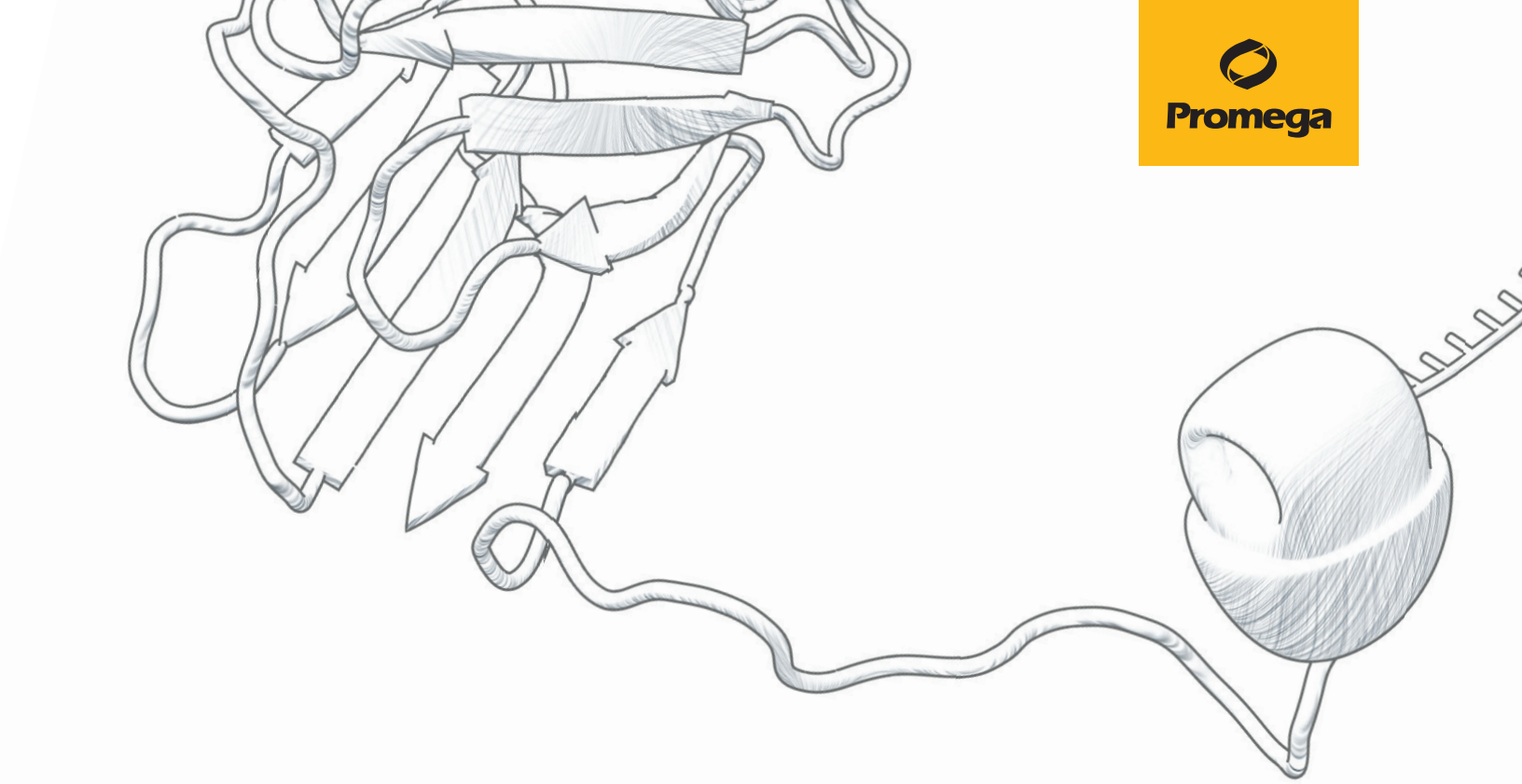
# 3 Cell-Free Protein Expression Systems



# Introduction

Cell-free protein synthesis is an important tool for molecular biologists in basic and applied sciences. It is increasingly being used in high-throughput functional genomics and proteomics, with significant advantages compared to protein expression in live cells. Cell-free protein synthesis is essential for the generation of protein arrays, such as nucleic acid programmable protein array (NAPPA) and enzyme engineering using display technologies. The cell-free approach provides the fastest way to correlate phenotype (function of expressed protein) to genotype. Protein synthesis can be performed in a few hours using either mRNA template in translational systems or DNA template (plasmid DNA or PCR fragments) in coupled transcription and translation systems. Furthermore, cell-free protein expression systems are indispensable for the expression of toxic proteins, membrane proteins, viral proteins and for proteins that undergo rapid proteolytic degradation by intracellular proteases.

## Origins of Cell-Free Expression Systems

Cell-free protein expression lysates are generated from cells engaged in a high rate of protein synthesis, such as immature red blood cells (reticulocytes). The most frequently used cell-free expression systems originate from rabbit reticulocytes, wheat germ and *E. coli*. There are two types of cell-free expression systems: Translation Systems and Coupled Translation and Transcription (TNT®) Systems **(Figure 3.1).** Both types of systems provide the macromolecular components required for translation, such as ribosomes, tRNAs, aminoacyl-tRNA synthetases, initiation, elongation and termination factors. To ensure efficient translation, each extract has to be supplemented with amino acids, energy sources (ATP, GTP), energy regenerating systems and salts ($Mg^{2+}$, $K^{+}$, etc.). For eukaryotic systems creatine phosphate and creatine phosphokinase serve as energy regenerating system, whereas prokaryotic systems are supplemented with phosphoenol pyruvate and pyruvate kinase. Coupled transcription and translation systems are supplemented with phage-derived RNA polymerase (T7, T3 or SP6) allowing the expression of genes cloned downstream of a T7, T3 or SP6 promoter.

## Selection of Cell-Free Protein Expression

Many different cell-free expression systems derived from prokaryotic and eukaryotic source are available. The choice of the system is dependent on several factors, including the origin of the template RNA and DNA, protein yield or whether the protein of interest requires post-translational modification (e.g., core glycosylation). We offer translation systems (mRNA-based) and coupled transcription/translation systems (DNA-based) from prokaryotic and eukaryotic sources. **Table 3.2** provides an overview of translational systems and **Table 3.3** provides an overview of coupled translation/transcription systems.

