Canine Pancreatic Microsomal Membranes

Promega

Technical Manual No. 231

INSTRUCTIONS FOR USE OF PRODUCTS Y4041, Y4061 AND Y4071.
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I. Description

Microsomal vesicles are used to study cotranslational and initial post-translational processing of proteins. Processing events such as signal peptide cleavage, membrane insertion, translocation, and core glycosylation can be examined by the translation of the appropriate mRNA in vitro in the presence of these microsomal membranes. In addition, processing and glycosylation events may be studied by the transcription/translation of the appropriate DNA in the TNT® Lysate Systems[a,b,c,d] when used with microsomal membranes. To assure consistent performance with minimal translational inhibition and background, microsomes have been isolated free from contaminating membrane fractions and stripped of endogenous membrane-bound ribosomes and mRNA (1). Membrane preparations are assayed for both signal peptidase and core glycosylation activities using two different control mRNAs. The two control mRNAs supplied with this system are the precursor for β-lactamase (or ampicillin resistance gene product) from *E. coli* and the precursor for α-mating factor (or α-factor gene product) from *S. cerevisiae*.

Potential applications of the Canine Pancreatic Microsomal Membranes[d] include:

- Signal peptide cleavage examination
- Membrane insertion and/or translocation analysis
- Core glycosylation analysis

II. Product Components

Product	Size	Cat.#
Canine Pancreatic Microsomal Membranes	50µl	Y4041

Includes:

- 50µl Canine Pancreatic Microsomal Membranes
- 1µg Signal Sequence Control mRNA
- 1µg Core Glycosylation Control mRNA
- 1 Protocol

Signal Sequence Control mRNA	1µg	Y4061
Core Glycosylation Control mRNA	1µg	Y4071

III. General Considerations

The synthesis of proteins in vitro is a popular tool in research today. Translation systems are used to rapidly characterize plasmid clones, study structural mutations and examine translational signals, among other applications.

Two basic approaches are available: i) in vitro systems programmed with RNA (translation systems) or ii) those programmed with DNA (coupled transcription/translation systems). Many other factors go into the choice of an in vitro translation system, particularly with the increasing number of commercially-available translation products. Some of these considerations are summarized below.

A. Translation Systems

Several cell-free protein synthesizing systems have been used in recent years for the translation of mRNA isolated from various sources. Of these, Promega offers several Rabbit Reticulocyte Lysate[a,c,d] and Wheat Germ Extract Systems. All are reliable, convenient, and easy-to-use systems to initiate translation and produce full-size polypeptide products. Reticulocyte Lysate is often favored for translation of larger mRNA species, and is recommended when microsomal membranes are to be added for cotranslational processing of translation products.

Promega's Flexi® Rabbit Reticulocyte Lysate[a,c,d] is recommended for those wishing to optimize translation of particular RNAs through adjustments to salt concentrations. Wheat Germ Extract readily translates certain RNA preparations, such as those containing low concentrations of double-stranded RNA (dsRNA) or oxidized thiols, which are inhibitory to reticulocyte lysate.

When appropriate, translation products may be further analyzed for cotranslational processing and core glycosylation by the addition of Canine Pancreatic Microsomal Membranes to a standard rabbit reticulocyte translation reaction. Processing events are generally detected as shifts in the apparent molecular weight of translation products.

In vitro translation reactions may be directed by either mRNAs isolated in vivo or by RNA templates transcribed in vitro using the RiboMAX™ Transcription System(c,d,e,f) and vectors such as the Riboprobe® System(d,e) Vectors and LambdaGEM® Vectors(g). Procedures for the rapid isolation and poly(A)+ selection of cellular mRNAs are provided in Promega's *Protocols and Applications Guide*, Third Edition (2). When using mRNA synthesized in vitro, the presence of a 5´-cap structure may enhance the translational activity in eukaryotic translational systems (3).

B. Coupled Transcription/Translation Systems

DNA sequences cloned in plasmid vectors may also be expressed directly using Promega's TNT® Rabbit Reticulocyte Lysate and Wheat Germ Extract Systems. These systems contain the components necessary for synthesis in vitro of either radioactively labeled or nonlabeled proteins. The TNT® Systems require plasmid constructs driven by SP6, T3 or T7 RNA polymerase promoters for the initiation of transcription, but translation in this system is under eukaryotic controls. The TNT® Systems also can be used with Canine Microsomal Membranes to study cotranslational processing and glycosylation events.

