TECHNICAL BULLETIN

E. coli S30 Extract System for Circular DNA

Instructions for Use of Product
L1020

Revised 3/15
TB092

E. coli S30 Extract System for Circular DNA

All technical literature is available at: www.promega.com/protocols/
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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The *E. coli* S30 Extract System for Circular DNA simplifies the transcription/translation of DNA sequences cloned in plasmid or lambda vectors, providing a powerful tool to identify and characterize polypeptides (Figure 1). The investigator needs only to supply the cloned DNA containing the appropriate prokaryotic promoter and ribosome binding sites. The *E. coli* S30 Extract System for Circular DNA is prepared by modifications of the method described by Zubay (1,2) from an *E. coli* strain B deficient in OmpT endoproteinase and lon protease activity. This results in greater stability of expressed proteins that would otherwise be degraded by proteases if expressed in vivo (3,4). The S30 in vitro system also allows higher expression of proteins that are normally expressed at low levels in vivo due to the action of host-encoded repressors (5).

The S30 System for Circular DNA 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, pBEST*luc*™ Vector (Figure 2), containing the eukaryotic firefly luciferase gene positioned downstream from the *tac* promoter and a ribosome binding site. Expression of the luciferase gene in the S30 System for Circular DNA can be easily assayed by non-radioactive methods using the provided Luciferase Assay Reagent (6–8). The assay produces high light output for several minutes, allowing the researcher to choose from several methods of detection (7–10), including simple visual observation of luminescence.

The most common application of the S30 Extract is the synthesis of small amounts of radiolabeled protein. Synthesis of a protein of the correct size is a useful means to verify the gene product of a particular DNA sequence. Proteins expressed in the *E. coli* S30 Extract System for Circular DNA also may be used for a variety of functional studies of transcription and translation (11). Some additional applications of the S30 Extract include synthesis of small amounts of radiolabeled protein for use as a tracer in protein purification (11), incorporation of unnatural amino acids into proteins for structural studies (12) and screening of compounds that affect translation.

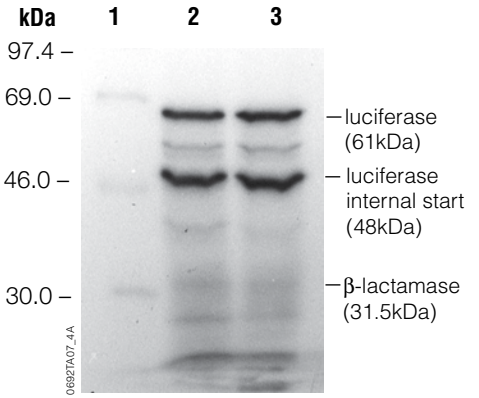

Figure 1. Coupled in vitro transcription/translation of circular DNA templates using the *E. coli* S30 Extract System for Circular DNA. Five microliters of each 50µl reaction mix was loaded in each lane. Lanes 2 and 3 show protein products synthesized from 2µg of pBEST*luc*™ DNA. Full-length luciferase migrates at 61kDa. An apparent internal translation start results in a second major gene product of 48kDa. β-lactamase migrates at 31.5kDa. Lane 1 shows molecular weight markers; lanes 2 and 3 are duplicate translation reactions.

2. Product Components and Storage Conditions

PRODUCT	CAT.#
***E. coli* S30 Extract System for Circular DNA**	**L1020**

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

- 175µl Amino Acid Mixture Minus Cysteine, 1mM
- 175µl Amino Acid Mixture Minus Methionine, 1mM
- 175µl Amino Acid Mixture Minus Leucine, 1mM
- 450µl S30 Extract, Circular (3 × 150µl)
- 750µl S30 Premix Without Amino Acids
- 250µl Luciferase Assay Reagent
- 1ml Luciferase Dilution Reagent
- 10µl pBEST*luc*™ DNA, Circular (1µg/µl)

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

Notes: Please see Related Products (Section 12.B) for information on Amino Acid Mixture, Complete (Cat.# L4461), and Amino Acid Mixture Minus Methionine and Cysteine (Cat.# L5511).

Bulk quantities (over 50ml) of S30 Extract and S30 Premix are available.

