TECHNICAL MANUAL

Canine Pancreatic Microsomal Membranes

Instructions for Use of Product
Y4041

Revised 11/18
TM231

Canine Pancreatic Microsomal Membranes

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. 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 when used with microsomal membranes. To assure consistent performance with minimal translational inhibition and background, microsomes have been isolated free of 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*.

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1. Description (continued)

Potential applications of the Canine Pancreatic Microsomal Membranes include:
- Signal peptide cleavage examination
- Membrane insertion and/or translocation analysis
- Core glycosylation analysis

Visit **www.promega.com/citations/** for a list of peer-reviewed publications that feature the use of Canine Pancreatic Microsomal Membranes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Canine Pancreatic Microsomal Membranes	**50µl**	**Y4041**

Each system contains sufficient reagents for 10 purifications. Includes:

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

Storage Conditions: Store at –70°C. After thawing, unused portions should be rapidly refrozen in liquid nitrogen. No detectable loss of activity results after two freeze-thaw cycles. See the product label for expiration information.

Note: The storage buffer for Canine Pancreatic Microsomal Membranes is 50mM triethanolamine, 2mM DTT and 250mM sucrose.

3. General Considerations

The synthesis of proteins in vitro is a popular tool in research. 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) systems 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.

3.A. Translation Systems

Several cell-free protein synthesizing systems have been used for the translation of mRNA isolated from various sources. Of these, Promega offers several Rabbit Reticulocyte Lysate Systems. All are reliable, convenient and easy-to-use systems to initiate translation and produce full-size protein products. Reticulocyte Lysate is recommended when microsomal membranes are to be added for cotranslational processing of translation products.

The Flexi® Rabbit Reticulocyte Lysate is recommended for those wishing to optimize translation of particular RNAs through adjustments to salt concentrations.

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 and vectors such as the Riboprobe® System Vectors. Procedures for the rapid isolation and poly(A)$^+$ selection of cellular mRNAs are provided in the *Protocols and Applications Guide*, Online 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).

3.B. Coupled Transcription/Translation Systems

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

This manual covers the basic Canine Pancreatic Microsomal Membrane system. For further information on the Rabbit Reticulocyte Lysate (#TM232), TnT® Coupled Reticulocyte Lysate (#TB126), Flexi® Lysate (#TB127) and TnT® Quick Coupled Transcription/Translation Systems (for T7 and SP6; #TM045), please contact Promega Technical Services. All technical documents are also available on our Web site at: **www.promega.com/protocols/**

4. 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 proteins synthesized.

4.A. Cotranslational Processing Protocol

Note: This protocol (Section 4.A) should be used with the Rabbit Reticulocyte Lysate System, Nuclease-Treated (Cat.# L4960). 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 Technical Manual* #TM045.

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

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- RNasin® Ribonuclease Inhibitor (Cat.# N2111)
- 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.
2. Denature the mRNA 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:

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

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

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 4.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 protein 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, protein synthesis in SP6 reactions is inhibited by higher concentrations of membranes.
3. Addition of 0.3–2.4µl of Canine Microsomal Membranes per 25µl of translation mix will process ≥50% of pre-β-lactamase to β-lactamase. The same amount of membranes will process ≥50% 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.

4.A. Cotranslational Processing Protocol (continued)

Endoglycosidase F also may 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 EasyTag™ L-[^{35}S]-Methionine (Perkin Elmer Cat.# NEG709A) will help prevent background labeling of the rabbit reticulocyte lysate 42kDa protein, which can occur using other grades of label. This [^{35}S]methionine may be stored at 4°C without dividing into aliquots. 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

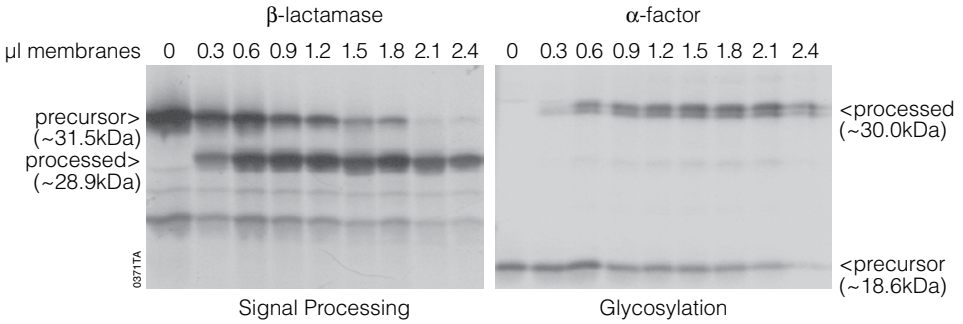

Figure 1. Processing and glycosylation activity of Canine Pancreatic Microsomal Membranes. The positive control mRNAs (0.1µg of *E. coli* β-lactamase and 0.1µg of *S. cerevisiae* α-factor) were translated using Rabbit Reticulocyte Lysate in a 25µl reaction for 60 minutes in the presence of the indicated amounts of Canine Microsomal Membranes. Aliquots (1.67µl) were then analyzed by SDS-PAGE and autoradiography of the ^{35}S-labeled proteins.

