Technical Bulletin

Transcend™ Non-Radioactive Translation Detection Systems

INSTRUCTIONS FOR USE OF PRODUCTS L5061, L5070 AND L5080.

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Transcend™ Non-Radioactive Translation Detection Systems

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1. Description

The Transcend™ Non-Radioactive Translation Detection Systems allow non-radioactive detection of proteins synthesized in vitro. Using these systems, biotinylated lysine residues are incorporated into nascent proteins during translation, eliminating the need for labeling 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 (Figure 1). After SDS-PAGE and electroblotting, the biotinylated proteins can be visualized by binding

either Streptavidin-Alkaline Phosphatase (Streptavidin-AP) or Streptavidin-Horseradish Peroxidase (Streptavidin-HRP), followed by either colorimetric or chemiluminescent detection. Typically, 0.5–5ng of protein can be detected by these methods within 3–4 hours after gel electrophoresis (Figure 2).

The use of Transcend™ tRNA offers several advantages:

- No radioisotope handling, storage or disposal is needed.
- The biotin tag is sensitive (0.5–5ng).
- The biotin tag is stable for 12 months, both as the Transcend™ tRNA reagent and within the labeled proteins. It is not necessary to periodically resynthesize biotin-labeled proteins, unlike ^{35}S-labeled proteins whose label decays over time.
- Results can be visualized quickly using either colorimetric or chemiluminescent detection.

The precharged *E. coli* lysine tRNAs provided in this system have been chemically biotinylated at the ε-amino group using a modification of the methodology developed by Johnson *et al.* (1). The biotin moiety is linked to lysine by a spacer arm, which greatly facilitates detection by avidin/streptavidin reagents (Figure 1). The resulting biotinylated lysine tRNA molecule (Transcend™ tRNA) can be used in either eukaryotic or prokaryotic in vitro translation systems such as the TNT® Coupled Transcription/Translation Systems, Rabbit Reticulocyte Lysate System or *E. coli* S30 Extract System (2,3). Lysine is one of the more frequently used amino acids. On average, lysine represents 6.6% of a proteins amino acid content, whereas methionine represents only 1.7% (4).

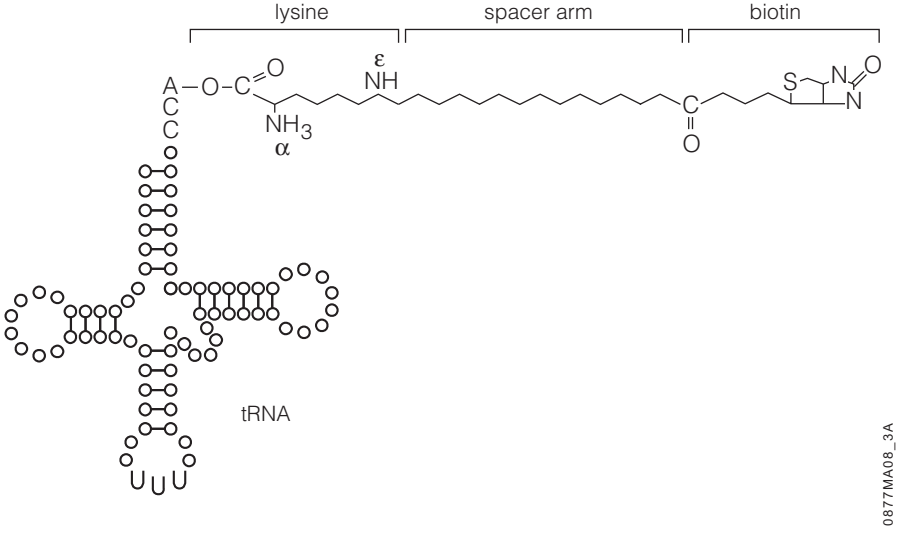

Figure 1. Structure of Transcend™ tRNA.

