TECHNICAL MANUAL

Wheat Germ Extract

Instructions for Use of Products
L4380 and L4330

Revised 1/19
TM230

Wheat Germ Extract

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1. Description

Cell-free extracts of wheat germ and rabbit reticulocyte lysate support the in vitro translation of a wide variety of viral, prokaryotic and eukaryotic mRNAs (1). Wheat Germ Extract is prepared by grinding wheat germ in an extraction buffer followed by centrifugation to remove cell debris. The supernatant is subjected to chromatography that separates endogenous amino acids and plant pigments from the extract. The extract is also treated with micrococcal nuclease to destroy endogenous mRNA and thus minimize background translation. Wheat Germ Extract and Rabbit Reticulocyte Lysate contain the cellular components necessary for protein synthesis (tRNA, ribosomes and initiation, elongation and termination factors). The extract is optimized further by the addition of the following:

- an energy-generating system consisting of phosphocreatine and phosphocreatine kinase
- spermidine to stimulate the efficiency of chain elongation and thus overcome premature termination
- magnesium acetate at a concentration recommended for the translation of most mRNA species

Only the addition of exogenous amino acids (including an appropriately labeled amino acid) and mRNA are necessary to stimulate translation. Potassium Acetate is supplied as a separate component so that the translational system may be optimized for a wide range of mRNAs.

Potential applications of Wheat Germ Extract translation products include:

- mutation detection and analysis (i.e., enzyme kinetics)
- protein:protein interactions
- immunoprecipitation of protein complexes
- protein dimerization assays
- ligand-binding region determination/confirmation/competition assays
- protein structure analysis
- electrophoretic mobility shift assays (EMSAs) for DNA-protein interactions
- DNA footprinting and protein cross-linking studies
- protein:RNA binding assays
- post-translational modification assays
- verification/characterization of cloned gene products

For peer-reviewed articles citing use of this product, please visit: **www.promega.com/citations/**

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wheat Germ Extract	**30 × 50µl reactions**	**L4380**

Wheat Germ Extract is provided in 200µl aliquots. Each system contains sufficient reagents to perform 30 × 50µl reactions. Includes:

- 1ml Wheat Germ Extract (5 × 200µl)
- 10µg Luciferase Control RNA
- 0.5ml Potassium Acetate (1M)
- 175µl Amino Acid Mixture Minus Methionine, 1mM
- 175µl Amino Acid Mixture Minus Leucine, 1mM
- 175µl Amino Acid Mixture Minus Cysteine, 1mM

PRODUCT	SIZE	CAT.#
Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System	**24 reactions**	**L4330**

Each system contains 2 × 200µl of both Rabbit Reticulocyte Lysate and Wheat Germ Extract; this is sufficient for 12 reactions using the Rabbit Reticulocyte Lysate and 12 reactions using the Wheat Germ Extract. Both the lysate and extract contain endogenous levels of all amino acids. Includes:

- 0.4ml Rabbit Reticulocyte Lysate, Nuclease-Treated (2 × 200µl)
- 0.4ml Wheat Germ Extract (2 × 200µl)
- 10µg Luciferase Control RNA
- 0.5ml Potassium Acetate (1M)
- 175µl Amino Acid Mixture Minus Methionine, 1mM
- 175µl Amino Acid Mixture Minus Leucine, 1mM
- 175µl Amino Acid Mixture Minus Cysteine, 1mM

Storage Conditions: Store at –70°C. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes, as these fluctuations can greatly alter product stability. Do not freeze-thaw the lysate more than two times. Prolonged exposure to dry ice can cause significant loss of lysate activity.

3. General Considerations

Cell-free eukaryotic translation systems are used to rapidly characterize plasmid clones, characterize protein: protein interactions, study structural mutations, examine translational signals and characterize mRNA populations. Two types of systems are available: 1) translation systems for RNA templates; and 2) coupled transcription/translation systems for DNA templates. Several factors may influence the choice of an in vitro translation system; some of these considerations are summarized below.