This manual covers the basic Canine Pancreatic Microsomal Membrane System offered by Promega. For further information on the Rabbit Reticulocyte Lysate (#TM232), Wheat Germ Extract (#TM230), TNT® (#TB126), Flexi® Lysate (#TB127), TNT® Quick Coupled(a,b,c,d,e) (for T7 and SP6; #TM045) Systems and three *E. coli* S30 Systems (#TB092, #TB102, #TB219), please contact Promega's Technical Services department. All technical documents are also available on Promega's Internet site at www.promega.com.

Note: An *Experienced User's Protocol* can be found at the end of this Technical Manual.

IV. Cotranslational Processing and Post-Translational Analysis Using Canine Pancreatic Microsomal Membranes

To test the processing efficiencies of the Canine Microsomal Membranes, the following procedure can be performed with the positive control mRNAs supplied. This protocol is used at Promega to assay the efficiency of protein processing by Microsomal Membranes. While these reaction conditions will be suitable for most applications, the efficiency of processing using alternate translation systems, mRNAs, or membranes may vary. Thus, reaction parameters may need to be altered to suit individual requirements. In general, increasing the amount of membranes in the reaction increases the proportion of polypeptides translocated into vesicles but reduces the total number of polypeptides synthesized.

Note: This protocol (Section IV.A) should be used with Promega's Rabbit Reticulocyte Lysate System, Nuclease Treated (Cat.# L4960) or with Wheat Germ Extract Systems (Cat.# L4330, L4380). Protocols for the use of Canine Microsomal Membranes with the TNT® Lysates are provided in the TNT® Coupled Reticulocyte Lysate Systems Technical Bulletin (TB126) or the TNT® Quick Coupled Systems (TM045).

A. Cotranslational Processing Protocol

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- RNasin® Ribonuclease Inhibitor(d) (Cat.# N2211)
- isotopically labeled amino acids, typically [^{35}S]methionine, [^{35}S]cysteine, [^{3}H]leucine or [^{14}C]leucine (see Table 1)

1. Remove the reagents from the freezer and allow them to thaw on ice. Thaw the Rabbit Reticulocyte Lysate by handwarming; immediately upon thawing place the lysate on ice.

Wheat Germ Extract should not be used with Canine Microsomal Membranes for glycosylation.

2. Denature the mRNA at 65°C for 3 minutes and immediately cool on ice. This step will increase the efficiency of translation, especially of GC-rich mRNA, by destroying local regions of secondary structure in the mRNA.

3. Mix the following components, in the order given, in a sterile microcentrifuge tube on ice:

Note: RNasin® Ribonuclease Inhibitor (approximately 40 units) may be added to this reaction (Step 3).

Component	Amount per Reaction
Nuclease-Treated Rabbit Reticulocyte Lysate	17.5µl
Amino Acid Mixture, Minus Methionine, 1mM	0.5µl
Canine Microsomal Membranes (see Notes 1 and 2)	1.5–2.4µl
RNA substrate in water (pre-β-lactamase and α-factor mRNA at 0.1µg/µl) (see Notes 3 and 4)	1.0µl
[^{35}S]methionine (1,200Ci/mmol) at 10mCi/ml (see Note 8)	2.0µl
Nuclease-Free Water to a final volume of	25.0µl

4. Incubate at 30°C for 60 minutes.

5. Analyze the results of translation and processing (see Note 4). Procedures are provided for gel analysis of translation products (Section IV.B). For information on assays of cotranslational processing, see Note 7.

Notes:

1. The amount of Canine Pancreatic Microsomal Membranes used in the reaction may need to be titrated. While these reaction conditions will be suitable for most applications, the efficiency of processing using membranes will vary. Thus, reaction parameters may need to be altered to suit individual requirements. In general, increasing the amount of membranes in the reaction increases the proportion of polypeptides that are processed but reduces the total amount of polypeptides synthesized.