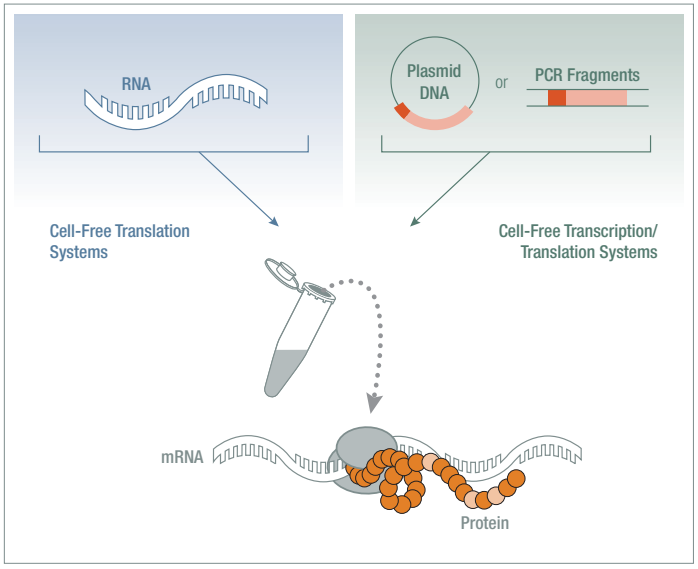



**Figure 3.1.** Cell-free protein expression systems are divided into mRNA-based translation systems and in DNA-based transcription/translation systems.

**Table 3.1.** Applications of Cell-Free Protein Synthesis

### Functional Genome/ Proteome Analysis

- Gene mutation/deletion analysis (e.g., enzyme kinetics)
- Protein domain mapping
- Characterization of protein interactions
- Gel Shift EMSA
- Generation of protein arrays

### Expression of Difficult-to-Express Proteins

- Cell-toxic proteins, membrane protein, viral proteins, kinases

### Protein Evolution/ Enzyme Engineering

- Display technologies (e.g., ribosome, mRNA display, in vitro compartmentalization)
- Evolution of antibodies in vitro by ribosome display

### Analysis of Transcriptional/ Translational Regulation

- Structural RNA analysis such as characterization of regulatory elements for translation (e.g., UTRs, Capping, IRES)
- RNA virus characterization

### Screenings

- Screening of chemical libraries for effect on translation
- Drug screening (e.g., antibiotics)

### Protein Labeling

- Open systems allow the incorporation of labeled amino acids

# 3.1 Translation Systems: mRNA-based

## OVERVIEW

Cell-free translation systems are used for protein expression of either in vitro transcribed mRNA or mRNA isolated from tissues or cells. These systems are used to express single proteins as well as multiple proteins in high-throughput applications such as display technologies. Furthermore, cell-free translation systems are useful for functional and structural RNA analysis, or to study aspects of the translational machinery. Eukaryotic translation systems originate from either rabbit reticulocyte lysates (RRL) or wheat germ extracts (WGE). We offer three mRNA-based translation systems. The extracts are treated with microccal nuclease to destroy endogenous mRNA and thus reduce background translation to a minimum **(Table 3.2)**.

The *Flexi® Rabbit Reticulocyte Lysate System* offers greater flexibility in reaction conditions by allowing translation reactions to be optimized for a wide range of parameters, including $Mg^{2+}$ and $K^{+}$ concentrations. The Wheat Germ Extract is a useful alternative to the RRL systems for expressing small proteins or for expressing proteins known to be abundant in RRL. Researchers expressing proteins from plants or yeasts or other fungi also may find WGE preferable to RRL.

**Table 3.2.** Overview of Cell-FreeTranslation Systems that use mRNA as a Template.

| Translation System | Nuclease-Treated | Signal Cleavage & Core Glycosylation with CMM* | Labeling Options** | Luciferase Control RNA | Protein Yield |
|---|---|---|---|---|---|
| Rabbit Reticulocyte Lysate System, Nuclease-Treated (Cat.# L4960) | + | + | Met,Cys,Leu, FluoroTect™; Transcend™ | + | 1–4 µg/ml |
| Flexi® Rabbit Reticulocyte Lysate (Cat.# L4540) *** | + | + | Met, Cys, Leu, FluoroTect™; Transcend™ | + | 1–4 µg/ml |
| Wheat Germ Extract (Cat.# L4380) | + | - | Met, Cys, Leu, FluoroTect™; Transcend™ | + | 0.6–3 µg/ml |

* CMM: Canine Microsomal Membranes

** The lysates are provided with three Amino Acid Mixtures for the incorporation of labeled amino acids like methionine, cysteine & leucine. Transcend™ tRNA (Cat.# **L5070; L5080**) and FluoroTect™ (Cat.# **L5001**) can be used to incorporate biotinylated or fluorescently labeled lysine residues.

*** The system provides greater flexibility of reaction conditions than standard rabbit reticulocyte lysate systems. The Flexi® Rabbit Reticulocyte Lysate System allows translation reactions to be optimized for a wide range of parameters, including $Mg^{2+}$ and $K^{+}$ concentrations and the option to add DTT.

# Rabbit Reticulocyte Lysate System, Nuclease-Treated

In vitro protein synthesis starting from mRNA.

## Description

Rabbit Reticulocyte Lysate (RRL), Nuclease-Treated, is optimized for mRNA translation by the addition of several supplements. These include hemin, which prevents activation of the heme-regulated eIF-2a kinase; an energy-generating system consisting of phosphocreatine kinase and phosphocreatine; and calf liver tRNAs to balance the accepting tRNA populations, thus optimizing codon usage and expanding the range of mRNAs that can be translated efficiently. In addition, the lysates are treated with micrococcal nuclease to eliminate endogenous mRNA. RRLs post-translationally modify proteins via phosphorylation, acetylation and isoprenylation. Signal peptide cleavage and core glycosylation also can be achieved by the addition of Canine Pancreatic Microsomal Membranes. See **Table 3.1** for additional applications.

## Principle

In RRL translation reactions, mRNA is used as template for translation. In general, optimal results will be achieved after an incubation time of 1.5 hours at 30°C. However, many template-related factors affect translation efficiency of specific mRNAs in the RRL 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) all may affect translation of a given mRNA.

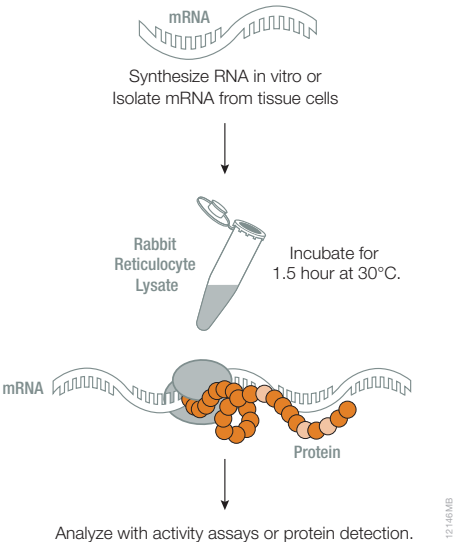



**Figure 3.2.** Flow chart of **in vitro** translation procedure using Rabbit Reticulocyte Lysate.

## Features and Benefits

- **Consistent:** Reliable and consistent translation with each lot.
- **Optimized and Ready-to-Use:** The treated Rabbit Reticulocyte Lysate is optimized for translation.
- **Convenient:** Luciferase Control RNA included.