3.A. Translation Systems

A number of cell-free translation systems have been developed for use with mRNA isolated from various sources. Promega offers several Rabbit Reticulocyte Lysate and Wheat Germ Extract Systems. All are reliable, convenient and easy-to-use systems to initiate translation and produce full-size polypeptide products. Rabbit Reticulocyte Lysate is generally recommended when microsomal membranes are to be added for cotranslational processing. Wheat Germ Extract can also be used to monitor cotranslational events, but signal sequence recognition and targeting require addition of signal recognition particle and microsomes (2–4) or semi-permeabilized cells (5). Cotranslational processing events are generally detected as shifts in the apparent molecular weight of translation products on SDS polyacrylamide gels. The Flexi® Rabbit Reticulocyte Lysate is recommended for those who wish to optimize translation of particular RNAs by adjusting salt and DTT concentrations.

In vitro translation reactions may be directed by mRNAs isolated in vivo or by RNA templates transcribed in vitro from vectors such as the pGEM® Vectors. Procedures for the rapid isolation and poly(A)+ selection of cellular mRNAs are provided in the *Protocols and Applications Guide* (6). When using mRNA synthesized in vitro, the presence of a 5´-cap structure may enhance the translational activity in eukaryotic translation systems (7).

3.B. Coupled Eukaryotic Transcription/Translation Systems

DNA sequences cloned in plasmid vectors also may be expressed directly using either the TnT® Quick Coupled Reticulocyte Lysate Systems, TnT® Coupled Wheat Germ Extract Systems or TnT® SP6 High-Yield Expression System (see Section 8.C). The TnT® Systems require plasmid constructs containing a phage RNA polymerase promoter (SP6, T3 or T7) for the initiation of transcription, but translation in this system is under eukaryotic controls. Optimal translation will occur if the AUG initiation codon is in a "Kozak consensus" context (A/GCCAUGG) (8) in the absence of inhibiting secondary structure.

4. Translation Protocol for Wheat Germ Extract

The translation protocol for use with Wheat Germ Extracts is provided in Section 4.A. Many factors affect translation efficiency of specific RNAs in the Wheat Germ Extract system and should be considered when designing in vitro translation experiments. The optimal mRNA concentration will vary for particular transcripts and should be determined empirically. In addition, the presence of certain nucleic acid sequence elements can have profound effects on initiation fidelity and translation efficiency. Poly(A)+ tails, 5´-caps, 5´-untranslated regions and the sequence context around the AUG start (or secondary AUGs in the sequence) (9) can all affect translation of a given mRNA. Lastly, optimal salt concentrations, particularly of K^+ and Mg^{2+}, vary for a given mRNA and should be determined empirically. Magnesium Acetate, 25mM (Cat.# L4581), and Potassium Chloride, 2.5M (Cat.# L4591) are available from Promega. For more information on these variables, please read the notes in Section 4.A.

Allow approximately 2 hours to perform the translation reaction (Figure 1). The SDS gel can conveniently be set up and cast (1 hour) during this time. Allow 2 hours to perform TCA precipitation and prepare filters for scintillation counting. Two hours should also be allowed to load and run the SDS polyacrylamide gel.

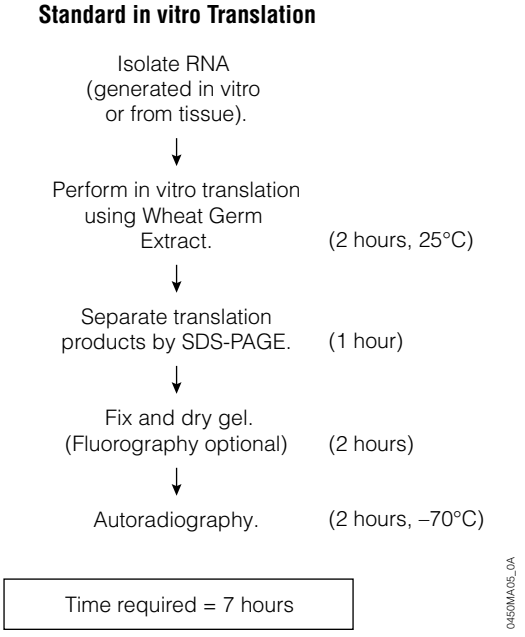

Figure 1. Flow chart of the in vitro translation procedure using Wheat Germ Extract.