2. We do not recommend exceeding 1.8µl of Canine Microsomal Membranes in the TNT® SP6 Coupled Reactions. The SP6 Polymerase is more sensitive to the presence of Canine Microsomal Membranes than are the T7 and T3 Polymerases. Thus, synthesis of polypeptides in SP6 reactions is inhibited by higher concentrations of membranes.

3. Addition of 1.5–2.4µl of Canine Microsomal Membranes per 25µl of translation mix will process 85% of pre-β-lactamase to β-lactamase. The same amount of membranes will process 75% of α-factor to core glycosylated forms of α-factor.

4. When analyzed by SDS gel electrophoresis, the precursor for β-lactamase migrates at 31.5kDa and the processed β-lactamase at 28.9kDa. The precursor for the α-factor migrates at 18.6kDa, and the core-glycosylated α-factor has a molecular weight of 32kDa but will migrate faster than the β-lactamase precursor. See Figure 1 for an example of SDS gel analysis of these translation products.

5. The amount of protein produced in lysates using Canine Microsomal Membranes will be less than the amount of protein produced in lysates alone. Depending on the construct used, translation efficiency can be expected to drop between 10–50% in the presence of Canine Microsomal Membranes.

6. The storage buffer for the Canine Microsomal Membranes consists of 50mM triethanolamine, 2mM DTT and 250mM sucrose.

7. In some cases, it is difficult to determine by gel analysis alone if efficient processing or glycosylation has occurred. The following references provide information on assays for detecting cotranslational and post-translational processing events.

   A general assay for cotranslational processing makes use of the protection afforded the translocated protein domain by the lipid bilayer of the microsomal membrane. In this assay, protein domains are judged to be translocated if they are observed to be protected from exogenously added protease (see references 4–7 for details).

   In a separate assay, endoglycosidase H can be used to determine the extent of glycosylation of translation products. In cell-free systems, N-linked glycosylation occurs only within intact microsomes. Endoglycosidase H cleaves the internal N-acetylglucosamine residues of high mannose carbohydrates resulting in a shift in apparent molecular weight on SDS gels to a position very close to that of the nonglycosylated species.

   Endoglycosidase F may also be used in a protease protection assay. Consult references 8 and 9 for more information on the uses of endoglycosidase F for monitoring glycosylation or membrane insertion of translation products.

8. The use of a grade of [^{35}S]methionine, such as Redivue™ L-[^{35}S]methionine (Amersham Pharmacia Biotech Ltd., Cat.# AG1094) will help prevent background labeling of the rabbit reticulocyte lysate 42kDa protein, which can occur using other grades of label. A stabilizer has been added to Redivue™ to increase the stability above that of conventional radiolabeled amino acids, so that the release of volatile gases is reduced substantially. This [^{35}S]methionine may be stored at 4°C without aliquoting. Other ^{35}S-labeled amino acids may be easily oxidized to translation-inhibiting sulfoxides and should be stored in aliquots at –70°C in the presence of 1mM dithiothreitol or β-mercaptoethanol.

Table 1. Recommended Concentrations of Alternative Radiolabeled Amino Acids.

Amino Acid	Final Concentration in Reaction	Volume to Add to Reaction
[^{3}H]leucine (100–200Ci/mmol)	0.5mCi/ml	5µl
[^{14}C]leucine (300mCi/mol)	5µCi/ml	5µl
[^{35}S]cysteine (600mCi/mol)	1mCi/ml	5µl

