity of the T7 S30 System may be inhibited by NaCl at ≥50mM, glycerol at ≥1%, and by small amounts of Mg^{2+} (1–2mM) or potassium salts (50mM). Ethanol precipitate the DNA template with sodium acetate rather than ammonium acetate. Protein yields from the *E. coli* T7 S30 Extract System vary with the template and the conditions used. Typical yields range from 50–250ng per 50µl reaction.

3.B. Detection Methods

The amino acid mixtures provided are compatible with the use of radiolabeled cysteine, methionine and leucine. Several amino acid mixtures are also available separately (see Section 11.C). Radiolabeled proteins can be detected by standard methods.

The Transcend™ Non-Radioactive Translation Detection Systems (Cat.# L5070 and L5080) provide an alternative to the use of radiolabeled amino acids. Using these systems, biotinylated lysine residues are incorporated into nascent proteins during translation, eliminating the need for labeling with radioactive amino acids. This biotinylated lysine is added to the translation reaction as a precharged ε-labeled biotinylated lysine-tRNA complex (Transcend™ tRNA) rather than a free amino acid. After SDS-PAGE and electroblotting, the biotinylated proteins are visualized by binding streptavidin alkaline phosphatase or streptavidin horseradish peroxidase, followed either by colorimetric or chemiluminescent detection. The biotin tag allows both detection and capture of the translated protein. As little as 0.5–5ng of protein can be detected using the Transcend™ method within 3–4 hours after gel electrophoresis. This sensitivity is equivalent to that achieved with [^{35}S]methionine incorporation and autoradiographic detection 6–12 hours after gel electrophoresis. For more information on the Transcend™ System, request the *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin*, #TB182.

Another non-radioactive detection method is the FluoroTect™ Green$_{Lys}$ in vitro Translation System (Cat.# L5001), which enables fluorescent labeling and detection of proteins synthesized in vitro. The system is based on a lysine-charged tRNA that is labeled at the ε position of the lysine with the fluorophore BODIPY®-FL. Fluorescent lysine residues will be incorporated into synthesized proteins during in vitro translation reactions, eliminating the need for radioactivity. Request the *FluoroTect™ Green$_{Lys}$ in vitro Translation System Technical Bulletin*, #TB285.

4. Coupled Transcription/Translation Procedure

4.A. Standard Protocol

An example of a standard reaction using [^{35}S]methionine is provided, but [^{35}S]cysteine, [^{3}H]leucine or [^{14}C]leucine can also be used. In general, add 1µl of the radiolabeled amino acid to a 50µl reaction.

If radiolabeled products are not required, omit the radiolabeled amino acid and use a complete amino acid mixture. To create a complete amino acid mixture, combine equal volumes of any two Minus Amino Acid Mixtures. Add 5µl of the complete amino acid mixture per 50µl reaction (see Note 4).

For multiple reactions, create a master mix by combining the appropriate volumes of Amino Acid Mixture Minus Methionine (or Cysteine or Leucine), S30 Premix Without Amino Acids, radiolabeled amino acid (optional), S30 Extract and Nuclease-Free Water. Divide the master mix into 1.5ml aliquots in microcentrifuge tubes and initiate the reactions by adding the DNA template to the tubes.

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- [^{35}S]methionine (1,200Ci/mmol at 15mCi/ml) [optional]

1. Set up the following reactions:

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

2. Vortex gently, then centrifuge in a microcentrifuge for 5 seconds to force the reaction mixture to the bottom of the tube.
3. Incubate the reaction(s) at 37°C for 1–2 hours (see Note 5).
4. Stop the reaction by placing the tubes in an ice bath for 5 minutes.
5. Analyze the results of the reaction. See Sections 5–9 for incorporation assays and gel analysis of proteins.

Notes:

1. Optimize the amount of DNA added. In general, reactions should not contain more than 4µg of DNA. An increased amount of DNA can result in higher incorporation of label but can also increase the number of internal translational starts or prematurely arrested translation products detected. Use the PinPoint™ Control Vector DNA for the positive control reaction. Refer to Section 3.A for a discussion on templates for the reaction.