4.A. Translation Procedure

The following is a general guideline for setting up a translation reaction. An example is provided for a standard reaction using [^{35}S]methionine (radioactive) detection. Transcend™ Non-Radioactive Translation Detection Systems (Cat.# L5070 and L5080) and FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Cat.# L5001) also can be used for detection of reaction products. Using the Transcend™ systems, biotinylated lysine residues are incorporated into nascent proteins during translation. The 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. The FluoroTect™ system is based on a lysine-charged tRNA that is labeled at the ε position of the lysine with the fluorophore BODIPY®-FL. For more information on non-radioactive detection, refer to the *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182 and *FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System Technical Bulletin* #TB285. These documents are available at: **www.promega.com/protocols/**

Wheat Germ Extract contains endogenously biotinylated proteins that may be difficult to distinguish from biotinylated translation products. When using Transcend™ tRNA and Transcend™ Translation Detection Systems, it is important to include a negative control reaction (without added RNA) to identify any endogenously biotinylated proteins in the Wheat Germ Extract.

4.A Translation Procedure (continued)

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- RNasin® Ribonuclease Inhibitor (Cat.# N2111) or RNasin® Plus RNase Inhibitor (Cat.# N2611)
- isotopically labeled amino acids, typically [^{35}S]methionine, [^{35}S]cysteine, [^{3}H]leucine, [^{14}C]leucine; Transcend™ Non-Radioactive Translation Detection System (Cat.# L5070 or L5080); or FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Cat.# L5001)

1. Remove the reagents from storage, and allow them to thaw slowly on ice.

   Notes:

   Except for the translation incubation, all Wheat Germ Extract components should be kept on ice while in use. Unused Wheat Germ Extract should be stored at –70°C.

   Do not freeze-thaw the lysate more than two times. Do not store lysate in the presence of dry ice.

2. Following the example that follows, assemble the reaction components appropriate for the label being used in a 0.5ml microcentrifuge tube. Gently mix the extract with a pipette tip upon addition of each component. If necessary, centrifuge briefly to return the sample to the bottom of the tube. Smaller scale reactions may be performed by reducing volumes proportionally.

   Notes:

   For use of other radiolabeled amino acids, see Table 1. When using other radiolabeled amino acids, be sure to substitute the appropriate amino acid mixture for the Amino Acid Mixture Minus Methionine, 1mM. When using radiolabeled leucine, use the Amino Acid Mixture Minus Leucine, 1mM, and when using radiolabeled cysteine, use the Amino Acid Mixture Minus Cysteine, 1mM.

   Include a control reaction containing no mRNA. This allows measurement of any background incorporation of labeled amino acids.

Component	Standard Reaction Using [^{35}S]methionine	Standard Reaction Using Transcend™ tRNA	Standard Reaction Using FluoroTect™ Green$_{Lys}$ tRNA
Wheat Germ Extract	25µl	25µl	25µl
Amino Acid Mixture Minus Methionine, 1mM	4µl	2µl	2µl
Amino Acid Mixture Minus Leucine, 1mM	–	2µl	2µl
RNA substrate in nuclease-free water (see Notes 1–6)	10µl of control RNA (10µg)	10µl of control RNA (10µg)	10µl of control RNA (10µg)
Potassium Acetate, 1M (see Note 3)	0–7µl	0–7µl	0–7µl
RNasin® Ribonuclease Inhibitor (40u/µl) (see Note 7)	1µl	1µl	1µl
[^{35}S]methionine (see Note 8)	2.5µl	–	–
Transcend™ tRNA (see Notes 9 and 10)	–	1–2µl	–
FluoroTect™ Green$_{Lys}$ tRNA (see Note 9)	–	–	1–2µl
nuclease-free water to a final volume of	50µl	50µl	50µl

Table 1. Recommended Concentrations of Alternative Radiolabeled Amino Acids.

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

3. Incubate the translation reactions at 25°C for 60–120 minutes.

4. Analyze the results of the translation. Procedures are provided for incorporation assays (Section 5.A) and SDS polyacrylamide gel analysis of translation products (Section 5.B). For analysis of reactions using Transcend™ tRNA, see Note 9 and refer to the *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182. For analysis of reactions using FluoroTect™ tRNA, refer to the *FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System Technical Bulletin* #TB285.