## Ordering Information

Rabbit Reticulocyte Lysate (RRL), Nuclease-Treated (Cat.# L4960)

# Flexi® Rabbit Reticulocyte Lysate System

In vitro protein synthesis starting from mRNA. Optimize translation for low-expressing mRNA.

## Description

The Flexi® Rabbit Reticulocyte Lysate System is widely used to identify mRNA species and characterize their products. It provides greater flexibility of reaction conditions than the Rabbit Reticulocyte Lysate, Nuclease-Treated, by allowing translation reactions to be optimized for a wide range of parameters, including $Mg^{2+}$ and $K^{+}$ concentrations. See **Table 3.1** for additional applications.

## Principle

As with the standard Rabbit Reticulocyte Lysate, the Flexi® Rabbit Reticulocyte Lysate System is optimized for translation by addition of the following supplements: hemin, to prevent inhibition of initiation factor eIF-2α; an energy-generating system consisting of pretested phosphocreatine kinase and phosphocreatine; calf liver tRNAs to balance the accepting tRNA populations, thus optimizing codon usage and expanding the range of mRNAs that can be translated efficiently; and micrococcal nuclease to eliminate endogenous mRNA, thus reducing background translation. This eukaryotic system has been reported to post-translationally modify proteins via phosphorylation, acetylation and isoprenylation. With the addition of Canine Pancreatic Microsomal Membranes signal peptide cleavage and core glycosylation can occur. The Flexi® Rabbit Reticulocyte Lysate System provides greater flexibility of reaction conditions than standard RRL systems.

## Features and Benefits

- **Consistent:** Reliable and consistent translation with each lot.
- **Easy Optimization:** To aid in optimizing magnesium concentrations, the endogenous magnesium concentration is provided for each lot of Flexi® Lysate.
- **Convenient:** Luciferase Control RNA and detection reagent included.

## Ordering Information

Flexi® Rabbit Reticulocyte Lysate System (Cat.# L4540)

# Wheat Germ Extract

In vitro protein synthesis starting from mRNA.

## Description

Wheat Germ Extract (WGE) is a well-defined processed and optimized extract from wheat germ. It contains the cellular components necessary for protein synthesis (tRNA, ribosomes and initiation, elongation and termination factors). The extract is supplemented with an energy-generating system (phosphocreatine/phosphocreatine kinase), and with spermidine to stimulate the efficiency of chain elongation. Only exogenous amino acids and mRNA are needed to initiate translation. Potassium acetate can be used to optimize translation for a wide range of mRNAs. See **Table 3.1** for additional applications.

## Principle

Wheat Germ Extract is a useful alternative to the Rabbit Reticulocyte Lysate (RRL) systems for expressing small proteins or for expressing proteins expected to be abundant in RRL. Researchers expressing proteins from plants, yeast or other fungi also may find Wheat Germ Extract preferable to RRL.

## Ordering Information

Wheat Germ Extract (Cat.# L4380)

## Features and Benefits

- **Optimized Extract:** Assists in expression of eukaryotic messages that do not express well in RRL.
- **Flexible:** Three Amino Acid Mixtures are provided, which enable different radioisotope choices.
- **Robust:** Potassium Acetate is provided to enhance translation for a wide range of mRNAs.
- **Convenient:** Luciferase Control RNA included.

# 3.2 Transcription and Translation Systems: DNA-based

## OVERVIEW

Coupled transcription and translation (TnT®) systems offer researchers time-saving alternatives for eukaryotic in vitro transcription and translation, by coupling these processes in a single tube format. TnT® Systems are used for a variety of applications in low- to high-throughput functional genome and proteome analyses, as summarized in **Table 3.1**. TnT® Systems are supplemented with T7, T3 or SP6 RNA polymerases, allowing protein expression from DNA cloned downstream of a T7, T3 or SP6 promoter.

We offer TnT® Systems originating from eukaryotic sources such as rabbit reticulocyte, wheat germ and insect cells as well as from prokaryotic *E. coli* extracts **(Table 3.3)**.

The highest production rates are normally achieved with *E. coli* extracts. However, eukaryotic systems often produce eukaryotic proteins with higher activity. Therefore, the origin of the protein of interest should be considered when selecting a cell-free expression system.

### DNA Template Consideration: Plasmids and PCR-Fragments

The performance of cell-free systems depends on the DNA template used. Basically, any vector containing T7, SP6 or T3 promoters can be used with TnT® Systems. However, there are several points to consider when engineering a DNA fragment or plasmid for optimal expression in a eukaryotic system: **(i)** the ATG start codon in the sequence should be the first ATG encountered following the transcription start site; **(ii)** ideally, following the promoter, the ATG is included in a Kozak consensus sequence; **(iii)** a stop codon should be included at the 3′- terminus of the sequence; and **(iv)** a synthetic poly(A) tail should be included following the stop codon. Additionally, vectors used in the TnT® T7 Coupled Wheat Germ System should either be linearized or have a T7 transcription terminator in a circular template.

In prokaryotic systems, the selection of a start codon generally depends on the presence of a ribosomal binding site (RBS; Shine-Dalgarno sequence), which contains a signal that marks the start of the reading frame. The presence of an optimal RBS can greatly increase expression in prokaryotic systems. The prokaryotic system does not recognize ATGs upstream of the ATG start codon unless they contain a properly positioned RBS.

Promega vectors approved for use with TnT® Systems can be found in **Table 9.1**.

The template considerations mentioned above are also valid for using PCR fragments as templates for the TnT® reaction. For the generation of the PCR fragments for protein expression in eukaryotic systems, the integration of a Kozak sequence downstream of a T7 or SP6 promoter is recommended **(Figure 3.4)**.

### Labeling of Proteins during in vitro Synthesis

All TnT® Systems are provided with three different Amino Acid Mixtures for the incorporation of radiolabeled amino acids like methionine, cysteine and leucine. Transcend™ tRNA and FluoroTect™ Systems can be used to incorporate biotinylated or fluorescently-labeled lysine residues (see Section 3.3).