2. For optimal results, store [^{35}S]methionine in aliquots at –70°C, use once and discard, as ^{35}S-labeled amino acids are easily oxidized to sulfoxides. If synthesizing unlabeled proteins, omit the radiolabeled amino acid and use a complete amino acid mixture. To create a complete amino acid mixture, combine equal volumes of any two Minus Amino Acid Mixtures. Add 5µl of the complete amino acid mixture per 50µl reaction.

3. Template DNA and water purity are extremely important. If efficiencies are low, examine the quality of the template DNA and water. Read the discussion concerning DNA templates in Section 3.A.

4. Use of the PinPoint™ Control Vector DNA template will result in the synthesis of the proteins shown in Figure 1. The largest molecular weight band corresponds to the PinPoint™/CAT fusion protein (39kDa). A prominent band corresponding to β-lactamase (28kDa) migrates below the PinPoint™/CAT fusion. Expression of β-lactamase is significantly higher in the T7 S30 Extract. This is the result of transcription from the T7 promoter upstream of the fusion protein, which reads through the ampicillin resistance gene. Some full-length CAT protein is also observed, which is 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 radiolabel incorporation (see Section 5.A).

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. Enhanced expression at lower temperatures for longer times appears to be gene-/protein-specific and may be tried if the standard reaction at 37°C for 1 hour does not produce the desired results.

6. Transcription by the endogenous *E. coli* RNA polymerase can be inhibited by the addition of the antibiotic rifampicin while transcription by the phage T7 RNA polymerase is unaffected. To inhibit the endogenous RNA polymerase, add 1µl of a 50µg/ml solution of rifampicin in water (see Section 11.A for recipe) prior to the addition of the DNA template to the reaction. Addition of excess rifampicin is unnecessary and may decrease protein synthesis in the extract.

4.A. Standard Protocol (continued)

7. The T7 S30 Extract contains nuclease activity, preventing the use of linear DNA templates such as PCR products in the reaction. PCR products containing a T7 promoter and a ribosome binding site can be expressed using TNT® T7 Quick for PCR DNA (Cat.# L5540). Alternatively, add a small amount (1µl) of the T7 S30 Extract to the *E. coli* S30 Extract for Linear DNA (Cat.# L1030).

4.B. Synthesis and Assay of Control DNA Template

Synthesis from the control template can be measured by a number of means. Incorporation of [^{35}S]methionine should result in the bands shown in Figure 1, lanes 3 and 4. The CAT fusion protein can also be detected by immunological and enzymatic assays.

For immunological and enzymatic assays, synthesize the CAT fusion protein without radiolabeled amino acids. Synthesize unlabeled CAT fusion protein using the positive control reaction from Section 4.A, Step 1, omitting the radiolabeled amino acid and using a complete amino acid mixture. To create a complete amino acid mixture, combine equal volumes of any two minus amino acid mixtures. Add 5µl of the complete amino acid mixture per 50µl reaction. The enzymatic assay should be performed as described in the *CAT Enzyme Assay System With Reporter Lysis Buffer Technical Bulletin* #TB084. Typically, approximately 15–20 units of CAT activity are produced from a standard 50µl reaction.

The immunoblotting assay should be performed as described in Section 6 using 5µl of the control reaction (Section 4.A). Detection with an anti-CAT antibody should detect a band of 39kDa corresponding to the CAT fusion protein and a band of 26kDa corresponding to an internal initiation site in the fusion sequence. Although the fusion protein codes for a sequence that is biotinylated in vivo, this sequence is not efficiently biotinylated in this in vitro system and is only weakly detected by avidin or streptavidin enzyme conjugates. A negative control reaction (i.e., no DNA) is useful to identify nonspecific binding of the antibodies to proteins in the extract.

4.C. Large-Scale Dialysis Reactions Using the *E. coli* S30 Extract Systems

Continuous systems have been described in which inhibitory translation byproducts are removed through a membrane by pumping a feed solution containing amino acids and ribonucleotides through the reaction vessel during the course of a 20- to 30-hour translation reaction (9).