4.A Translation Procedure (continued)

Notes:

1. An unfractionated cytoplasmic RNA preparation is 90–95% rRNA and, as a result, translates poorly. Usually such preparations yield no better than 20–30% of the maximum incorporation attainable, and final concentrations of 100–200µg/ml RNA are needed to stimulate translation. Viral RNAs and poly(A)+ mRNAs (including mRNA transcribed in vitro) should be titrated to determine the optimal RNA template concentration, which will vary with the RNA template. We recommend a 100–200µg/ml final concentration of Poly(A) or viral RNA.
2. The optimal RNA concentration for translation should be determined prior to performing definitive experiments. In determining the optimal concentration, serially dilute the RNA template first, then add the same volume of RNA to each reaction to ensure that other variables are kept constant. Also see Note 1 above.
3. Optimum potassium concentration varies from 50mM to 200mM, depending on the mRNA used. If this concentration of potassium results in poor translation of your sample mRNA, potassium levels should be reduced or increased to an optimum concentration. Certain mRNAs may also require altered magnesium concentrations. The optimum magnesium concentration for the majority of mRNAs is expected to fall in the range of 2–5mM. See Table 2 for the concentrations of key exogenous components of Wheat Germ Extract.
4. Avoid adding calcium to the translation reaction. Calcium may reactivate the micrococcal nuclease used to destroy endogenous mRNA in the extract and result in degradation of the mRNA template.
5. Residual ethanol should be removed from mRNA preparations and labeled amino acids before they are added to the translation reaction.
6. Some template RNAs may require denaturation prior to translation. Incubate the RNA template at 67°C for 10 minutes, and immediately cool on ice. This increases the efficiency of translation, especially of GC-rich mRNA, by destroying local regions of secondary structure.
7. The addition of RNasin® Ribonuclease Inhibitor to the translation reaction is recommended but not required. RNasin® Ribonuclease Inhibitor acts to inhibit degradation of sample mRNAs by contaminating RNase.
8. We recommend PerkinElmer EasyTag™ L-[^{35}S]methionine (PerkinElmer Cat.# NEG709A). This grade of [^{35}S]methionine does not cause the background labeling of a 42kDa product that can occur using other grades of label.
9. The amount of Transcend™ tRNA or FluoroTect™ Green $_{Lys}$ tRNA that is added to the reaction can be increased up to 4µl to allow more sensitive detection of proteins that contain few lysines or are poorly expressed.
10. Commonly used translation extracts frequently contain endogenously biotinylated proteins, which may be detected when translation products are analyzed by SDS-PAGE, electroblotting and streptavidin-AP or streptavidin-HRP detection. Rabbit Reticulocyte Lysate contains one biotinylated protein, which migrates as a faint band at 100kDa and, in some lots, an additional faint band at 47kDa. Wheat Germ Extract contains 5 major endogenous biotinylated proteins, migrating at 200kDa, 80kDa, 32kDa and a doublet at 17kDa. Comparison to a control reaction without template will enable distinction between endogenous biotinylated proteins and newly synthesized biotinylated translation products.

11. Each batch of Wheat Germ Extract contains 30–50mg/ml of endogenous protein.

Table 2. Final Concentrations of Wheat Germ Extract Components in a 50µl Translation Reaction.

Component	Final Concentration
creatine phosphate	10mM
creatine phosphokinase	50µg/ml
DTT	5mM
magnesium acetate	2.1mM
potassium acetate	53mM*
spermidine	0.5mM
ATP	1.2mM
GTP	0.1mM

*You may need to add additional Potassium Acetate to optimize translation for each RNA sample (see Note 3, above).

5. Post-Translational Analysis

Materials to Be Supplied by the User

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

- 1M NaOH
- 25% TCA/2% casamino acids (Difco brand, Vitamin Assay Grade)
- 5% TCA
- separating gel 4X buffer
- stacking gel 4X buffer
- SDS polyacrylamide running 10X buffer
- SDS sample buffer
- acetone
- SDS polyacrylamide gel suitable for resolving the protein of interest
- Whatman GF/C glass fiber filters
- Whatman 3MM filter paper
- fixing solution
- X-ray film