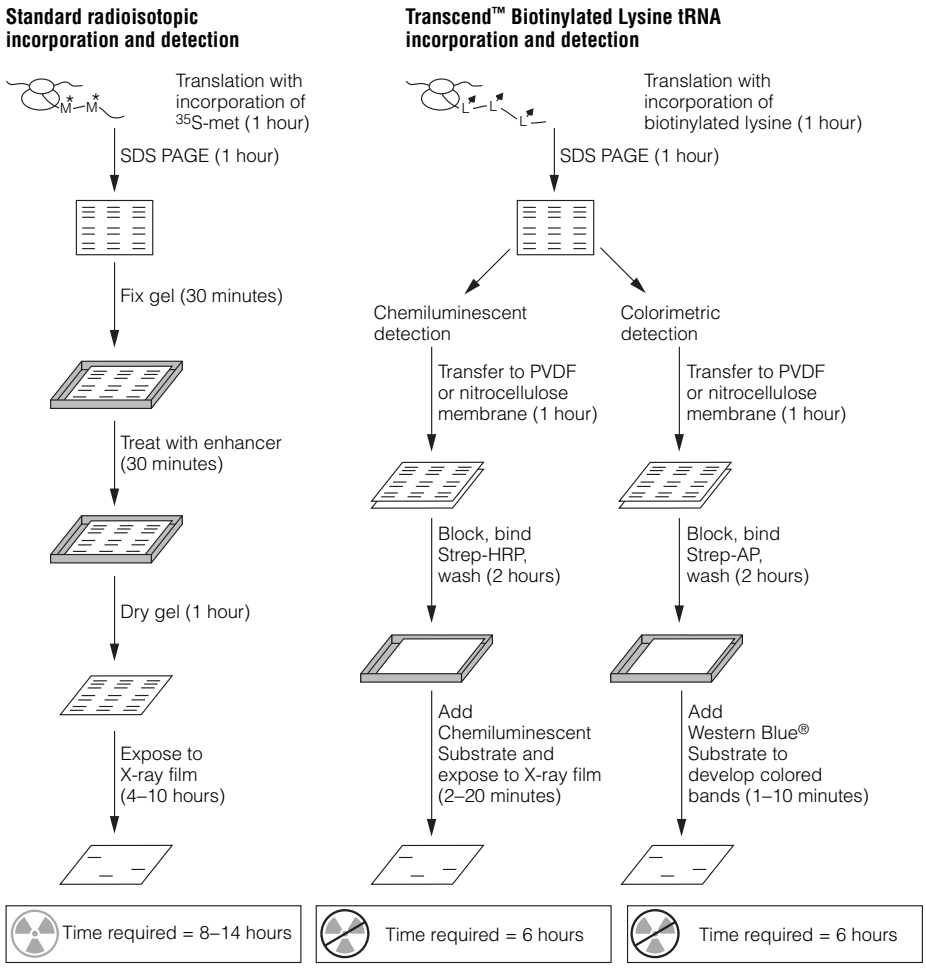

Figure 2. Comparison of incorporation and detection protocols using radiolabeled amino acids or Transcend™ tRNA.

2. General Considerations

2.A. Effect of Biotinylated Lysine Incorporation on Expression Levels and Enzyme Activity

Lysine residues are common in most proteins and usually are exposed at the aqueous-facing exterior. The presence of biotinylated lysines may or may not affect the function of the modified protein. For example, the enzymatic activity of β-galactosidase is reduced by the incorporation of biotinylated lysines, but luciferase and chloramphenicol acetyltransferase (CAT) are less affected (Figure 3 and Table 1). In gel shift experiments, c-Jun synthesized in TNT® Coupled Reticulocyte Lysate reactions and labeled with Transcend™ tRNA performed identically to unlabeled c-Jun (3).

2.B. Estimating Incorporation Levels of Biotinylated Lysine in Transcend™ Reactions

The incorporation of radioactively labeled amino acids into proteins is typically quantitated as a percent incorporation of the label added. This value can include incorporation of radioactivity into spurious gene products such as truncated polypeptides. Thus, percent incorporation values provide only a rough estimate of the amount of full-length protein synthesized and do not provide any information on translation fidelity. With Transcend™ reactions, it is difficult to determine the percent incorporation of biotinyl-lysines into a translated protein directly. An alternative means of estimating translation efficiency and fidelity in Transcend™ reactions is to determine the minimum amount of product detectable after SDS-polyacrylamide gel electrophoresis. We have been able to detect proteins in 1µl of translation reaction using as little as 0.5µl of Transcend™ tRNA per 50µl reaction (Figure 3). The amount of biotin incorporated into translation products increases linearly with the amount of Transcend™ tRNA added to the reaction, up to a maximum of about 2µl.