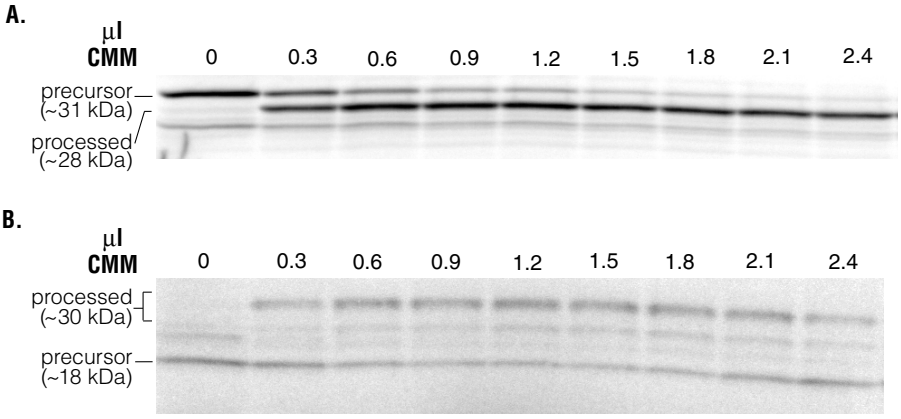

Figure 1. Processing and glycosylation activity of Canine Pancreatic Microsomal Membranes. The positive control mRNAs (**Panel A**, 0.1µg of *E. coli* β-lactamase; **Panel B**, 0.1µg of *S. cerevisiae* α-factor) were translated using Promega's Rabbit Reticulocyte Lysate in a 25µl reaction for 60 minutes in the presence of the indicated amounts of Canine Microsomal Membranes (CMM). Aliquots (1µl) were analyzed by SDS-PAGE on a 4–20% Novex™ gel, transferred to a sheet of PVDF (Bio-Rad®, Sequi-Blot) and exposed to a PhosphorImager® cassette plate for 12 hours.

B. Post-Translational Analysis

For information on the preparation of SDS-polyacrylamide gels and separation of proteins by electrophoresis, refer to Promega's *Protocols and Applications Guide,* Third Edition (2), available from Promega Corporation. Alternatively, precast polyacrylamide gels are available from a number of manufacturers. For protein analysis, NOVEX™ and Bio-Rad® Laboratories, Inc., offer a variety of precast mini-gels, which are compatible with their vertical electrophoresis and blotter systems. These companies offer Tris-Glycine, Tricine and Bis-Tris gels for resolution of proteins under various conditions and over a broad spectrum of protein sizes. The NOVEX™ 4–20% Tris-Glycine gradient gels (Cat.# EC6025, EC60355) and the Bio-Rad® Ready Gel 4–20% Tris-Glycine Gel, 10-well (Cat.# 161-0903) are convenient for resolving proteins over a wide range of molecular weights. In addition to convenience and safety, precast gels provide consistent results. If using precast gel preparations, follow the manufacturer's recommendations.

Materials to Be Supplied by the User

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

- 30% acrylamide solution
- separating gel 4X buffer
- stacking gel 4X buffer
- SDS polyacrylamide 10X running buffer
- SDS sample buffer
- fixing solution
- Whatman® GF/C glass fiber filters (optional)
- Whatman® 3MM filter paper (optional)
- X-ray film
- **optional:** precast gels (e.g., NOVEX™ 4–20% Tris-Glycine gradient gels, Cat.# EC6025, EC60355, and Bio-Rad® Ready Gel 4–20% Tris-Glycine Gel, 10-well, Cat.# 161-0903)

1. When the 25µl translation reaction is complete (or at any desired timepoint), remove a 5µl aliquot and add it to 20µl SDS sample buffer. The remainder of the reaction may be stored at –20°C.

2. Cap the tube and heat at 100°C for 2 minutes to denature the proteins.

3. Load 5µl of the denatured sample onto an SDS-polyacrylamide gel or store at –20°C.

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

4. 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 has run off the bottom of the gel. Because the dye front also contains the free labeled amino acids, disposal of unincorporated label may be easier if the gel is stopped while the dye front remains in the gel. Proceed to Step 8 for Western blotting analysis.

5. Place the polyacrylamide gel in a plastic box and cover the gel with fixing solution (as prepared in Section VI.A) for 30 minutes. Agitate slowly on an orbital shaker. Pour off the fixing solution. Proceed to Step 6 (gel drying prior to film exposure). Fixing the gel should reduce background counts.

   Optional: 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, such as Amplify™ Reagent (Amersham Pharmacia Biotech Ltd.) can conveniently be used for fluorographic enhancement of signal. Alternatively, the fixed gel can be exposed to a phosphorimaging screen. These screens provide greater sensitivity, speed and the ability to quantitate the radioactive bands.

Note: Occasionally, denaturation at 100°C results in the formation of large molecular weight species that do not migrate well on gels. Denaturing at 80–90°C avoids formation of these large molecules.