5.A. Determination of Percent Incorporation of Radioactive Label

1. After the 50µl translation reaction is complete, remove 2µl from the reaction, and add it to 98µl of 1M NaOH.
2. Vortex briefly, and incubate at 37°C for 10 minutes.
3. At the end of the incubation add 900µl of ice-cold 25% TCA/2% casamino acids to precipitate the translation product. Incubate on ice for 30 minutes.
4. Wet a Whatman GF/C glass fiber filter with a small amount of cold 5% TCA. Collect the precipitate by vacuum filtering 250µl of the TCA reaction mix. Rinse the filter three times with 1–3ml of ice-cold 5% TCA. Rinse once with 1–3ml of acetone. Allow the filter to dry at room temperature or under a heat lamp for at least 10 minutes.
5. For determination of ^{35}S-incorporation, put the filter in 1–3ml of appropriate scintillation fluid, invert to mix and count in a liquid scintillation counter.
6. To determine total counts present in the reaction, spot a 5µl aliquot of the TCA reaction mix directly onto a filter. Dry the filter for 10 minutes. Count in a liquid scintillation counter as in Step 5. The measured counts per minute (cpm) are the cpm in 5µl of the TCA reaction mix.
7. To determine background counts, remove 2µl from a 50µl translation reaction containing no RNA, and proceed as described in Steps 1–5 above.
8. Perform the following calculation to determine percent incorporation:

$$\frac{\text{cpm of washed filter (Step 4)}}{\text{cpm of unwashed filter (Step 6)} \times 50} \times 100 = \text{percent incorporation}$$

9. Perform the following calculation to determine the fold stimulation over background:

$$\frac{\text{cpm of washed filter (Step 4)}}{\text{cpm of “no RNA control reaction” washed filter (Step 7)}} = \text{fold stimulation}$$

5.B. Denaturing Gel Analysis of Translation Products

For protein analysis, Invitrogen Corporation 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 Invitrogen Novex® 4–20% Tris-Glycine gradient gels (Cat.# EC6028) and the Bio-Rad Ready Gel 4–20% Tris HCl linear gradient 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.

1. Once the 50µl translation reaction is complete (or at any desired time point), 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. Close the tube, and heat at 70°C for 15 minutes to denature the proteins.

3. A 10µl aliquot of the denatured sample can then be loaded on an SDS polyacrylamide gel or stored at −20°C. It is not necessary to separate labeled polypeptides from free amino acids by acetone precipitation.

   Note: Gel banding patterns may be improved by loading unlabeled samples of Wheat Germ Extract 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. Because the dye front also contains unincorporated labeled amino acids, disposal of unincorporated label may be easier if the gel is stopped while the dye front remains in the gel. If transferring the gel to a membrane filter for Western blot analysis, proceed to Step 8.

5. Place the polyacrylamide gel in a plastic box, and cover the gel with fixing solution for 30 minutes. Agitate slowly on an orbital shaker. Pour off the fixing solution.

   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 thus increases the proportion of energy that may be detected by X-ray film. Commercial reagents, such as GE Healthcare Bio-sciences Amplify® Reagent, 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.

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, and cover the top of the gel with plastic wrap. It is often advantageous to cut or mark one corner of the filter paper to help discern the gel orientation on the filter.

   Dry at 80°C for 30–90 minutes under a vacuum using a conventional gel dryer; dry completely. The gel may also be dried overnight using the 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 1–6 hours at −70°C (for fluorography) or 6–15 hours at room temperature (for autoradiography).

5.B. Denaturing Gel Analysis of Translation Products (continued)

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 the *Protocols and Applications Guide, Online Edition* (6). For detection of biotinylated protein using the Transcend™ Non-Radioactive Translation Detection Systems, see Technical Bulletin #TB182. For detection of BODIPY®-FL-labeled protein using the FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System, see Technical Bulletin #TB285.

6. Related Procedures

6.A. Synthesis of Milligram Quantities of in vitro Transcripts

In vitro transcription reactions are widely used to synthesize microgram amounts of RNA probes from recombinant DNA templates. An important consideration in preparing DNA templates for transcription is to avoid linearizing the DNA with restriction enzymes that leave 3´, 4-base overhangs (PstI, KpnI, SacI, SacII, BstXI, NsiI, ApaI and AatII) because aberrant transcription products can be produced (16). If no alternative enzyme is available, the 3´-overhang can be removed to produce blunt ends using DNA Polymerase I Large (Klenow) Fragment (Cat.# M2201) prior to transcription.

The RiboMAX™ Large Scale RNA Production Systems and T7 RiboMAX™ Express Large Scale RNA Production System (Cat.# P1320) have been used to produce milligram amounts of RNA for use in in vitro translation systems.