4.B. Post-Translational Analysis

For information on the preparation of SDS-polyacrylamide gels and separation of proteins by electrophoresis, refer to the *Protocols and Applications Guide*, Online Edition (2), available from Promega. Alternatively, precast polyacrylamide gels are available from a number of manufacturers. For protein analysis, Novex® (GE Healthcare) 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.# EC6025BOX, EC60355BOX) and the Bio-Rad Ready Gel 4–20% Tris-Glycine Gel, 10-well (Cat.# 161-1105EDU) 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 6.B.)

- 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, GE Healthcare, Cat.# EC6025BOX, EC60355BOX, and Bio-Rad Ready Gel 4–20% Tris-Glycine Gel, 10-well, Cat.# 161-1105EDU)

1. When the 25µl translation reaction is complete (or at any desired timepoint), remove a 5µl aliquot and add it to 20µl of SDS sample buffer. The remainder of the reaction may be stored at –20°C.
2. Cap the tube and heat at 70°C for 15 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 6.B) 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.

4.B. Post-Translational Analysis (continued)

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 (GE Healthcare) can be conveniently 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.

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 the Promega Gel Drying Kit (Cat.# V7120). To decrease the likelihood of cracking gradient gels, dry them upside down (with wells pointing down).

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

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.

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

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 10–14. 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 then may be subjected to immunodetection analyses. For more information, refer to the *Protocols and Applications Guide*, Online Edition (2).

5. Troubleshooting

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

Symptoms	Causes and Comments
The control reactions did not work	Reaction components are no longer viable: • Do not use membranes after their expiration date. • Do not use membranes 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. These contaminants can inhibit translation.
The translation efficiency of sample mRNA is low	RNA concentration not optimized. The final RNA concentration 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.
	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 may be present in the translation sample reaction. To determine whether inhibitors are present in the mRNA preparation, mix the mRNA with Luciferase Control RNA 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).

5. Troubleshooting (continued)

Symptoms	Causes and Comments
The translation efficiency of sample mRNA is low (continued)	Calcium is present in the translation reaction. Calcium should be avoided in the translation reaction, because it 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.
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].
	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.
Unexpected bands are present in the control reaction lane	Oxidized β-mercaptoethanol is present or there is not enough SDS in the loading buffer. Use a loading buffer that contains 2% SDS and 100mM DTT.

Symptoms	Causes and Comments
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 sample loaded onto the gel and the amount of loading buffer. Loading too much protein can cause smearing.
	Acrylamide concentration too low to resolve proteins. Acrylamide concentration can be increased to 12%.

6. Appendix

6.A. References

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6.A. References (continued)

11. Burnette, W.N. (1981) "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein. *Anal. Biochem.* **112**, 195–203.
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15. Hicks, D. *et al.* (1986) Immobilon™ PVDF transfer membrane: A new membrane substrate for Western blotting of proteins. *BioTechniques* **4**, 272.
16. Jackson, R.J. and Hunt, T. (1983) Preparation and use of nuclease-treated rabbit reticulocyte lysates for the translation of eukaryotic messenger RNA. *Methods Enzymol.* **96**, 50–4.
17. Snyder, R.D. and Edwards, M.L. (1991) Effects of polyamine analogs on the extent and fidelity of in vitro polypeptide synthesis. *Biochem. Biophys. Res. Comm.* **176**, 1383–92.

6.B. Composition of Buffers and Solutions

separating gel 4X buffer

18.17g	Tris base
4ml	10% SDS

Adjust pH to 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-HCl (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.

6.C. Related Products

Rabbit Reticulocyte Lysate

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease Treated	5 × 200µl	L4960
Rabbit Reticulocyte Lysate, Untreated	1ml	L4151

Other Translation Systems

Product	Size	Cat.#
Flexi® Rabbit Reticulocyte Lysate System	5 × 200µl	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	40 × 50µl reactions	L1170
TnT® T7 Quick Coupled Transcription/Translation System Trial Size	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	5 × 200µl	L4380

Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination Systems

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

E. coli S30 Extract System Templates

Product	Size	Cat.#
E. coli S30 Extract System for Linear Templates	30 × 50µl reactions	L1030
E. coli S30 Extract System for Circular DNA	30 × 50µl reactions	L1020
E. coli T7 S30 Extract System for Circular DNA	30 × 50µl reactions	L1130

RNA Purification Systems

Product	Size	Cat.#
RiboMAX™ Large Scale RNA Production System—SP6	1 system	P1280
RiboMAX™ Large Scale RNA Production System—T7	1 system	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

Includes 1 pair of Gel Drying Frames, 20 sheets of Gel Drying Film (25.5 x 28cm) and 12 clamps.

RNasin® Ribonuclease Inhibitor

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

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6.D. Summary of Changes

The following change was made to the 11/18 revision of this document:

1. The document design was updated.