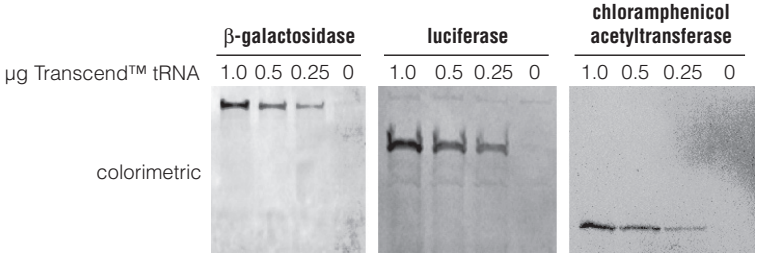

Figure 3. Effects of Transcend™ tRNA concentration on detection of proteins synthesized in vitro. TNT® Coupled Transcription/Translation reactions were performed according to the directions (5). The indicated amounts of Transcend™ tRNA (equivalent to 2.0, 1.0, 0.5 or 0µl) were added to the translation reactions prior to incubation at 30°C for 1 hour. One microliter of the reaction was used for SDS-PAGE. The separated proteins were transferred to PVDF membrane (100V for 1 hour). The membrane was blocked in TBS + 0.5% Tween® 20 (TBST) for 1 hour, probed with Streptavidin-AP (45 minutes), washed twice with TBST and twice with water, and incubated with Western Blue® Substrate for 2 minutes.

Although we have not determined the ratio of moles of biotin incorporated per mole of protein, experiments by Crowley *et al.* using fluorescently labeled tRNA (ε-NBD-[^{14}C]lysine tRNA) indicate that 25–33% of lysine residues are biotinylated in translation products that contain 4–6 lysine residues (6). Although proteins vary in lysine composition and each molecule synthesized in the translation reaction will contain a different number of biotinylated lysines, it is reasonable to expect that similar levels of labeling (20–35% of lysines) will be obtained with other proteins.

Table 1. Effect of Biotinylated Lysine Incorporation on Levels of Synthesis and Enzymatic Activity of Three Reporter Genes Expressed in the TNT® Reticulocyte Lysate System.

Transcend™ tRNA Added to Reaction	Level of Synthesis[1] (% of Control Reaction)	Biotin Signal Strength[2] (Colorimetric)	Enzymatic Activity[3] (% of Control Reaction)
Luciferase (40 total lysines)			
0.5µl	101	44	101
1.0µl	93	136	93
2.0µl	78	158	94
CAT (12 total lysines)			
0.5µl	95	13	105
1.0µl	92	70	–
2.0µl	73	136	115
β-galactosidase (20 total lysines)			
0.5µl	82	42	53
1.0µl	70	82	61
2.0µl	70	184	61

These data, taken from a single experiment, are representative of results routinely obtained in our laboratories.

[1] [^{35}S]methionine incorporation as a percent of control translation reaction containing no Transcend™ tRNA.

[2] Western Blue® Stabilized Substrate (BCIP/NBT) colorimetric detection of bound Streptavidin-AP expressed in arbitrary intensity units obtained with an AMBIS™ Image Analysis System.

[3] Expressed as a percent of activity obtained with a control translation reaction containing no Transcend™ tRNA. Luciferase activity was determined according to reference 7. CAT activity was determined according to reference 8. β-galactosidase activity was quantitated using a chemiluminescent assay (9). Equal volumes of TNT® translation reaction products were used in all assays.

3. Product Components and Storage Conditions

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

Each system contains sufficient reagents to label 30 × 50µl translation reactions and perform colorimetric detection of biotinylated proteins on 6 blots (7 × 9cm) using Streptavidin-AP Conjugate and Western Blue® Substrate. Includes:

- 30µl Transcend™ tRNA
- 36µl Streptavidin-AP
- 35ml Western Blue® Stabilized Substrate for Alkaline Phosphatase

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

Each system contains sufficient reagents to label 30 × 50µl translation reactions and perform chemiluminescent detection of biotinylated proteins on 6 blots (7 × 9cm) using Streptavidin-HRP Conjugate and Transcend™ Chemiluminescent Substrate. Includes:

- 30µl Transcend™ tRNA
- 15µl Streptavidin-HRP, 0.25mg/ml
- 25ml Transcend™ Chemiluminescent Substrate A
- 25ml Transcend™ Chemiluminescent Substrate B

Product	Size	Cat. #
Transcend™ tRNA	30µl	L5061

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

Note: Transcend™ tRNA (Cat.# L5061) is shipped separately from the other components due to differences in storage temperatures.

Storage Conditions: Store Transcend™ tRNA at –70°C. Do not subject Transcend™ tRNA to more than 5 freeze-thaw cycles and quickly return it to –70°C after use.