### Signal Peptide Cleavage and Core Glycosylation

Rabbit reticulocyte lysate has been reported to post-translationally modify proteins via phosphorylation, acetylation and isoprenylation. However, the addition of Canine Pancreatic Microsomal Membranes (CMM), to RRLs is required to achieve signal peptide cleavage and core glycosylation of the translation product.

**Table 3.3.** Overview of Transcription and Translation Systems

| | System | Plasmid DNA (Circular or Linearized) | PCR-generated DNA | RBS Required[1] | Kozak Preferred[2] | Labeling Options | Signal cleavage & glycosylation with CMM[3] | Control DNA & Detection Reagent[4] | Yield |
|---|---|---|---|---|---|---|---|---|---|
| **Rabbit** | TnT® Coupled Reticulocyte Lysate System (T7, T3, or SP6 RNA Polymerase; Cat.# L4610, L4950, L4600)[5] | +[6] | +[7] | – | + | Met, Cys, Leu, FluoroTect™, Transcend™ | + | + | 3–6µg/ml |
| | TnT® Quick Coupled Transcription/ Translation (T7 or SP6 RNA Polymerase; Cat.# L1170, L2080) | +[6] | +[7] | – | + | Met, FluoroTect™, Transcend™ | + | + | 3–6µg/ml |
| | TnT® T7 Quick for PCR DNA (Cat.# L5540) | NR | + | – | + | Met, FluoroTect™, Transcend™ | + | – | 3–6µg/ml |
| **Wheat Germ** | TnT® Coupled Wheat Germ (T7 or SP6 RNA Polymerase) (Cat.# L4130, L4140)[4] | +[8] | +[7] | – | + | Met, Cys, Leu, FluoroTect™, Transcend™ | – | + | 3–6µg/ml |
| | TnT® SP6 High-Yield Wheat Germ Protein Expression System (Cat.# L3260) | + | + | – | + | Met, Cys, Leu, FluoroTect™, Transcend™ | – | – | 10–100µg/ml |
| **Insect** | TnT® Insect Cell Extract Protein Expression System (Cat.# L1101) | + | NR | – | + | Met, Cys, Leu, FluoroTect™, Transcend™ | – | Control DNA | 15–75µg/ml |
| ***E. coli*** | *E coli* S30 for Linear DNA (Cat.# L1030) relies on endogenous RNA polymerases | +[9] | + | + | – | Met, Cys, Leu, FluoroTect™, Transcend™ | – | + | 1–5µg/ml |
| | S30 T7 High-Yield Protein Expression System (Cat.# L1110) | + | NR | + | – | Met, Cys, Leu, FluoroTect™, Transcend™ | – | Control DNA | 200–500µg/ml |

NR: Not Recommended

[1] DNA templates for TnT® *E.coli* Systems requires the Shine Dalgarno ribosomal binding site (RBS).

[2] DNA templates for eukaryotic TnT® Systems should preferably contain the Kozak consensus sequence for translation initiation.

[3] CMM: Canine Microsomal Membranes.

[4] Control DNA contains the firefly luciferase gene. Luciferase activity is detected by the Luciferase Assay Reagent (Cat.# E1500).

[5] Translation reactions can be further optimized by adding $Mg^{2+}$ and $K^{+}$.

[6] SP6 circular plasmids give higher yields than T7 or T3 circular plasmids; T7 or T3 linearized plasmid may be considered as templates; SP6 linearized plasmids are not recommended.

[7] Not recommended for SP6 containing template.

[8] For T7 circular plasmids include the T7 terminator sequence; otherwise linearized plasmids are preferred; for SP6 templates only circular plasmids.

[9] Only linearized templates.

# TnT® Coupled Reticulocyte Lysate Systems

Robust eukaryotic cell-free expression systems for the expression of functional mammalian proteins in a simple one-step procedure.

## Description and Principle

We offer two types of Rabbit Reticulocyte Lysate Transcription and Translation (TnT®) Systems: The TnT® Coupled (T7, T3, SP6) System and the TnT® Quick Coupled (T7, SP6) System. The main difference between these systems is that the TnT® Quick Coupled System provides a master mix containing all the reaction components required in one tube, whereas the TnT® Coupled System has all the reaction components provided in separate tubes **(Figure 3.3)**. TnT® T7 Quick for PCR DNA is a rapid and convenient coupled TnT® System designed for expression of PCR-generated DNA templates. The system is robust and able to express a variety of proteins ranging in size from 10–150kDa. The lysates are supplied with all reagents needed for TnT® reactions including RNA polymerases. To use these systems, DNA is added directly to TnT® Lysate and incubated in a 50µl reaction for 60–90 minutes at 30°C. See **Table 3.1** for additional applications.

## Features and Benefits

- **Use in Multiple Applications:** The TnT® Systems are widely used for functional genomics and proteomics analyses.
- **Save Time:** The TnT® Reaction is completed in a maximum of 1.5 hours.
- **Complete System:** All reagents for the TnT® Reaction are provided (except for labeled amino acids).
- **Reliable:** Can eliminate solubility issues by using an in vitro mammalian system.

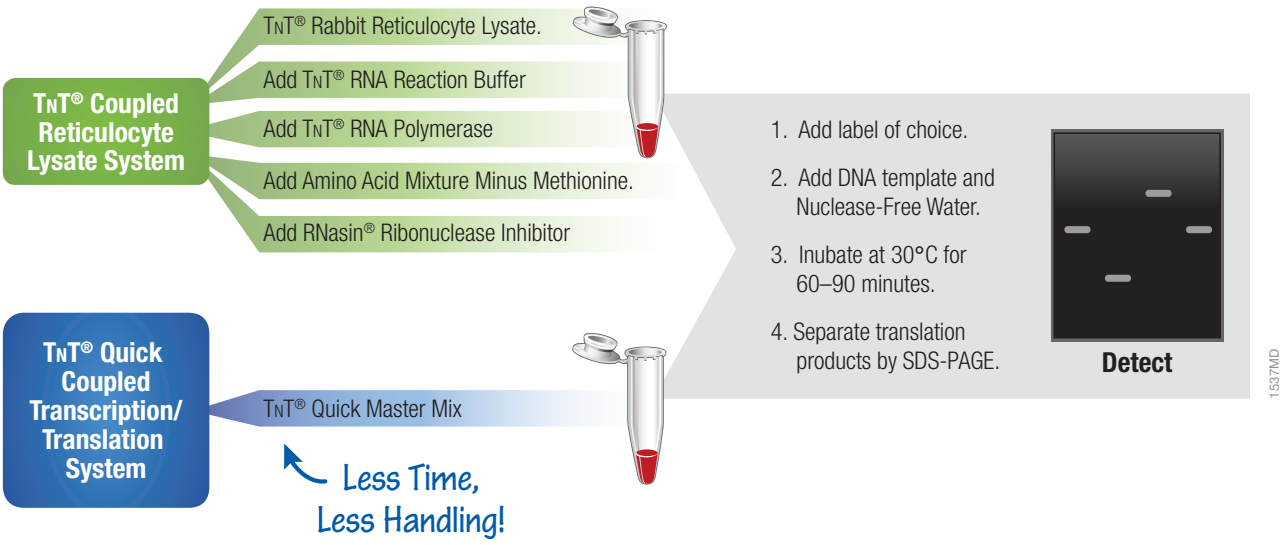



**Figure 3.3.** Comparison of the TnT® Coupled Reticulocyte Lysate System and the TnT® Quick Coupled Transcription/Translation System protocols.