5. TCA Protein Precipitation Assay for Amino Acid Incorporation

Use the following protocol to determine the amount of radiolabeled amino acid incorporated into protein during a typical coupled transcription/translation reaction.

5.A. TCA Precipitation Procedure

Materials to Be Supplied by the User
(Solution compositions are provided in Section 11.A.)

- Whatman® GF/A glass fiber filters
- 1M NaOH (freshly prepared)
- 25% TCA/2% casamino acids (ice-cold)
- 5% TCA (ice-cold)
- acetone

1. Vortex the translation reaction gently. Remove a 5µl aliquot and add it to 245µl of 1M NaOH in a 1.5ml microcentrifuge tube. Mix and incubate at 37°C for 10 minutes. To determine background counts, remove 5µl from a negative control reaction (see Section 4.A, Note 4) and proceed with Steps 2–5.

   Note: The NaOH hydrolyzes aminoacyl tRNAs and prevents labeled tRNA from being included in the incorporation calculation.

2. After 10 minutes, add 1.0ml of ice-cold 25% TCA/2% casamino acids to precipitate the translation products. (The casamino acids act as carriers.) Incubate on ice for 30 minutes.

3. Collect the precipitate by filtering under vacuum on Whatman® GF/A glass fiber filters. Wet the filter with a small amount of ice-cold 5% TCA. Filter the sample and rinse the filter 3 times with 3ml of ice-cold 5% TCA. Rinse once with 1–3ml of acetone. Dry the filter completely at room temperature or at 75°C for 10 minutes.

4. For determination of ^{35}S or ^{14}C incorporation, place the filter in 1–3ml of an appropriate scintillation mixture, invert to mix and count.

   For ^{3}H, put the filter in 1–3ml of an appropriate scintillation mixture, invert to mix and then leave the scintillation vials in the dark for 30 minutes at room temperature prior to counting.

5. To determine total counts present in the translation reaction, spot 5µl of the reaction mix directly onto a Whatman® GF/A glass fiber filter and allow it to dry. Count in a liquid scintillation counter as in Step 4.

5.B. Sample Calculations to Determine Translation Efficiency

1. Calculate total counts in the translation reaction (typical volume is 50µl):

   Total counts in the reaction =

   $$\frac{\text{cpm from Section 5.A, Step 5}}{\text{volume spotted on filter from Section 5.A, Step 5}} \times \text{total reaction volume}$$

   Example: $\frac{1 \times 10^7 \text{ cpm}}{5\mu l} \times 50\mu l = 1 \times 10^8 \text{ cpm}$

2. Calculate the total number of counts incorporated into protein in a standard reaction:

   Total TCA precipitable counts =

   $$\frac{\text{cpm of TCA precipitate on filter (Section 5.A, Step 4)}}{\mu l \text{ of reaction used for TCA precipitation}} \times \text{ total volume}$$

   Example: $\frac{1 \times 10^6 \text{ cpm}}{5\mu l} \times 50\mu l = 1 \times 10^7 \text{ cpm}$

3. Calculate percent incorporation:

   $$\% \text{ incorporation} = \frac{\text{total TCA precipitable counts}}{\text{total counts in reaction}} \times 100$$

   Example: $\frac{1 \times 10^7 \text{ cpm}}{1 \times 10^8 \text{ cpm}} \times 100 = 10\%$ incorporation

6. SDS Polyacrylamide Gel Analysis of Translation Products

The most widely applicable and versatile method for analysis of in vitro translation products is polyacrylamide slab gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (SDS) and a discontinuous buffer system. Precast gels are available from a number of manufacturers. In addition to convenience and safety, precast gels provide consistent results.

6.A. Sample Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 11.A.)

- acetone
- SDS polyacrylamide sample buffer
- Precast SDS polyacrylamide gels (e.g., NOVEX® 14% Tris-Glycine [Invitrogen Cat.# EC64852] and 4–20% Tris-Glycine gradient gels [Invitrogen Cat.# EC60252])

1. Once the S30 Extract reaction is complete (or at any desired time point), remove a 5µl aliquot, add it to 20µl of acetone in a microcentrifuge tube, and place it on ice for 15 minutes. The unused portion of the reaction may be stored at –20°C.