Store all other components at 4°C. **Do not freeze Western Blue® Substrate or Streptavidin-Alkaline Phosphatase.**

4. Biotinylated Lysine Incorporation Using Transcend™ tRNA

4.A. Translation Protocol

Materials to Be Supplied by the User

- RNasin® Ribonuclease Inhibitor (Cat.# N2111) or Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511)
- Nuclease-Free Water (Cat.# P1193)
- translation extract (e.g., Rabbit Reticulocyte Lysate, *E. coli* S30 Extract, TNT® Coupled Transcription/Translation Systems; see Note 1 at the end of this section)
- salts, DTT and other components as needed to optimize translation reaction
- complete amino acid mix or a combination of two minus amino acid mixes

Use the following protocol as a guideline for setting up a translation reaction using Transcend™ tRNA. In general, Transcend™ tRNA may be used in an in vitro translation protocol at a concentration of 1–2µl Transcend™ tRNA per 50µl reaction. An example of a standard reaction for Rabbit Reticulocyte Lysate is provided. For more information on specific in vitro translation systems, please see Section 4.B.

1. Remove the translation and Transcend™ tRNA reagents from storage at –70°C. Thaw the Transcend™ tRNA on ice. Thaw the translation lysate by hand warming and immediately place on ice. The other components can be thawed at 37°C and then stored on ice as soon as they thaw.

   Note: Do not subject Transcend™ tRNA to more than 5 freeze-thaw cycles. If necessary, store Transcend™ tRNA in multiple aliquots at –70°C.

2. On ice, set up 50µl translation reactions as you would for radioactive amino acid incorporation, with the following exception: Add 1µl of a complete amino acid mix (containing 1mM of each amino acid) or a combination of two minus amino acid mixtures (such as 0.5µl of minus methionine and 0.5µl of minus leucine).

   Note: In the *E. coli* S30 Extract Systems, the use of a minus lysine amino acid mixture may significantly reduce the yield of translation product.

Example of a Standard Rabbit Reticulocyte Lysate Reaction Using Transcend™ tRNA.

Rabbit Reticulocyte Lysate	35µl
Nuclease-Free Water	10µl
RNasin® Ribonuclease Inhibitor (40u/µl)	1µl
1mM complete amino acid mixture (or mixture of two minus amino acid mixtures)	1µl
RNA template in Nuclease-Free Water (see Note 3 at the end of this section)	2µl
Transcend™ tRNA (see Note 2 at the end of this section)	1–2µl
final volume	50µl

3. Add all components except the Transcend™ tRNA and gently mix by pipetting the reaction while stirring the reaction with the pipette tip. If necessary, spin briefly in a microcentrifuge to return the sample to the bottom of the tube. Add the Transcend™ tRNA.

   Note: We recommend including a control reaction containing Transcend™ tRNA but no added nucleic acid template. This allows measurement of any background incorporation contributed by endogenous mRNA and also reveals any endogenous biotinylated protein(s) in the translation extract (see Note 1 at the end of this section). If using a wheat germ extract, this is especially important.

4. Immediately incubate the translation reaction at 30°C for 60 minutes (see Note 4).

5. Terminate the reaction by placing on ice. If necessary, the translation reaction mix can be stored for several months at –20 to –70°C.

6. Analyze the results of translation. Procedures for gel analysis of translation products are provided in Section 5.

Notes:

1. Commonly used translation extracts contain endogenous biotinylated proteins, which may be detected when translation products are analyzed by SDS-PAGE, electroblotting and Streptavidin-AP/Streptavidin-HRP detection. Rabbit Reticulocyte Lysate contains one biotinylated protein, which migrates as a faint band at 100kDa and, in some lots, an additional very faint band at 47kDa. *E. coli* S30 Extract contains one endogenous protein, migrating at 22.5kDa. Wheat Germ Extract contains five major endogenous biotinylated proteins, migrating at 200kDa, 80kDa, 32kDa and a doublet at 17kDa. A comparison to a no-template control will distinguish the endogenous biotinylated protein(s) from the newly synthesized biotinylated translation product.

2. Biotin labeling of poorly expressed proteins or proteins containing few lysines can be increased by doubling the amount of Transcend™ tRNA added per 50µl translation reaction (Table 1 and Figure 3).

3. For maximal expression of your protein, optimize the amount of template added to the reaction and use highly purified RNA or DNA, depending on the translation system used.