## Eukaryotes

**SP6**

5'$(N)_{6–10}$- TATTTAGGTGACACTATAG$(N)_{3–6}$ - CCACC**ATG**G - $(N)_{17–22}$-3'

**SP6 Promoter** | **Kozak region** | **Sequence-specific Nucleotides**

**T7**

5'$(N)_{6–10}$- TAATACGACTCACTATAGGG$(N)_{3–6}$ - CCACC**ATG**G - $(N)_{17–22}$-3'

**T7 Promoter** | **Kozak region** | **Sequence-specific Nucleotides**

**Figure 3.4.** Forward primers used to generate PCR fragments for protein expression in TnT® Systems.

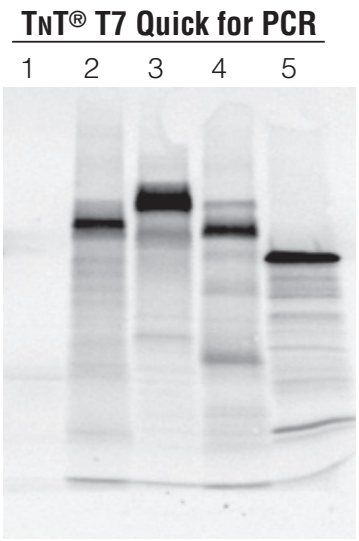



**Figure 3.5.** TnT® T7 Quick for PCR was used to express variants of the APC gene and BRCA1 gene. PCR fragments were used as starting material for the TnT® reaction. Transcend™ tRNA was included in the reaction for the incorporation of biotinylated lysine residues. **Lane 1** contains the no DNA controls; **lane 2**, APC Seg 2 PCR fragment; **lane 3**, APC Seg 3 PCR DNA fragment; **lane 4**, BRCA1 Seg 3 PCR fragment; **lane 5**, the Luciferase T7 Control DNA.

## Ordering Information

**TnT® Coupled Reticulocyte Lysate Systems:**

TnT® SP6 Coupled Reticulocyte Lysate System (Cat.# L4600)

TnT® T7 Coupled Reticulocyte Lysate System (Cat.# L4610)

TnT® T3 Coupled Reticulocyte Lysate System (Cat.# L4950)

**TnT® Quick Coupled Transcription/ Translation Systems:**

TnT® T7 Quick Coupled Transcription/Translation System (Cat.# L1170)

TnT® SP6 Quick Coupled Transcription/Translation System (Cat.# L2080)

TnT® T7 Quick for PCR DNA (Cat.# L5540)

# TnT® SP6 High-Yield Wheat Germ Protein Expression System

In vitro protein synthesis starting from DNA.

## Description

The TnT® SP6 High-Yield Wheat Germ Protein Expression System is a convenient, quick, single-tube, coupled transcription/translation system designed to express up to 100µg/ml of protein. The TnT® SP6 High-Yield Wheat Germ Protein Expression System expresses genes cloned downstream of an SP6 RNA polymerase promoter. This cell-free expression system is prepared from an optimized wheat germ extract and contains all the components (tRNA, ribosomes, amino acids, SP6 RNA polymerase, and translation initiation, elongation and termination factors) necessary for protein synthesis directly from DNA templates. See **Table 3.1** for additional applications.

## Principle

The TnT® SP6 High-Yield Wheat Germ Protein Expression System can be used with standard plasmid DNA or PCR-generated templates containing the SP6 promoter. However, to achieve optimal yield, specialized vectors designed for Wheat Germ Extracts such as pF3A WG (BYDV) Flexi® Vector or pF3K WG (BYDV) Flexi® Vector are recommended. DNA templates are directly added to the SP6 High Yield Master Mix and incubated in a 50µl reaction for 2 hours at 25°C. Expressed proteins can be used directly or purified for related applications.

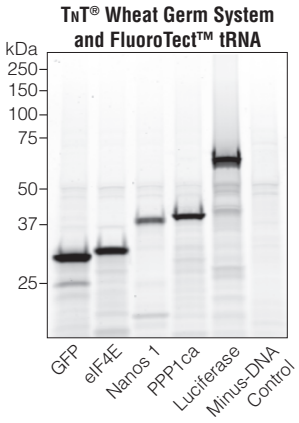



**Figure 3.6.** Proteins of different size and origin were expressed using TnT® SP6 High-Yield Wheat Germ Protein Expression System in the presence of FluoroTect™ tRNA for lysine residue labeling. Samples were separated by SDS-PAGE and imaged using a fluorescence scanner.

## Features and Benefits

- **Save Time:** Generate proteins in two hours, compared to days when using cell-based (*E. coli*) systems.
- **Choose Your Format:** Use plasmid- or PCR-generated templates.
- **Generate Full-Length Protein:** Generate soluble, full-length protein and avoid problems associated with *E. coli* systems.

## Ordering Information

TnT® SP6 High-Yield Wheat Germ Protein Expression System (Cat.# L3260, L3261)

# TnT® T7 Insect Cell Extract Protein Expression System

In vitro protein synthesis starting from a DNA template.

## Description

The TnT® T7 Insect Cell Extract Protein Expression System is a convenient, quick, single-tube, coupled transcription and translation system for the cell-free expression of proteins. See **Table 3.1** for additional applications.

## Principle

The extract is made from the commonly used *Spodoptera frugiperda* Sf21 cell line. All components necessary for transcription and translation are present in the TnT® T7 Insect Cell Extract (ICE) Master Mix. Proteins are expressed from genes cloned downstream of the T7 promoter in ICE vectors such as pF25A or pF25K ICE T7 Flexi® Vector **(Table 9.1)**. These vectors contain 5´- and 3´-untranslated (UTR) sequences from the baculovirus polyhedrin gene to enhance translation efficiency. After addition of the DNA template, protein synthesis is initiated. The reactions are incubated at 28–30°C and are complete within 4 hours. Approximately 15–75µg/ml of functional protein can be produced using the TnT® T7 Insect Cell Extract Protein Expression System.