For a protocol and further information, the *T7 RiboMAX™ Express Large Scale RNA Production System Technical Bulletin* #TB298 or *RiboMAX™ Large Scale RNA Production Systems Technical Bulletin* #TB166, are available at: **www.promega.com/protocols/**

6.B. In vitro Synthesis of Capped RNA Transcript

Most eukaryotic mRNAs contain a m7G(5´)ppp(5´)G cap at the 5´-end, which is important for the binding of translation initiation factors and contributes to mRNA stability. The use of capped RNA is suggested for programming particular translation systems, such as Xenopus oocytes. Many transcripts do not require a cap structure for efficient translation in either Wheat Germ Extract or Rabbit Reticulocyte Lysate Systems. However, enhanced translation of certain capped transcripts has been observed (7,17).

Increasingly, uncapped messages are being used effectively in the Wheat Germ Extract and Rabbit Reticulocyte Lysate Systems. We have found that comparable levels of protein synthesis can be achieved by simply increasing the amount of uncapped RNA added to the translation reaction (18).

For further information or if capping is desired, contact Promega and request the *RiboMAX™ Large Scale RNA Production Systems Technical Bulletin* #TB166, which includes a protocol for synthesis of capped RNA transcripts using the Ribo m^7G Cap Analog (Cat.# P1711) and is available at: **www.promega.com/protocols/**

7. 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
Sample reactions and control reactions did not produce detectable amount of protein	Reaction components were used after the expiration date. date. Check the expiration date of the reaction components.
	Reaction components were damaged by multiple freeze-thaw cycles. For optimal activity, the reaction components should not be exposed to more than two freeze-thaw cycles.
The control reaction worked, but the sample reaction did not	Ethanol or salt was present in the translation reaction. Ethanol or salt may inhibit translation.
	Final concentration of RNA is outside of the appropriate range. The final concentration of RNA is important and may need to be optimized. Optimal RNA levels are usually between 100–200µg/ml.
Translation efficiency of sample mRNA is low	RNA concentration was not optimized. The optimal RNA concentration for translation should be determined prior to performing definitive experiments. To determine the optimal concentration, serially dilute your RNA 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 was not optimized. Optimum potassium concentration varies from 50mM to 200mM, 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. Adding 0.5–2.5mM of magnesium, in addition to that endogenously present in the lysate, is generally sufficient for the majority of mRNAs used.
	Ethanol was 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 caused decreased protein synthesis. Incubate translation reaction at 30°C.

Symptoms	Causes and Comments
Translation efficiency of sample mRNA is low (continued)	Inhibitors are present in the translation reaction. Oxidized thiols, low concentrations of double-stranded RNA and polysaccharides are typical inhibitors of translation (19).
	Reaction time may need to be optimized. Increase the reaction time to 90–120 minutes.
	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 extract and result in degradation of the mRNA template.
	The addition of spermidine and certain diamines has been shown to stimulate translation (20). The addition of spermidines and diamines may change the optimal Mg^{2+} concentration; thus, these components need to be co-optimized.
Unexpected bands are present on the gel	More than one peptide was translated from the RNA template. The RNA template may have more than one start site.
	There could be a contaminating translation product. Run the reaction with no RNA to see if the additional band is present.
	^{35}S-labeled amino acid was beyond its expiration date. Older ^{35}S may dissociate from the amino acid and label other proteins in the lysate. Use fresh ^{35}S-labeled amino acids.
	The [^{35}S]methionine used was not translation-grade. There are reports of a 42kDa band with some grades of [^{35}S]-methionine (21). We recommend PerkinElmer EasyTag™ L-[^{35}S]methionine (PerkinElmer Cat.# NEG709A) and have not seen a 42kDa band with this label.
	Aminoacyl tRNAs may produce background bands. Add RNase A to the Wheat Germ Extract reaction (after completion) to a final concentration of 0.2mg/ml. Incubate for 5 minutes at 30°C.

Symptoms	Causes and Comments
There is smearing on the gel	The gel was not clean. The gel must be washed before placing onto film. Once 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 onto the gel can cause smearing. Check the amount of samples loaded on the gel and the amount of loading buffer.
	Acrylamide concentration was too low to resolve proteins. The acrylamide concentration in the SDS polyacrylamide gel can be increased to 12%.
	Ethanol was present in the sample. Ethanol can cause smearing on the gel.