   Translation: An unfractionated cytoplasmic RNA preparation is 60–70% rRNA and, as a result, translates poorly. Usually such preparations yield no better than 20–30% of the maximum incorporation attainable, and concentrations of 100–200µg/ml (final concentration) are needed to stimulate translation. In contrast, viral RNAs and poly(A)+ mRNAs (including mRNA transcribed in vitro) can be used at much lower concentrations (5–80µg/ml final concentration).

Increased expression levels may be obtained by optimizing the Mg^{2+} and K^+ concentrations in the translation reaction or by using a TNT® Coupled Transcription/Translation System or a TNT® Quick Coupled Transcription/Translation System.

Coupled Transcription/Translation: In *E. coli* S30 reactions, increased band intensities often can be obtained by using 2–3 times the DNA concentration normally recommended.

4. The appropriate incubation temperature will vary from one translation system to another. Please refer to the appropriate Promega protocol (see Section 4.B) for specific reaction conditions.

4.B. In Vitro Translation Systems

For more information on specific in vitro translation systems, please request the appropriate protocol from Promega.

- TNT® Quick Coupled Transcription/Translation System Technical Manual (#TM045)
- TNT® Coupled Reticulocyte Lysate Systems Technical Bulletin (#TB126)
- TNT® Coupled Wheat Germ Extract Systems Technical Bulletin (#TB165)
- Flexi® Rabbit Reticulocyte Lysate System Technical Bulletin (#TB127)
- Wheat Germ Extract System Technical Manual (#TM230)
- *E. coli* S30 Extract System for Circular DNA Technical Bulletin (#TB092)
- *E. coli* S30 Extract System for Linear Templates Technical Bulletin (#TB102)
- *E. coli* T7 S30 Extract System for Circular DNA Technical Bulletin (#TB219)
- TNT® Quick for PCR DNA Technical Manual (#TM235)

The number of lysines in the translated polypeptide and the efficiency of translation are the two most important factors affecting the SDS-PAGE band intensity of the translation product. To increase the intensity of weak bands, increase the amount of Transcend™ tRNA to up to twice the standard amount (see Figure 3).

To reduce the chance of RNase contamination, wear gloves throughout the experiment and use microcentrifuge tubes and pipet tips that have been autoclaved and handled only with gloves. Addition of RNasin® Ribonuclease Inhibitor to the translation reaction is recommended but not required. RNasin® Ribonuclease Inhibitor prevents degradation of sample mRNAs by many contaminating RNases.

If the amount of translation product must be estimated, add radioactive amino acid(s), in addition to the Transcend™ tRNA, to either a control translation reaction or all translation reactions. The percent incorporation of the radioactive amino acid can be used in combination with knowledge of the protein's amino acid composition to estimate the amount of translation product produced.

5. Post-Translational Analysis

The biotin-containing translation product can be analyzed by either of two approaches. The product can be resolved directly on an SDS-PAGE gel, transferred to an appropriate membrane and detected by either a colorimetric (Section 5.C) or chemiluminescent (Section 5.D) reaction.

5.A. Denaturing Gel Analysis of Translation Products

Biotinylated protein standards (Bio-Rad Cat.# 161-0319) can be used to determine the apparent molecular weight of the translated biotinylated protein. Alternatively, fluorescently labeled size standards can be observed after transfer and marked with a pencil under UV irradiation. The positions of unlabeled size standards also can be determined by staining the blot after transfer (see Section 5.C or 5.D).

1. Once the 50µl translation reaction is complete (or at any desired time point), remove a 1µl aliquot and add it to 15µl of SDS sample buffer. The remainder of the reaction may be stored at –20°C.
2. Close the tube and heat at 70°C for 15 minutes to denature the proteins.
3. Load the denatured sample on an SDS-polyacrylamide gel. (Protocols for SDS polyacrylamide gel electrophoresis may be found in the Promega *Protocols and Applications Guide* (10) or in the technical literature provided with Promega translation systems, which is listed in Section 4.B.)
4. Perform electrophoresis using standard conditions for your apparatus. Typically, electrophoresis is carried out at a constant current of 20mA. Electrophoresis usually is performed until the bromophenol blue dye has run off the bottom of the gel.