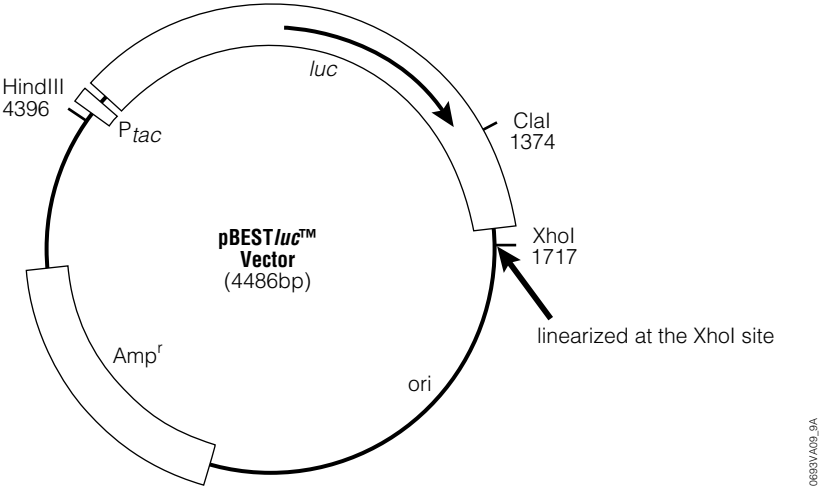

Figure 2. Circle map of pBEST*luc*™ Vector.

3. General Considerations

3.A. Template Considerations

Expression of cloned DNA fragments in the *E. coli* S30 Extract System for Circular DNA requires that the gene be under the control of a good *E. coli* promoter. Examples of such promoters include the lambda P_R, lambda P_L, *tac*, *trc* and *lac*UV5.

It is important to emphasize that large differences in gene expression result from the context of the gene. Changes in the position of the gene relative to the ribosomal binding site (RBS) will affect expression levels. 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 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).

Use highly purified DNA templates (e.g., CsCl- or gel-purified). Avoid adding excessive salts or glycerol with the DNA template. The activity of the S30 System may be inhibited by NaCl ≥50mM, glycerol ≥1% or very small amounts of magnesium or potassium salts. Precipitate the DNA template with sodium acetate rather than ammonium acetate. Protein yields from the S30 System vary with the template and conditions used. Typical yields are 50–250ng per 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 also are available separately (see Section 12.B). Radiolabeled proteins can be detected by standard methods, such as autoradiography or detection with a phosphorimaging instrument.

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 to label with [^{35}S]methionine or other 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 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 within 6–12 hours after gel electrophoresis. For more information on the Transcend™ Systems, consult Technical Bulletin #TB182.

Another non-radioactive option, the FluoroTect™ System, uses a charged lysine tRNA labeled with the fluorophore, BODIPY®-FL, to incorporate fluorescently labeled lysine residues into the in vitro translation product. For more information on the FluoroTect™ System, consult Technical Bulletin #TB285.

Note: Technical Manuals and Bulletins are available online at: **www.promega.com/protocols/**

4. Standard Coupled Transcription/Translation Protocol

An example of a standard reaction using [^{35}S]methionine is provided. [^{35}S]cysteine, [^{3}H]leucine or [^{14}C]leucine also an 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 3).

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. Dispense the master mix into aliquots in 1.5ml 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) (PerkinElmer EasyTag™ L-[^{35}S]methionine, Cat.# NE-G709A) [optional]

1. Set up the following reactions:

Component	Standard Reaction	Positive Control (See Note 3)
DNA template (see Notes 1 and 2)	≤2µg	2µl
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) (See Notes 3 and 4)	1µl	1µl
S30 Extract, Circular (mix gently prior to use)	15µl	15µl
Nuclease-Free Water to a final volume of: (See Note 2)	**50µl**	**50µl**

2. Vortex gently, then centrifuge in a microcentrifuge for 5 seconds to bring the reaction mixture to the bottom of the tube.
3. Incubate the reactions at 37°C for 60 minutes (see Note 5).
4. Stop the reactions by placing the tubes in an ice bath for 5 minutes.
5. Analyze the results of the reaction. See Sections 6–10 for incorporation assays and gel analysis of proteins.

Notes: (continued on next page)

4. Standard Coupled Transcription/Translation Protocol (continued)

Notes:

1. Optimize the amount of DNA added. In general, reactions should not contain more than 2µg of DNA. An increased amount of DNA can result in higher incorporation of label but also can increase the number of internal translational starts or prematurely arrested translation products. Use pBEST*luc*™ DNA for the positive control reaction. Refer to Section 3.A for a discussion on templates for the reaction.
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 (see Figure 2 for a plasmid map). Luciferase migrates at 61kDa. An apparent internal translation start results in a second major gene product of 48kDa. The control plasmid also contains the gene for ampicillin resistance (β-lactamase). β-lactamase may appear as a faint band migrating at 31.5kDa. See Figure 1 for typical results.