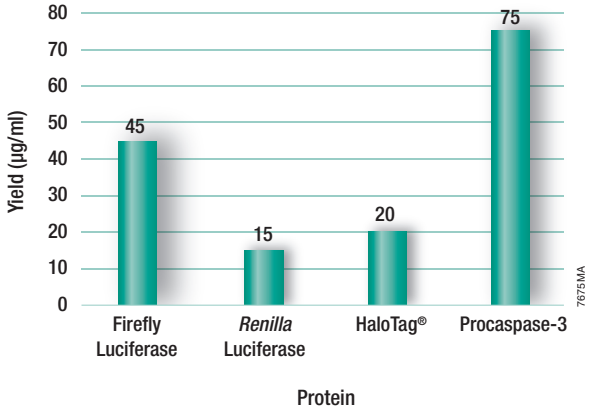



**Figure 3.7.** Typical protein yields using the TnT® T7 Insect Cell Extract Protein Expression System.

## Features and Benefits

- **Obtain Data Faster:** Protein is expressed in only 4 hours.
- **Achieve High Protein Yields:** Express up to 75µg/ml of protein.
- **Convenient:** Luciferase Control DNA included.

## Ordering Information

TnT® T7 Insect Cell Extract Protein Expression System (Cat.# L1102, L1101)

# *E. coli* S30 Extract System for Linear Templates

In vitro protein synthesis starting from DNA.

## Description

The *E. coli* S30 Extract System for Linear Templates allows successful transcription/translation of linear DNA templates. You need only to provide linear DNA containing a prokaryotic *E. coli*-like promoter (such as *lac*UV5, *tac*, λPL (con) and λ-$P_R$). A ribosome binding site is required to direct the synthesis of proteins in vitro. In vitro-generated RNA from DNA templates lacking an *E. coli* promoter may also be used in this system, but protein yields produced from linear DNA templates will be decreased 1–10%.

## Principle

The S30 Extract for Linear Templates is prepared from an *E. coli* B strain (SL119), which is deficient in *Omp*T endoproteinase, lon protease and exonuclease V (*rec*BCD). The absence of protease activity results in greater stability of expressed proteins. The *rec*D mutation of the SL119 strain produces a more active S30 Extract for Linear DNA than the previously described nuclease-deficient, *rec*BC-derived S30 extracts. However, the S30 Extract for Linear Templates is less active than the S30 Extract System for Circular DNA. An easy-to-perform, nonradioactive positive control reaction using the Luciferase Assay Reagent provided, allows detection of luciferase gene expression in the *E. coli* S30 System for linear templates. The control reaction produces high light output for several minutes, allowing the researcher to choose from several detection methods, including simple visual observation of luminescence.

## Features and Benefits

- **Flexible:** Various templates can be used: DNA fragments, PCR-synthesized DNA, ligated overlapping oligonucleotides, in vitro-generated RNA and prokaryotic RNA.
- **Complete:** Contains all necessary components for coupled transcription/translation.
- **Optimized:** Premix is optimized for each lot of S30 Extract.
- **Control DNA:** Easy detection of firefly luciferase expression using (included) Luciferase Assay Reagent.

## Ordering Information

*E. coli* S30 Extract System for Linear Templates (Cat.# L1030)

# *E. coli* S30 T7 High-Yield Protein Expression System

In vitro protein synthesis starting from DNA.

## Description

The S30 T7 High-Yield Protein Expression System is an *E. coli* extract-based protein synthesis system. It simplifies the transcription and translation of DNA sequences cloned in plasmid or lambda vectors containing a T7 promoter, by providing an extract that contains T7 RNA polymerase for transcription and all necessary components for translation.

## Principle

The *E. coli* S30 T7 High-Yield Protein Expression System is designed to express up to 500µg/ml of protein in one hour from plasmid vectors containing a T7 promoter and a ribosome binding site. The protein expression system provides an extract that contains T7 RNA polymerase for transcription and is deficient in OmpT endoproteinase and lon protease activity. All other necessary components in the system are optimized for protein expression. This results in greater stability and enhanced expression of target proteins. Control DNA expression results in production of *Renilla* luciferase, which can be detected by Coomassie® Blue staining following SDS-PAGE or assayed with *Renilla* Luciferase Assay System (Cat.# E2810).

## Features and Benefits

- **Obtain Data Faster:** Protein expression in only one hour.
- **Achieve High Protein Expression:** Express up to 500µg/ml of protein for multiple applications.
- **Scalable:** Convenient screening protocol for high-throughput protein expression.

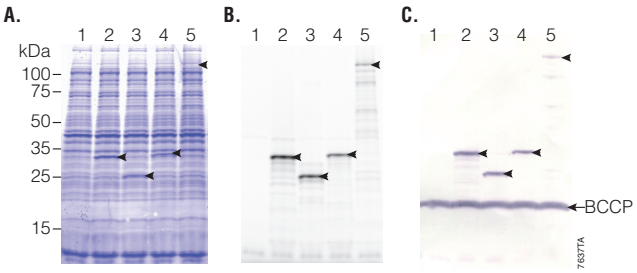



**Figure 3.8.** Coupled **in vitro** transcription/translation of circular DNA templates using the S30 T7 High-Yield Protein Expression System. The protein-coding sequences cloned into pFN6A (HQ) Flexi® Vector were expressed as described in the *S30 T7 High-Yield Protein Expression System Technical Manual* #TM306, resolved by SDS-PAGE (4–20% Tris-glycine) and visualized by Coomassie® blue staining **(Panel A)**, fluorescence scanning **(Panel B)**, or transferred to PVDF membrane, treated with Streptavidin Alkaline Phosphatase (Cat.# V5591) and stained with Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat..# S3841; **Panel C**). For each gel: lane 1, no DNA; lane 2, *Renilla* luciferase; lane 3, Monster Green® Fluorescent Protein; lane 4, HaloTag® protein; lane 5, α-galactosidase (BCCP = *E. coli* biotin carboxyl carrier protein).