Note: If a gene product is weakly expressed or contains few lysines, up to 2µl of the translation reaction (Reticulocyte Lysate) can be loaded on an SDS gel without the loss of resolution observed with autoradiography. **However, loading more of the translation reaction can result in high background on the blot.** When loading more than 1µl of an S30 Extract translation reaction, first precipitate the proteins by adding 20µl of acetone per 5µl of extract and incubate on ice for 15 minutes. Centrifuge the acetone-precipitated S30 reaction at 12,000 × *g* for 5 minutes. Remove the supernatant and dry the pellet. Resuspend the pellet in 5µl of water, add 10µl of SDS sample buffer, heat at 90–100°C for 5 minutes and load 5–15µl of the sample on the gel.

5.B. Electroblotting of Proteins to Membrane

For colorimetric detection (see Section 5.C), the translation reaction products can be blotted from the SDS-polyacrylamide gel to (in decreasing order of preference) PVDF, nitrocellulose or another membrane using any standard apparatus and protocol, including semi-dry systems. Detailed procedures for electrophoretic blotting are usually included with commercial devices. We routinely transfer at a constant voltage of 100V for 60 minutes using a minigel-size electroblotting unit or 15 minutes using a semi-dry system. PVDF membrane must be prewet in methanol before it is equilibrated in transfer buffer.

5.C. Colorimetric (BCIP/NBT) Detection

Materials to Be Supplied by the User
(Solution compositions are provided in Section 8.A.)

- PVDF or nitrocellulose membrane
- Tris-buffered saline (TBS)
- TBS + 0.5% Tween® 20 (TBST)
- Optional: Ponceau S stain (Sigma Cat.# P7170)

Do not allow the membranes to dry out during any of the subsequent steps. Perform all of the washing and incubation steps at room temperature with gentle shaking. Use a shallow container that is slightly larger than the membrane.

For the Streptavidin-AP incubation and the color development reaction, use just enough solution to submerge the membrane, protein side up. Usually, this volume is about 0.24ml/cm^2 of membrane surface (15ml for a 7 × 9cm membrane). Use at least twice this volume for blocking and washing steps.

Determination of Apparent Molecular Weight of Biotinylated Proteins

If you are not using biotinylated or fluorescently labeled protein size standards, you may still detect the positions of protein standards by staining the blot after transfer. Immediately after transfer, incubate the blot for 30 seconds in Ponceau S and then destain the blot for 1 minute with water. Mark the locations of the markers with a pencil.

Another approach to determining the molecular weights of biotinylated proteins is to run duplicate sets of samples on the left and right halves of the gel. After transfer to a membrane, analyze one set with Streptavidin-AP and stain the other with Amido Black (for nitrocellulose membranes) or Coomassie® blue (for PVDF membranes).

Notes:

1. Perform all steps at room temperature.
2. Wear gloves when handling the membrane. Do not let the membrane dry out between steps.
3. After transferring proteins to PVDF membrane, it can be dried and stored. The PVDF membrane must be rewet with 100% methanol and washed twice with deionized water prior to blocking the membrane.

Blocking the Membrane

1. Add 15ml of TBST and incubate at room temperature for 60 minutes.

Streptavidin-AP Binding

2. Immediately prior to use, dilute 6µl of Streptavidin-AP into 15ml of fresh TBST. Pour off the TBST and add this Streptavidin-AP solution to the blot. Rock or agitate gently for 45–60 minutes.
3. Pour off the Streptavidin-AP solution. Wash two times for 1 minute each with 15ml of TBST and then two more times (1 minute each) with 15ml of water.

Color Development

4. Start the color reaction by incubating the membrane in Western Blue® Stabilized Substrate for Alkaline Phosphatase (or another BCIP/NBT reagent) until the bands of interest have reached the desired intensity. One 7 × 9cm blot requires about 5ml of substrate solution. Protect the solution from strong light. Reactive areas will turn purple, usually within 1–15 minutes.
5. 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.
6. Air-dry the membrane. Protect the membrane from light during prolonged storage.

5.D. Detection Using Transcend™ Chemiluminescent Substrate

Materials to Be Supplied by the User

(Solution compositions are provided in Section 8.A.)

- PVDF or nitrocellulose membrane
- Tris-buffered saline (TBS)
- TBS + 0.5% Tween® 20 (TBST)

Notes:

1. Perform all steps at room temperature.
2. Wear gloves when handling the membrane. Do not let the membrane dry out between steps.
3. After transferring proteins to PVDF membrane, it can be stored and dried. The PVDF membrane must be rewet with 100% methanol and washed twice with deionized water prior to blocking the membrane.
4. Ensure that no buffers contain sodium azide, as this will inhibit peroxidase activity.