   Note: In Step 1, an acetone precipitation is required to remove PEG from the extract to avoid background staining. However, if 1µl is loaded onto the gel, acetone precipitation is not necessary. The PEG in a 1µl sample is too little to cause background staining.

2. Centrifuge the acetone-precipitated S30 sample at 12,000 × *g* for 5 minutes.

3. Remove the supernatant and dry the pellet for 15 minutes under vacuum.

4. When the pellet is dry, add 20µl of SDS polyacrylamide sample buffer and heat at 100°C for 2–5 minutes. A small aliquot (1–5µl) of the sample may be loaded onto an SDS polyacrylamide gel, or the sample may be stored at –20°C.

6.B. Preparation and Running of SDS Polyacrylamide Gels

For instructions on the preparation and running of SDS polyacrylamide gels, consult references 10 and 11.

Note: Gel banding patterns can be improved by loading unlabeled samples of S30 Extract in the lanes adjacent to the radioactive sample lanes.

1. Load 10µl of the heated sample into the bottom of the wells.

2. Typically, electrophoresis is carried out at a constant current of 15mA in the stacking gel and 30mA in the separating gel. Electrophoresis is usually performed until the bromophenol blue dye front has run off the end of the gel.

6.C. Transfer of Proteins from SDS Polyacrylamide Gels

Materials to Be Supplied by the User
(Solution compositions are provided in Section 11.A.)

- Towbin transfer buffer
- nitrocellulose or PVDF membrane

Transfer proteins from polyacrylamide gels to the membrane by passive diffusion or electroelution. Detailed procedures for electroelution are usually included with commercial devices and also can be found in references 11, 12 and 13. We transfer by electroelution at 4°C at 100V (constant voltage) for 1 hour in 1 liter of transfer buffer (Towbin buffer; 13). SDS can be added to the transfer buffer to a final concentration of 0.5% to facilitate the protein transfer from the gel. After transfer, nitrocellulose (but not PVDF) membranes may be stained with Ponceau S (Sigma Cat.# P7767) to detect molecular weight markers.

Note: To moisten a nitrocellulose membrane, float it in a container of TBS until it is evenly wet, submerge it briefly, and place it on a piece of dry filter paper. Let the excess buffer drain for approximately 5 minutes before use.

6.D. Immunodetection of CAT

Do not allow the membranes to dry out during any of the following steps. Use a shallow container that is slightly larger than the membrane and perform all washing and incubation steps at room temperature with gentle shaking. For antibody incubations and color development reactions, use just enough solution to submerge the membrane, protein side up. Use a larger volume for the washing steps.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 11.A.)

- TBS
- TBS/Tween® 20 (TBST)
- Blot-Qualified BSA (Cat.# W3841)
- anti-CAT primary antibody
- anti-IgG alkaline phosphatase (AP) conjugated antibody
- Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841)

Blocking of Membranes

1. To saturate nonspecific protein binding sites, incubate the membrane in TBST + 1% Blot-Qualified BSA for 30 minutes for nitrocellulose membranes and 60 minutes for PVDF membranes.

Primary Antibody Binding

2. Primary antibodies to CAT are available from several commercial sources. To bind primary antibody, replace the blocking solution (which can be reused several times) with TBST containing the appropriate dilution of primary antibody and incubate for 60 minutes with gentle agitation. Often a 30-minute incubation is sufficient.

3. To remove unbound antibody, wash the membrane three times in TBST for 5–10 minutes each.

Secondary Antibody Binding

4. Transfer the membrane to TBST containing the appropriate dilution of anti-IgG alkaline phosphatase (AP) conjugate and incubate for 30 minutes with gentle agitation (1:5,000 dilution is recommended).