   Unlabeled luciferase is used in a luminescence assay to monitor the efficiency of the S30 reaction (Section 5). To generate unlabeled luciferase, use a complete amino acid mixture rather than a minus amino acid mixture and omit the radiolabeled amino acid (see Section 5).

   For a negative control, omit the DNA from the reaction. Use the negative control to determine background radiolabel incorporation (see Section 6.A).
4. We recommend using a grade of [^{35}S]methionine, such as PerkinElmer EasyTag™ L-[^{35}S]methionine, Cat.# NEG709A). This [^{35}S]methionine may be stored at 4°C without dispensing into aliquots. Other types of ^{35}S-labeled amino acids may be oxidized easily to translation-inhibiting sulfoxides and should be stored in aliquots at –70°C in buffer containing DTT.
5. Reactions 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. 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.

5. Synthesis and Assay of Luciferase Control

The gene encoding firefly luciferase is highly effective to study gene function and cellular events. Luciferase assays are extremely sensitive, rapid, easy to perform and relatively inexpensive. For information on the Luciferase Assay System, consult Technical Bulletin #TB281.

A total luciferase assay volume of 60–70µl is convenient for most light-measurement devices. For some methods, such as using film to detect luciferase activity, a larger volume may be desired. Also, many scintillation counters and luminometers may operate well using a smaller volume. Changes in the total assay volume, while maintaining the ratio of sample volume to assay reagent volume, should not affect the properties of the assay.

5.A. Synthesis of Luciferase Control

1. Synthesize unlabeled luciferase using the following reaction:

Component	Volume
pBEST*luc*™ DNA (1µg/µl)	2µl
Complete Amino Acid Mixture* (mix gently prior to use)	5µl
S30 Premix Without Amino Acids (mix gently prior to use)	20µl
S30 Extract, Circular (mix gently prior to use)	15µl
Nuclease-Free Water** to a final volume of:	**50µl**
*To create a complete amino acid mixture, combine equal volumes of any two Minus Amino Acid Mixtures. **Water purity is extremely important. If translation efficiencies are low, examine the water quality.	

2. Vortex gently, then centrifuge in a microcentrifuge for 5 seconds to bring the reaction mixture to the bottom of the tube.
3. Incubate the reaction at 37°C for 60 minutes (see Section 4, Note 5).
4. Stop the reaction by placing the tube in an ice bath for 5 minutes.
5. Prepare a dilution series as follows:
   a. At room temperature, add 50µl of Luciferase Dilution Reagent to each of four microcentrifuge tubes. If samples are to be quantitated in a scintillation counter, further dilutions (five- to tenfold) using Luciferase Dilution Reagent may be needed, as these instruments experience signal saturation at high light intensities.
   b. Add 50µl of the luciferase S30 control reaction to the first tube (this results in a twofold dilution), 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 (e.g., Thermo Electron Microlite™ plate). The remaining dilutions may be stored at –20°C for several months with little loss of luciferase activity.
7. Measure luminescence by luminometry or scintillation counting (Section 5.B), photography (Section 5.C) or visual detection (Section 5.D).

5.B. Standard Luciferase Assay (Luminometer or Scintillation Counter)

Luminometer

Because of the constant light output with the Luciferase Assay System, an automated injection device is not required. In many luminometers, the photo-multiplier tube requires 1–2 seconds to stabilize after a sample is introduced. Therefore allow an initial delay of at least 3 seconds, then measure luminescence for 10 seconds to 5 minutes.

Promega offers several luminometers in single-tube and microplate format. The features of these luminometers make them an excellent choice to measure luminescence both in cell extracts and living cells expressing luciferase.

Scintillation Counter

Scintillation counters should be used in the manual mode because light generated by the luminescent reaction decays slowly ($t_{½}$ = approximately 5 minutes). Samples should be introduced into the counting chamber shortly after light production is initiated. Because the enzymatic reaction produces light at all wavelengths, samples to be quantitated in a scintillation counter should be measured with all channels open ("open window"). Measure light produced for a period of 10 seconds to 6 minutes. The light intensity of the reaction is nearly constant for about 20 seconds, then decays slowly, with a half-life of about 5 minutes.

Ideally, the coincidence circuit of the scintillation counter should be turned off. Usually this is achieved through an option of the programming menu or by a switch on the instrument. If the circuit cannot be turned off, a linear relationship between luciferase concentration and cpm can be produced by calculating the square root of the measured cpm minus background cpm (i.e., $[\text{sample} - \text{background}]^{½}$). To measure background cpm, read a water blank. To measure activity in your sample, place the sample in a microcentrifuge tube, then place the tube in the scintillation vial. Do not add scintillant; it will inactivate the luciferase.