Blocking the Membrane

1. Block the membrane by incubation in 15ml of TBST for 1 hour with gentle shaking.

Streptavidin-HRP Binding

2. Prepare 15ml of a 1:10,000 dilution of the Streptavidin-HRP conjugate in TBST (add 1.5µl to 15ml). This dilution will give less background than a 1:5,000 dilution; however, if the signal is weak a 1:5,000 dilution is recommended.
3. Incubate the membrane in the diluted Streptavidin-HRP conjugate for 45–60 minutes with gentle shaking.
4. Wash the membrane by incubation in 15ml of TBST for 5 minutes with gentle shaking. Repeat the wash two more times using TBST, then three times using 15ml of water.

Preparation of Chemiluminescent Substrate

5. Mix the substrates in dim light and allow the mixture to warm to room temperature before using. Mix 2.5ml of Substrate A with 2.5ml of Substrate B in a 15ml Corning® centrifuge tube.
6. Incubate the membrane with the chemiluminescent substrate mixture for 1 minute on a shaker in dim light. Use 5ml of substrate mix for a 7 × 9cm blot.

Development

7. Place the membrane on transparency film (3M Cat.# PP2500). Place a second piece of transparency film on top of the membrane. Squeeze out the excess substrate mix from between the films using a paper towel. Place the membrane and transparency film inside a film cassette.

8. Expose the blot to Kodak® X-Omat AR X-ray film for 2–20 minutes. Develop the film.

Figure 4 shows typical examples of results obtained using the Transcend™ Chemiluminescent Translation Detection System with either PVDF (Panel A) or nitrocellulose (Panel B) membranes.

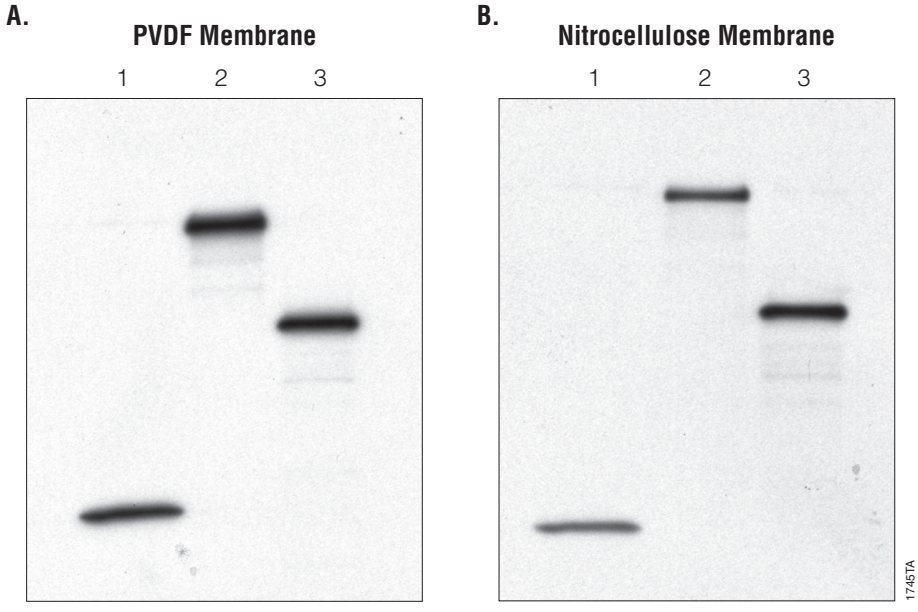

Figure 4. The Transcend™ Chemiluminescent Translation Detection System. Chloramphenicol acetyltransferase (lanes 1), β-galactosidase (lanes 2) and luciferase (lanes 3) were separated on a 4–20% SDS-PAGE gel and electroblotted onto PVDF (**Panel A**) or nitrocellulose (**Panel B**) membranes. The membranes were probed with a 1:20,000 dilution of Streptavidin-HRP and processed according to the protocol given in Section 5.D.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
No signal develops	Poor transfer of proteins to the membrane. Make sure the protocol steps (Section 5.C and 5.D) were followed correctly. Use a protein stain on unblocked membrane to confirm good protein transfer.
	Incorrect orientation of membrane during film exposure. Ensure that the correct orientation is maintained during film exposure.
	Poor translation reaction: • If possible, verify translation by examining the enzyme activity. • If endogenous biotinylated protein can be detected but not the protein of interest, too few lysine residues may be in the target protein.
Signal development is weak	Insufficient amount of target protein present on the gel. Increase the amount of lysate loaded onto the gel or increase the amount of Transcend™ tRNA used in the reaction.
	Insufficient incubation time: • Increase the incubation time for the Streptavidin-HRP conjugate. • Increase film exposure time.
	Excessive washing. Excessive washing can reduce signal intensity. Wash as recommended in Section 5.D, Step 4.
	Reagents used were too cold. All reagents must be at room temperature before using.
	Streptavidin-HRP conjugate is too dilute. Try using a 1:5,000 dilution of the Streptavidin-HRP conjugate.
Too much background	Too much lysate loaded onto the gel. Decrease the amount of lysate loaded onto the gel.
	Blot is exposed to film for too long. Decrease exposure time. If the target protein cannot be detected in a shorter exposure, it may be due to poor translation/labeling (see "No signal develops" above).