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

Note: When detecting proteins by phosphorimaging, transfer the protein to a membrane to sharpen the bands.

6. Dry the gel prior to exposure to film as follows: Soak the gel in 7% acetic acid, 7% methanol and 1% glycerol for 5 minutes to prevent cracking during drying. Place the gel on a sheet of Whatman® 3MM filter paper, cover with plastic wrap and dry at 80°C under a vacuum using a conventional gel dryer, until the gel is dried completely. The gel may also be dried overnight using Promega's Gel Drying Kit (Cat.# V7120). To decrease the likelihood of cracking gradient gels, dry them upside down (with wells pointing down).

7. Expose the gel on X-ray film for 1–15 hours at –70°C (for fluorography), or 6–15 hours at room temperature (for autoradiography) or expose to a phosphorimaging screen for 2–15 hours.

8. For Western blot analysis of proteins, transfer (immobilize) the protein from the gel onto nitrocellulose or PVDF membrane (10,11). Usually Western blots are made by electrophoretic transfer of proteins from SDS-polyacrylamide gels. Detailed procedures for electrophoretic blot analysis are often included with commercial devices and can be found in references 10, 12, 13 and 14. A general discussion of Western blot analysis with PVDF membranes is found in reference 15. PVDF membranes must be prewet in methanol or ethanol before equilibrating in transfer buffer. The blot may then be subjected to immunodetection analyses. For more information, refer to Promega's *Protocols and Applications Guide*, Third Edition (2). For detection of biotinylated protein using the Transcend™ Non-Radioactive Translation Detection Systems, see Promega's Technical Bulletin #TB182.

V. 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	Possible Causes	Comments
The control reactions did not work	Reaction components were used after the expiration date	The membranes should not be used after their expiration date.
	Reaction components were used after multiple freeze-thaw cycles	The membranes should not be used after more than two freeze-thaw cycles.
	Reaction volume was scaled down	Use the recommended reaction volume.
	Ethanol or salt was present in the translation reaction	Ethanol or salt may inhibit translation.
The translation efficiency of sample mRNA is low	RNA concentration is not optimized	The final concentration of RNA is important. For poly(A), in vitro transcripts and viral RNA, use 5–20µg/ml. For total RNA add 100–200µg/ml to a reaction containing CMMs. The optimal RNA concentration for translation should be determined prior to performing definitive experiments. To determine the optimal concentration, serially dilute your template **first** and then add the same **volume** of RNA to each reaction to ensure that other variables are kept constant.

V. Troubleshooting (continued)

Symptoms	Possible Causes	Comments
The translation efficiency of sample mRNA is low (continued)	Potassium or magnesium concentration is not optimized	Optimum potassium concentration varies from 40–100mM depending on the mRNA used. Additional potassium can be added if the initial translation results are poor. Similarly, specific mRNAs may require altered magnesium concentrations. Addition of 0.5–2.5mM magnesium is generally sufficient for the majority of mRNAs utilized.
	Inhibitors are present in the translation reaction	To determine if inhibitors are present in the mRNA preparation, mix the mRNA with Luciferase Control RNA[c,d] and determine if Luciferase Control RNA translation is inhibited relative to a control translation containing Luciferase Control RNA alone. Oxidized thiols, low concentrations of double-stranded RNA, and polysaccharides are typical inhibitors of translation (16).
	Calcium is present in the translation reaction	Avoid adding calcium to the translation reaction. Calcium may reactivate the micrococcal nuclease used to destroy endogenous mRNA in the membranes and result in degradation of the mRNA template.
	Ethanol is present in the translation reaction	Residual ethanol should be removed from mRNA preparations and labeled amino acids before they are added to the translation reaction.
	Incubation of the reaction at 37°C causes decreased protein synthesis	Incubate the translation reaction at 30°C.
	The addition of spermidine and certain diamines has been shown to stimulate translation (17)	Add spermidine to a final concentration of approximately 0.4mM. Add Mg^{2+} to a final concentration of 2mM.
	Reaction time may need to be optimized	Increase reaction time to 90–120 minutes.