5. Wash the membrane in TBST three times for 5–10 minutes each to remove unbound secondary antibody.

6. Rinse briefly in two changes of TBS to remove Tween® 20 from the membrane surface. Residual Tween® 20 can affect deposition of the precipitated substrate and lead to smearing of bands. Deionized water can be substituted for TBS if it is neutral in pH.

7. Transfer the membrane to 20ml of Western Blue® Stabilized Substrate and incubate until the bands have reached the desired intensity. Reactive areas will turn purple, usually within 1–15 minutes.

8. When the color has developed to the desired intensity, stop the reaction by washing the membrane in deionized water for several minutes, changing the water at least once. The membrane can be photographed while still moist by placing it on top of a damp piece of filter paper on a glass plate. For storage, the membrane should be air-dried on filter paper. The bands and background will fade slightly upon drying. Protect the membrane from light during prolonged storage.

7. Fluorography

Following electrophoresis, labeled protein bands in gels may be visualized by autoradiography or fluorography. Fluorography dramatically increases the sensitivity of detection of ^{35}S-, ^{14}C- and ^{3}H-labeled proteins and is recommended for the analysis of in vitro translation products.

The increased detection sensitivity of fluorography is obtained by infusing an organic scintillant into the gel. The scintillant converts the emitted energy of the isotope to visible light and so increases the proportion of energy that may be detected by X-ray film. Commercial reagents are available that can conveniently be used for fluorographic enhancement of signal. Follow the manufacturer's recommended procedure. After the gel is dried, a 1–4-hour exposure to film (Kodak® X-OMAT® AR) at –70°C will detect the ^{35}S-labeled translation products.

8. Gel Drying

Following staining and the optional treatment for fluorography, dry the gel as follows: Cut a sheet of Whatman® 3MM paper a little larger than the gel. Place the paper under the gel after destaining or fluorography is completed. Transfer the gel and 3MM paper to a vacuum gel drier. Place plastic wrap over the gel and dry for 1 hour at 60°C followed by 1 hour at room temperature.

Note: It is advantageous to cut or mark one corner of the filter to help in discerning the gel orientation on the filter.

Alternatively, the gel may be air-dried using the Gel Drying Kit (Cat.# V7120). Soak the gel in 10% glycerol for 30 minutes to prevent the gel from cracking during drying. Place the gel between two sheets of thoroughly moistened cellulose gel drying film and clamp in the frames. Allow the gel to dry overnight.

9. Autoradiography

Following electrophoresis and drying, labeled protein bands in gels may be visualized by autoradiography. Autoradiography is sufficiently sensitive to detect ^{35}S-labeled translation products using an overnight exposure to film (Kodak® X-OMAT® AR).

9. References

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11. Appendix

11.A. Composition of Buffers and Solutions

destaining solution (per liter)

70ml	glacial acetic acid
930ml	deionized water

Store at room temperature.

1M NaOH

0.4g	NaOH

Bring to a final volume of 10ml with deionized water.

rifampicin stock solution (5ml of 10mg/ml)

50mg	rifampicin

Add DMSO to a final volume of 5ml. Aliquot and store at –20°C. For a working solution, dilute the stock to 50µg/ml in water.

SDS polyacrylamide sample buffer (per 10ml)

2.0ml	glycerol
2.0ml	10% SDS
0.25mg	bromophenol blue
2.5ml	stacking gel 4X buffer
0.5ml	β-mercaptoethanol

Bring to a final volume of 10ml with deionized water. Store at room temperature.

stacking gel 4X buffer (per 100ml)

6.06g	Tris base
4ml	10% SDS

Adjust to pH 6.8 with 12N HCl and add deionized water to a final volume of 100ml. Store at room temperature.

staining solution (per liter)

250ml	isopropanol
100ml	glacial acetic acid
650ml	deionized water
2.5g	Coomassie® brilliant blue R250

Store at room temperature.