It may be necessary to make a significant dilution of the sample (for a first check, dilute the sample 1:10,000 in water containing 1mg/ml BSA). If the photomultiplier tubes are saturated by too much light output, the scintillation counter may either produce no reading or identical readings from dilution to dilution.

Protocol

1. Add 50µl of **room-temperature** Luciferase Assay Reagent (LAR) to each aliquot, and mix quickly by pipetting. We recommend that the sample to be assayed also be at room temperature.

   Note: Light intensity is a measure of the rate of catalysis by luciferase and is dependent upon temperature. Reproducible luciferase assay readings will result with an assay temperature of 20–25°C.

2. Place the reaction in the luminometer or scintillation counter (place the microcentrifuge tube inside the scintillation vial). Consult the appropriate operator's manual for operation of the luminometer or scintillation counter.

   Important: Fully equilibrate the LAR to 25°C before beginning measurements. This can be accomplished by placing the tube containing the LAR into a container of room-temperature water for 30 minutes before use.

5.C. Photographic Luciferase Assay

1. Prepare a Polaroid® camera for a 6-minute exposure. Either a hand-held (IBI Quickshooter model QSP) or an overhead-positioned Polaroid® camera is acceptable. Position the camera over a white 96-well plate, and focus on the top rim of the well. Open the aperture as wide as possible (e.g., f4.5), and set the shutter speed to the bulb, or B, setting. Make sure the camera is loaded with Polaroid® 667 (ISO 3,000) film.
2. Add 50µl of **room-temperature** Luciferase Assay Reagent to each sample, and mix quickly by pipetting.
3. Immediately turn off all lights (including red darkroom lights), and set the camera for a 6-minute exposure.
4. The photographic assay is sensitive in the 1–10ng luciferase range using these conditions. The sensitivity of this assay is directly related to the size, depth and "whiteness" of the reaction chamber.

5.D. Qualitative Visual Detection of Luciferase Activity

1. Add 50µl of **room-temperature** Luciferase Assay Reagent to each sample, and mix quickly by pipetting.
2. Turn off all lights. For qualitative determination of luciferase activity, the reactions may be visualized by eye in a dark room after acclimation to the dark.

   Note: Most individuals should be able to see the reaction after a minute or two of acclimation, although individuals may differ in their ability to detect these low light levels. The visual intensity of the luciferase reaction is increased by using a white reaction chamber.

6. 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.

6.A. TCA Precipitation Procedure

Materials to Be Supplied by the User
(Solution compositions are provided in Section 12.A.)

- 1M NaOH (freshly prepared)
- 25% TCA/2% casamino acids (ice-cold)
- 5% TCA (ice-cold)
- Whatman® GF/C glass fiber filter (Whatman® Cat.# 1822A021)
- 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.

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

   To determine background counts, remove 5µl from a negative control reaction (see Section 4, Note 3), and proceed with Steps 2–5.
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.

6.A. TCA Precipitation Procedure (continued)

3. Collect the precipitate by filtering under vacuum on Whatman® GF/C 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. To determine ^{35}S or ^{14}C incorporation, put the filter in 1–3ml of an appropriate scintillation mixture, invert to mix and count.

   To measure ^{3}H incorporation, put the filter in 1–3ml of an appropriate scintillation mixture, invert to mix, 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/C glass fiber filter, and allow it to dry. Count in a liquid scintillation counter as in Step 4.

6.B. Sample Calculations to Determine Translation Efficiency

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

$$\text{Total counts in reaction} = \frac{\text{cpm from Section 6A, Step 5}}{\text{volume spotted on filter from Section 6A, Step 5}} \times \text{total reaction volume}$$

   Example:

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

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

$$\text{Total TCA-precipitable counts} = \frac{\text{cpm of TCA precipitated on filter (Secion 6A, Step 4)}}{\mu\text{l of reaction used for TCA precipitation}} \times \text{total volume}$$

   Example:

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

3. Calculate percent incorporation:

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

   Example:

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

7. SDS-PAGE Analysis of Translation Products

The most widely applicable and versatile method to analyze cell-free translation products is polyacrylamide slab gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (SDS) and a discontinuous buffer system. A 15% acrylamide separating gel gives good separation of peptide mixtures between 20,000 and 100,000kDa, with peptides between 55,000 and 60,000kDa migrating halfway down the length of the gel.