V. Troubleshooting (continued)

Symptoms	Possible Causes	Comments
Unexpected bands are present on the gel	More than one peptide is translated from the RNA template	The RNA template may have more than one translation initiation start site (at internal methionines).
	There could be a contaminating translation product	Run the reaction with no RNA to see if the additional band is present.
	[^{35}S] is old	Older [^{35}S] may dissociate from the amino acid and label other proteins in the membrane preparations. Use fresh [^{35}S].
	The [^{35}S]methionine used is not of translational grade	There are reports of a 42kDa band with some grades of [^{35}S]methionine (16). We recommend Redivue™ (Amersham Pharmacia Biotech Ltd., Cat.# AG1094).
	Aminoacyl tRNAs may produce background bands	Add RNase A to the membrane reaction (after completion) to a final concentration of 0.2mg/ml. Incubate for 5 minutes at 30°C.
There are unexpected bands in the control reaction lane	Oxidized β-mercapto-ethanol is present or not enough SDS in the loading buffer	Use a loading buffer that contains 2% SDS and 100mM DTT.
There is smearing on the gel	The gel is not clean	The gel must be washed before placing onto film. Once gel electrophoresis is complete, soak the gel in either a standard Coomassie® destaining solution (50% methanol, 7.5% glacial acetic acid) or in water for 15–30 minutes prior to drying.
	Too much protein loaded on the gel	Check the amount of samples loaded onto the gel and the amount of loading buffer. Too much protein loaded onto the gel can cause smearing.
	Acrylamide concentration too low to resolve proteins	Acrylamide concentration can be increased to 12%.
	Ethanol present in the sample	Ethanol present in the sample can cause smearing on the gel.

VI. Appendix

A. Composition of Buffers and Solutions

separating gel 4X buffer

18.17g	Tris base
4ml	10% SDS

Adjust to pH 8.8 with 12N HCl and add water to a final volume of 100ml. Store at room temperature.

30% acrylamide solution

30g	acrylamide
0.8g	bisacrylamide

Add water to a final volume of 100ml. Store at 4°C.

stacking gel 4X buffer

6.06g	Tris base
4ml	10% SDS

Add water to a final volume of 80ml. Adjust to pH 6.8 with 12N HCl and add water to a final volume of 100ml. Store at room temperature.

fixing solution

50%	methanol
10%	glacial acetic acid
40%	water

SDS sample buffer

50mM	Tris-Cl (pH 6.8)
10%	glycerol
2%	SDS
0.1%	bromophenol blue
100mM	dithiothreitol

SDS sample buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

SDS polyacrylamide running 10X buffer

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

Bring to a final volume of 1 liter.

staining solution

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

Store at room temperature.

B. Related Products

Rabbit Reticulocyte Lysate

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease Treated	30 × 50µl reactions	L4960
Rabbit Reticulocyte Lysate, Untreated	30 × 50µl reactions	L4151

Bulk Rabbit Reticulocyte Lysate is available from Promega.

Other Translation Systems

Product	Size	Cat.#
Flexi® Rabbit Reticulocyte Lysate System	30 × 50µl reactions	L4540

Bulk Flexi® Rabbit Reticulocyte Lysate is available from Promega.

TNT® Quick Coupled Transcription/Translation Systems

Product	Size	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System[a,b,c,d,e]	40 × 50µl reactions	L1170
TNT® T7 Quick Coupled Transcription/Translation System Trial Size[a,b,c,d,e]	5 × 50µl reactions	L1171

TNT® Coupled Reticulocyte Lysate System

Product	Size	Cat.#
TNT® SP6 Coupled Reticulocyte Lysate System	40 × 50µl reactions	L4600
TNT® T7 Coupled Reticulocyte Lysate System	40 × 50µl reactions	L4610
TNT® T3 Coupled Reticulocyte Lysate System	40 × 50µl reactions	L4950
TNT® T7/T3 Coupled Reticulocyte Lysate System	40 × 50µl reactions	L5010
TNT® T7/SP6 Coupled Reticulocyte Lysate System	40 × 50µl reactions	L5020
TNT® SP6 Coupled Reticulocyte Lysate System, Trial Size	8 × 50µl reactions	L4601
TNT® T7 Coupled Reticulocyte Lysate System, Trial Size	8 × 50µl reactions	L4611