TBS/Tween® 20 solution

20mM	Tris-HCl (pH 7.6)
150mM	NaCl
0.5% (v/v)	Tween® 20

25% TCA/2% casamino acids

25% (w/v)	trichloroacetic acid (TCA)
2% (w/v)	casamino acids (Difco Cat.# 0231-17-2)

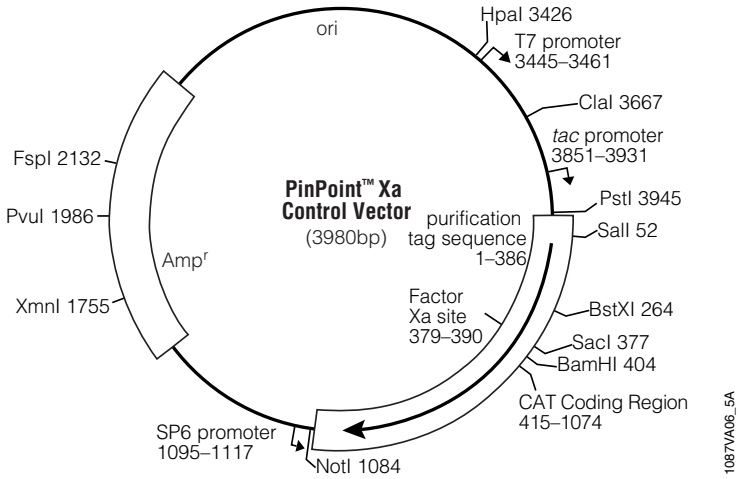
Prepare 500ml and store at 4°C.

Towbin buffer

25mM	Tris-HCl (pH 8.0)
192mM	glycine
20%	methanol

Adjust the final pH to 8.3.

11.B. PinPoint™ Control Vector Circle Map



11.C. Related Products

Product	Size	Cat. #
E. coli S30 Extract System for Linear Templates	30 reactions	L1030
E. coli S30 Extract System for Circular Templates	30 reactions	L1020

Each system contains sufficient reagents to perform 30 × 50µl coupled reactions.

Product	Size	Cat.#
Amino Acid Mixture Minus Leucine (1mM)	175µl	L9951
Amino Acid Mixture Minus Methionine (1mM)	175µl	L9961
Amino Acid Mixture Minus Cysteine (1mM)	175µl	L4471
Amino Acid Mixture Minus Methionine and Cysteine (1mM)	175µl	L5511
Amino Acid Mixture, Complete (1mM)	175µl	L4461

For Laboratory Use.

Product	Cat.#
Transcend™ Colorimetric Non-Radioactive Translation Detection System	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System	L5080

For Laboratory Use. Each system contains sufficient reagents to label 30 × 50µl translation reactions and perform detection of biotinylated proteins on 6 blots (7 × 9cm).

Product	Size	Cat.#
Transcend™ tRNA	30µl	L5061

For Laboratory Use. Thirty microliters of Transcend™ tRNA is sufficient for 30 × 50µl translation reactions.

Product	Size	Cat.#
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System	40 reactions	L5001

For Laboratory Use.

Product	Size	Cat.#
TNT® SP6 Quick Coupled Transcription/ Translation System	40 reactions	L2080
	5 reactions	L2081
TNT® T7 Quick Coupled Transcription/ Translation System	40 reactions	L1170
	5 reactions	L1171
TNT® T7 Quick for PCR DNA	40 reactions	L5540
TNT® T3 Coupled Reticulocyte Lysate System	40 reactions	L4950
TNT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610
	8 reactions	L4611
TNT® SP6 Coupled Reticulocyte Lysate System	40 reactions	L4600
	8 reactions	L4601
TNT® T7/SP6 Coupled Reticulocyte Lysate System	40 reactions	L5020
TNT® T7/T3 Coupled Reticulocyte Lysate System	40 reactions	L5010
TNT® T3 Coupled Wheat Germ Extract System	40 reactions	L4120
TNT® T7 Coupled Wheat Germ Extract System	40 reactions	L4140
TNT® SP6 Coupled Wheat Germ Extract System	40 reactions	L4130
TNT® T7/SP6 Coupled Wheat Germ Extract System	40 reactions	L5030
TNT® T7/T3 Coupled Wheat Germ Extract System	40 reactions	L5040

For Laboratory Use.

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

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