7.A. Sample Preparation

Materials to Be Supplied by the User
(Solution compositions are provided in Section 12.A.)

- acetone
- SDS-PAGE sample buffer

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: An acetone precipitation is required to remove PEG from the extract; PEG will result in background staining and "wavy" banding patterns.

2. Centrifuge the acetone-precipitated 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-PAGE sample buffer, and heat at 100°C for 2–5 minutes. A small aliquot of the sample may be loaded onto an SDS gel, or the sample may be stored at –20°C.

7.B. Preparation and Running of SDS Polyacrylamide Gels

For instructions to prepare and run an SDS-PAGE gels, consult references 13 and 14.

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 bottom of the gel.

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

7.C. Staining SDS Polyacrylamide Gels

Materials to Be Supplied by the User
(Solution compositions are provided in Section 12.A.)
- staining solution
- destaining solution

After electrophoresis, protein bands in the gel may be visualized by staining with Coomassie® blue dye. However, Coomassie® staining is usually not sensitive enough to detect translation products and thus need not be performed before analyzing gel results by fluorography. The staining and destaining steps do, however, help to wash out unincorporated labeled amino acids at the gel dye front.

1. Stain with gentle agitation until the dye has penetrated the gel (15–30 minutes).
2. Dye that is not bound to protein is removed by transferring the gel to destaining solution. Add a wad of laboratory tissues to absorb excess stain, and gently agitate in this solution until bands are clearly visible (changing the solution may be required).

8. 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 to analyze 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- to 4-hour exposure to film (Kodak® X-OMAT®) at –70°C will detect the ^{35}S-labeled translation products.

9. 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 itself. Place this under the gel once destaining or fluorography is complete. Transfer the gel and 3MM paper to a vacuum gel drier. Place plastic wrap over the gel, and dry it for 1 hour at 60°C followed by 1 hour at room temperature.

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.

10. Autoradiography

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

11. References

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12. Appendix

12.A. Composition of Buffers and Solutions

1M NaOH

0.4g	NaOH

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

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.

destaining solution (per liter)

70ml	glacial acetic acid
930ml	deionized water

Store at room temperature.

Luciferase Dilution Reagent

25mM	Tris-phosphate (pH 7.8)
2mM	DTT
2mM	1,2-diaminocyclohexane N, N, N´, N´-tetraacetic acid
10%	glycerol
1%	Triton® X-100
1mg/ml	BSA

SDS-PAGE sample buffer

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

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.

12.B. Related Products

Product	Size	Cat.#
E. coli S30 Extract System for Linear Templates	450µl	L1030
E. coli T7 S30 Extract System for Circular DNA	450µl	L1130

Sufficient reagents are provided for 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

Product	Size	Cat.#
Streptavidin-Alkaline Phosphatase	0.5ml	V5591

Product	Size	Cat.#
TnT® SP6 High-Yield Protein Expression System	40 reactions	L3260
	10 reactions	L3261
TnT® T7 Quick for PCR DNA	40 reactions	L5540
TnT® SP6 Quick Coupled Transcription/Translation System	40 reactions	L2080
TnT® SP6 Quick Coupled Transcription/Translation System Trial Size	5 reactions	L2081
TnT® T7 Quick Coupled Transcription/Translation System	40 reactions	L1170
TnT® T7 Quick Coupled Transcription/Translation System Trial Size	5 reactions	L1171
TnT® T3 Coupled Reticulocyte Lysate System	40 reactions	L4950
TnT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610
TnT® T7 Coupled Reticulocyte Lysate System Trial Size	8 reactions	L4611
TnT® SP6 Coupled Reticulocyte Lysate System	40 reactions	L4600
TnT® SP6 Coupled Reticulocyte Lysate System Trial Size	8 reactions	L4601
TnT® T7/SP6 Coupled Reticulocyte Lysate System	20 reactions of each	L5020
TnT® T7/T3 Coupled Reticulocyte Lysate System	20 reactions of each	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

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

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

Thirty microliters of Transcend™ tRNA are sufficient for 30 × 50µl translation reactions.

Product	Size	Cat.#
FluoroTect™ GreenLys in vitro Translation Labeling System	40 reactions	L5001

12.B. Related Products (continued)

Product	Cat.#
Gel Drying Kit, 17.5 × 20cm capacity	V7120
Gel Drying Film, 25.5 × 28cm	V7131

13. Summary of Changes

The following change was made at the 3/15 revision of this document:

The patent information was updated to remove expired statements.