Wheat Germ Extract Systems

Product	Size	Cat.#
Wheat Germ Extract	30 × 50µl reactions	L4380

Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination Systems

Product	Size	Cat.#
Rabbit Reticulocyte/Wheat Germ Extract Combination System	30 reactions	L4330

E. coli S30 Extract System for Linear Templates

Product	Size	Cat.#
E. coli S30 Extract System for Linear Templates[a,c]	30 × 50µl reactions	L1030

E. coli S30 Extract Systems for Circular DNA Templates

Product	Size	Cat.#
E. coli S30 Extract for Circular DNA[a,c]	30 × 50µl reactions	L1020
E. coli T7 S30 Extract for Circular DNA	30 × 50µl reactions	L1130

RNA Purification Systems

Product	Size	Cat.#
RiboMAX™ Large Scale RNA Production System - SP6	50 reactions	P1280
RiboMAX™ Large Scale RNA Production System - T3	50 reactions	P1290
RiboMAX™ Large Scale RNA Production System - T7	50 reactions	P1300

Amino Acid Mixtures

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

Gel Drying Kit

Product	Cat.#
Gel Drying Kit, 17.5 × 20cm capacity	V7120

The Gel Drying Kit includes 1 pair of Gel Drying Frames, 20 sheets of Gel Drying Film (25.5 x 28cm), 12 clamps and a Technical Bulletin.

RNasin® Ribonuclease Inhibitor

Product	Size	Cat.#
RNasin® Ribonuclease Inhibitor	2,500u	N2111
	10,000u	N2115
Recombinant RNasin® Ribonuclease Inhibitor[d,e]	2,500u	N2511
	10,000u	N2515

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(a)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, Australian Pat. No. 649289, and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Certain applications of this product may require licenses from others.

(b)U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

(c)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(d)U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

(e)U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217 and Australian Pat. No. 646803 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

(f)The RiboMAX™ Large Scale RNA Production Systems—T7 and T3 (Cat.# P1290 and P1300) are covered by U.S. Pat. No. 5,256,555 and are sold under a license from Ambion, Inc. They are intended for research use only. Parties wishing to use these products for other applications should contact Ambion, Inc.

(g)U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

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Canine Pancreatic Microsomal Membranes: *Experienced User's Protocol*

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Section IV) the first time you use the Canine Pancreatic Microsomal Membranes.

Before You Begin

Remove the reagents from storage at –70°C and allow them to thaw slowly on ice.

Preparation of Sample RNA

Heat the mRNA at 65°C for 3 minutes and immediately cool on ice.

Translation Procedure (Section IV.A)

1. Assemble the reaction components, appropriate for the label being used, in a 0.5ml polypropylene microcentrifuge tube. After adding all components, gently mix by pipetting and stirring the reaction with the pipette tip. If necessary, centrifuge briefly to return the sample to the bottom of the tube.

Component	Amount per Reaction
Nuclease-Treated Rabbit Reticulocyte Lysate	17.5µl
Amino Acid Mixture, Minus Methionine, 1mM	0.5µl
Canine Microsomal Membranes (see Notes 1 and 2, Section IV.A)	1.5–2.4µl
RNA substrate in Nuclease-Free Water (pre-β-lactamase and α-factor mRNA at 0.1µg/µl) (see Notes 3 and 4, Section IV.A)	1.0µl
[^{35}S]methionine (1,200Ci/mmol) at 10mCi/ml (see Note 8, Section IV.A)	2.0µl
Nuclease-Free Water to a final volume of	25.0µl

Note: RNasin® Ribonuclease Inhibitor (approximately 40 units) may be added to the above reaction.

Small-scale reactions may be performed by reducing volumes proportionally. To prepare 12.5µl reactions, use one-half of the stated volumes for all reaction components listed above.

2. Incubate at 30°C for 60 minutes.
3. Analyze the results of translation and processing (see Note 4, Section IV.A). Procedures are provided for gel analysis of translation products (Section IV.B). For information on analysis of cotranslational processing, see Note 7, Section IV.A.

Control Translation Reactions

1. We recommend including a control reaction without added mRNA. This allows measurement of any background incorporation of labeled amino acids.