## Ordering Information

S30 T7 High-Yield Protein Expression System (Cat.# L1110, L1115)

# 3.3 Cell-Free Protein Labeling Reagents

## OVERVIEW

Labeling and detection of proteins expressed using cell-free systems is necessary for most applications such as protein:protein interaction and protein:nucleic acid interaction studies. FluoroTect™ Detection and Transcend™ Detection Systems were developed for non-radioactive protein labeling during cell-free protein synthesis. Both labeling products are based on the incorporation of labeled lysine residues into the polypeptide chain. The labeled protein products can be easily detected either by fluorescent imaging after SDS-PAGE or by western blotting using streptavidin conjugates either to horseradish peroxidase (Strep-HRP) or Alkaline Phosphatase (Strep-AP).

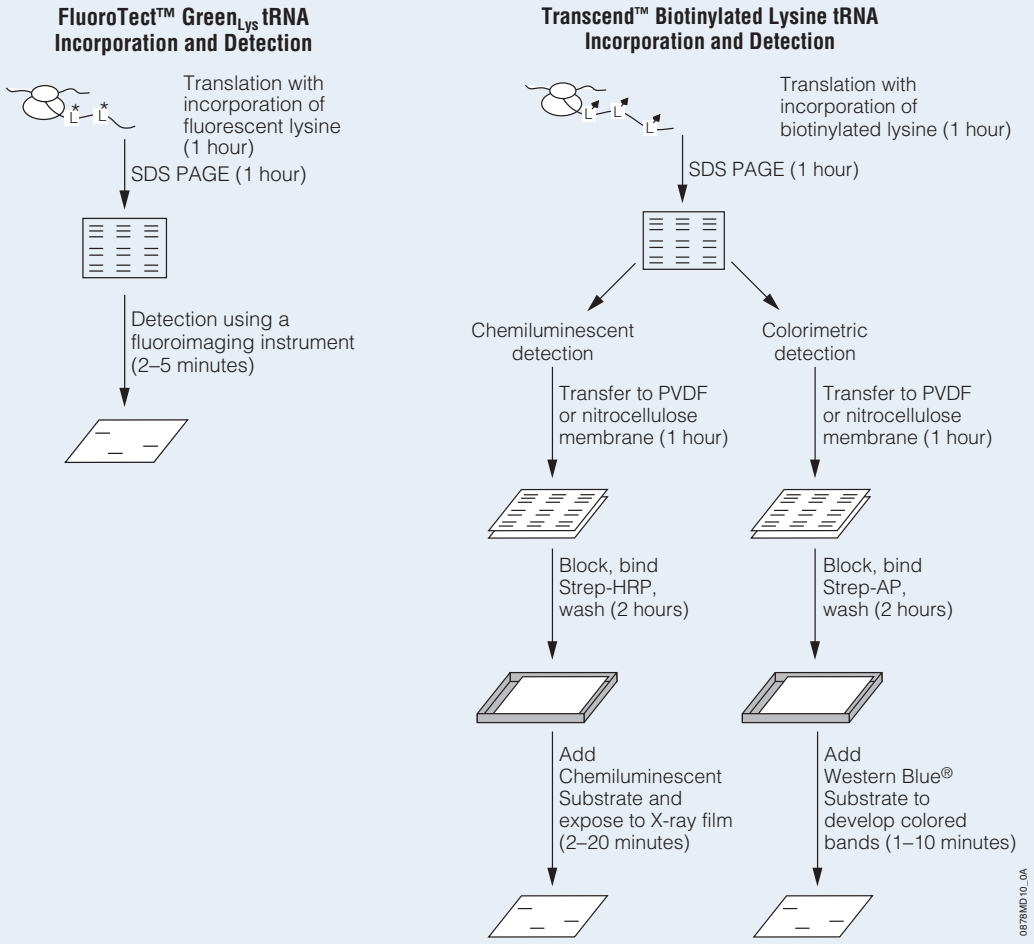



**Figure 3.9.** Detection protocols using FluoroTect™ Green$_{Lys}$ tRNA and Transcend™ tRNA.

# FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System

Labeling and detection of in vitro synthesized proteins.

## Description

The FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System allows fluorescent labeling and detection of proteins synthesized in vitro. The system is based on a lysine-charged tRNA, which is labeled at the ε position of the lysine with the fluorophore BODIPY®-FL. Fluorescent lysine residues will be incorporated into synthesized proteins during in vitro translation reactions, eliminating the need for radioactivity.

## Principle

Detection of the labeled proteins is accomplished in 2–5 minutes directly "in-gel" by use of a fluorescence gel scanner. This eliminates any requirements for protein gel manipulation, such as fixing/drying or any safety, regulatory or waste disposal issues associated with the use of radioactively-labeled amino acids. The convenience of "in-gel" detection also avoids the time-consuming electroblotting and detection steps of conventional non-isotopic systems.

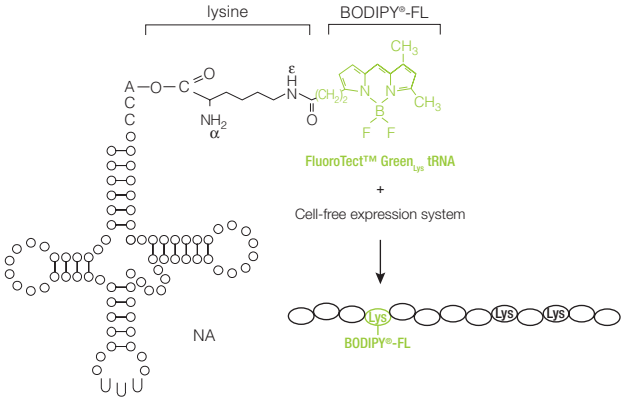



**Figure 3.10.** Schematic diagram of the incorporation of FluoroTect™ Green$_{Lys}$-labeled lysine into nascent protein.

## Features and Benefits

- **Fast:** Data can be obtained in minutes. No requirement to transfer, fix or dry gels.
- **Nonradioactive:** No safety, regulatory or waste disposal issues associated with radioactivity.
- **Flexible:** The modified charged tRNA can be used with: Rabbit Reticulocyte Lysate, TNT® Coupled Transcription/Translation System, Wheat Germ Extract and *E. coli* S30 Extract.

## Ordering Information

FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Cat.# L5001)

# Transcend™ Nonradioactive Translation Detection Systems

Labeling and detection of in vitro synthesized proteins.

## Description

The Transcend™ Nonradioactive Translation Detection Systems allow nonradioactive detection of proteins synthesized in vitro. Using these systems, biotinylated lysine residues are incorporated into nascent proteins during translation, eliminating the need for labeling with [$^{35}$S]methionine or other radioactive amino acids

## Principle

This biotinylated lysine is added to the translation reaction as a precharged ε-labeled biotinylated lysine-tRNA complex (Transcend™ tRNA) rather than a free amino acid. After SDS-PAGE and blotting, the biotinylated proteins can be visualized by binding either Streptavidin-Alkaline Phosphatase (Streptavidin-AP) or Streptavidin-Horseradish Peroxidase (Streptavidin-HRP), followed either by colorimetric or chemiluminescent detection (see Chapter 8). Typically, these methods can detect 0.5–5ng of protein within 3–4 hours after gel electrophoresis. This sensitivity is equivalent to that achieved with [$^{35}$S]methionine incorporation and autoradiographic detection 6–12 hours after gel electrophoresis.