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Too much background (continued)	Insufficient blocking or washing. Make sure the protocol is followed correctly.
	Peroxidase activity from hemoglobin in the sample. Soak the membrane in TBS containing 10% ImmunoPure Peroxidase Suppressor (Pierce Cat.# 35000) for 30 minutes prior to blocking.
Bands are poorly defined (fuzzy)	Poor transfer of proteins to the membrane. Ensure that the protein transfer was performed correctly.
	Excess substrate present on the blot. Remove excess substrate from the blot prior to film exposure (Section 5.D, Step 7).
	Membrane moved during development. Do not allow membranes or film to shift position during development.

7. References

1. Johnson, A.E. *et al.* (1976) N-epsilon-acetyllysine transfer ribonucleic acid: A biologically active analogue of aminoacyl transfer ribonucleic acids. *Biochemistry* **15**, 569–75.
2. Kurzchalia, T.V. *et al.* (1988) tRNA-mediated labeling of proteins with biotin. A nonradioactive method for the detection of cell-free translation products. *Eur. J. Biochem.* **172**, 663–8.
3. Beckler, G.S. and Hurst, R. (1993) tRNA^{nscendTM} non-radioactive detection of in vitro translation products labeled using biotinylated lysine tRNA. *Promega Notes* **43**, 24–31.
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7. *Luciferase Assay System Technical Bulletin* #TB281, Promega Corporation.
8. *CAT Enzyme Assay System With Reporter Lysis Buffer Technical Bulletin* #TB084, Promega Corporation.
9. *Galacto-Light™ β-Galactosidase Reporter Assay System Instruction Manual*, Tropix, Inc.
10. *Protocols and Applications Guide*, Online Edition (2004) Promega Corporation.

8. Appendix

8.A. Composition of Buffers and Solutions

Tris-buffered saline (TBS)

20mM	Tris-HCl (pH 7.5)
150mM	NaCl

TBS + 0.5% Tween® 20 (TBST)

20mM	Tris-HCl (pH 7.5)
150mM	NaCl
0.5%	Tween® 20

SDS polyacrylamide running 10X buffer

30g	Tris base
144g	glycine
100ml	10% SDS

Add water to a final volume of 1L.

8.B. Related Products

Eukaryotic Transcription/Translation Systems

Product	Size	Cat.#
TNT® SP6 High-Yield Protein Expression System	40 × 50µl reactions	L3260
	10 × 50µl reactions	L3261
TNT® T7 Quick Coupled Transcription/ Translation System	40 reactions	L1170
TNT® SP6 Quick Coupled Transcription/ Translation System	40 reactions	L2080
TNT® T3 Coupled Reticulocyte Lysate System	40 reactions	L4950
TNT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610
TNT® SP6 Coupled Reticulocyte Lysate System	40 reactions	L4600
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® T7 Quick for PCR DNA	40 reactions	L5540

E. coli S30 Extract Systems

Product	Size	Cat.#
E. coli S30 Extract System for Circular DNA	30 reactions	L1020
E. coli S30 Extract System for Linear DNA	30 reactions	L1030
E. coli T7 S30 Extract System for Circular DNA	30 reactions	L1130

Rabbit Reticulocyte Lysate Translation Systems

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease Treated	1ml	L4960
Flexi® Rabbit Reticulocyte Lysate System	1ml	L4540

Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System

Product	Size	Cat.#
Rabbit Reticulocyte Lysate/ Wheat Germ Extract Combination System	12 reactions	L4330

Available Separately

Product	Size	Cat.#
Streptavidin Alkaline Phosphatase	0.5ml	V5591
Western Blue® Stabilized Substrate for Alkaline Phosphatase	100ml	S3841
Amino Acid Mixture, Complete	175µl	L4461

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