8. Appendix

8.A. Composition of Buffers and Solutions

Coomassie® destaining solution

50% methanol
7.5% glacial acetic acid

fixing solution

50% methanol
10% glacial acetic acid
40% water

Store at room temperature.

SDS polyacrylamide running 10X buffer

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

separating gel 4X buffer

18.17g Tris base
4ml 10% SDS

Bring the volume to approximately 80ml with deionized water. Adjust to pH 8.8 with 12N HCl, and add deionized water to a 100ml final volume. Store at room temperature.

SDS sample buffer

2ml glycerol
2ml 10% SDS
0.25mg bromophenol blue
2.5ml stacking gel 4X buffer
0.5ml ß-mercaptoethanol

Combine all components except ß-mercaptoethanol, and add deionized water to a final volume of 9.5ml. Store at room temperature. Add the ß-mercaptoethanol to the sample buffer immediately before use.

stacking gel 4X buffer

6.06g Tris base
4ml 10% SDS

Bring the volume to approximately 80ml with deionized water. Adjust to pH 6.8 with 12N HCl, and add deionized water to a 100ml final volume. Store at room temperature.

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8.B. References

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8.C. Related Products

TnT® Coupled Wheat Germ Extract Systems

Product	Size	Cat.#
TnT® SP6 High-Yield Wheat Germ Protein Expression System	40 × 50µl reactions	L3260
	10 × 50µl reactions	L3261
TnT® SP6 Coupled Wheat Germ Extract System	40 × 50µl reactions	L4130
TnT® T7 Coupled Wheat Germ Extract System	40 × 50µl reactions	L4140
TnT® T7/SP6 Coupled Wheat Germ Extract System	40 × 50µl reactions	L5030

TnT® Quick Coupled Transcription/Translation Systems

Product	Size	Cat.#
TnT® T7 Quick Coupled Transcription/Translation System	40 × 50µl reactions	L1170
TnT® SP6 Quick Coupled Transcription/Translation System	40 × 50µl reactions	L2080

Trial size (5 × 50µl reactions) also available.

TnT® Coupled Reticulocyte Lysate Systems

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

*Trial size (8 × 50µl reactions) also available.

8.C Related Products (continued)

Translation Systems

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease Treated	30 × 50µl reactions	L4960
Rabbit Reticulocyte Lysate, Untreated	30 × 50µl reactions	L4151
Flexi® Rabbit Reticulocyte Lysate System	30 × 50µl reactions	L4540

Non-Radioactive Translation Detection Systems

Product	Size	Cat.#
Transcend™ Colorimetric Non-Radioactive Translation Detection System	30 × 50µl reactions	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System	30 × 50µl reactions	L5080
Transcend™ tRNA	30µl	L5061
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System	40 reactions	L5001

E. coli S30 Extract Systems

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

Canine Pancreatic Microsomal Membranes

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

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

Translation-Related Products

Product	Size	Cat.#
Potassium Chloride, 2.5M	200µl	L4591
Magnesium Acetate, 25mM	100µl	L4581
Ribo m^7G Cap Analog	10 A_{254} units	P1711
	25 A_{254} units	P1712
RNasin® Ribonuclease Inhibitor*	10,000u	N2115
Recombinant RNasin® Ribonuclease Inhibitor*	10,000u	N2515
RNasin® Plus RNase Inhibitor*	10,000u	N2615

*Additional size (2,500u) also available.

RNA Production Systems

Product	Size	Cat.#
T7 RiboMAX™ Express Large Scale RNA Production System	1 system	P1320
RiboMAX™ Large Scale RNA Production System—SP6	1 system	P1280
RiboMAX™ Large Scale RNA Production System—T7	1 system	P1300
Riboprobe® System—SP6	1 system	P1420
Riboprobe® System—T3	1 system	P1430
Riboprobe® System—T7	1 system	P1440
Riboprobe® Combination System—T3/T7 RNA Polymerase	1 system	P1450
Riboprobe® Combination System—SP6/T7 RNA Polymerase	1 system	P1460

9. Summary of Changes

The following change was made to the 1/19 revision of this document:

1. Corrected the amount of Luciferase Control RNA listed in Section 2, Product Components and Storage Conditions.