## Features and Benefits

- **Sensitive:** The biotin tag allows detection of 0.5–5ng of translated protein.
- **Safe:** No radioisotope handling, storage or disposal is required.
- **Flexible:** Results can be visualized by using colorimetric or chemiluminescent detection.

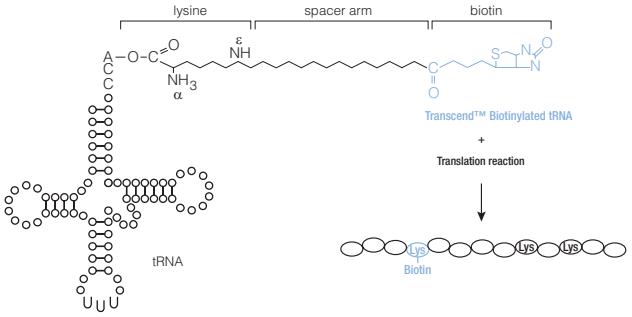



**Figure 3.11.** Schematic diagram of the incorporation of Transcend™ labeled lysine into nascent protein.

## Ordering Information

Transcend™ Colorimetric Translation Detection System (Cat.# L5070)

Transcend™ Chemiluminescent Translation Detection System (Cat.# L5080)

# 3.4 Membrane Vesicles for Signal Peptide Cleavage and Core Glycosylation

## OVERVIEW

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.

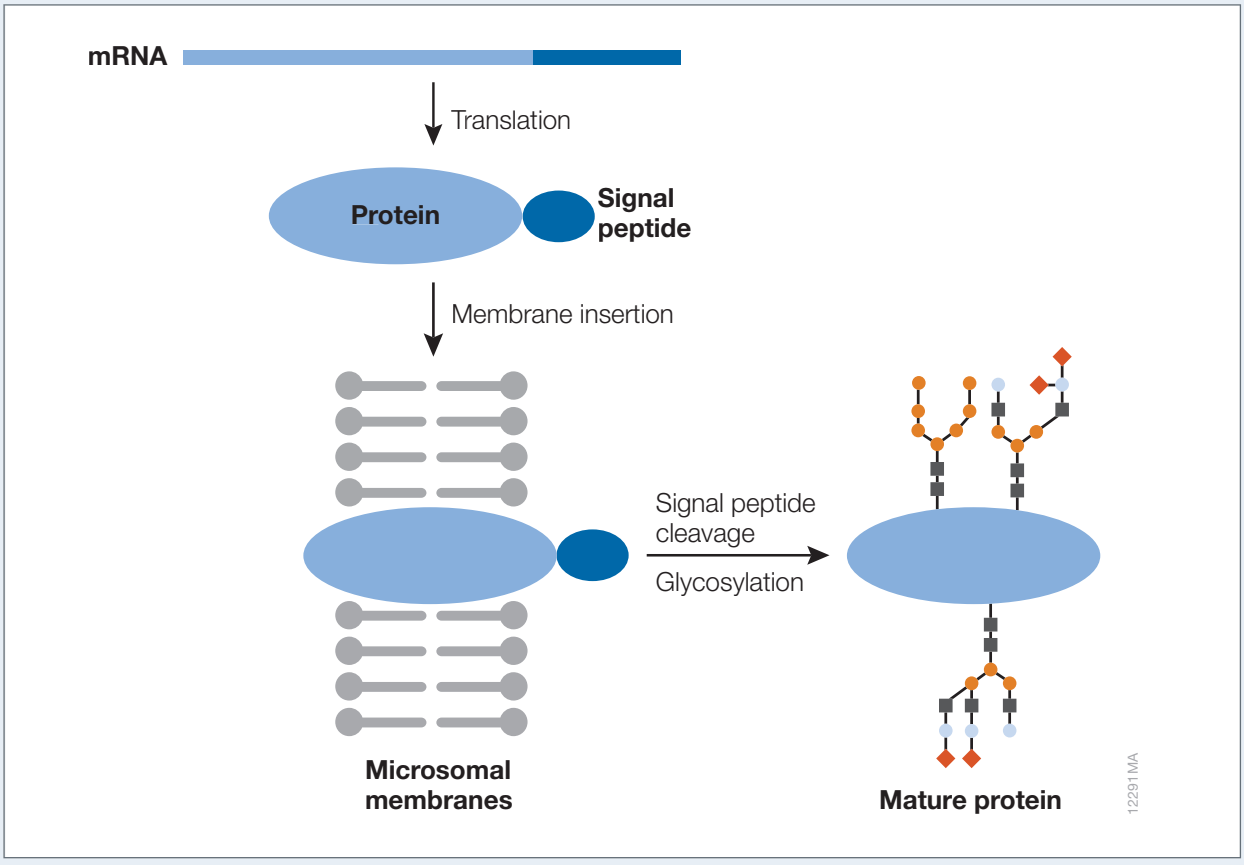



**Figure 3.12.** Schematic of signal peptide cleavage and introducing core glycosylation by use of canine microsomal membranes in combination with rabbit reticulocyte lysate cell-free protein expression system.

# Canine Pancreatic Microsomal Membranes

Examination of signal peptide cleavage, membrane insertion, translocation and core glycosylation of in vitro expressed proteins.

## Description

Canine Pancreatic Microsomal Membranes are used to study cotranslational and initial posttranslational processing of proteins in combination with in vitro expressed protein using Rabbit Reticulocyte Systems (RRLs). 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 transcription/translation of the appropriate DNA in TnT® RRL Systems.

## Principle

Processing and glycosylation events can be studied with Rabbit Reticulocyte Lysate Cell-free expression systems. 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. 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*.

## Features and Benefits

- **Minimal Translational Inhibition, Minimal Background:** Microsomes are stripped of endogenous membrane-bound ribosomes and mRNA.
- **Compatible:** Can be used with TnT® RRL Systems, Rabbit Reticulocyte Lysate and Flexi® Lysate.
- **Reliable Results:** Control mRNAs are supplied.

## Ordering Information

Canine Pancreatic Microsomal Membranes (Cat.# Y4041)

# Chapter 3 References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### Characterization of Protein Interactions

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