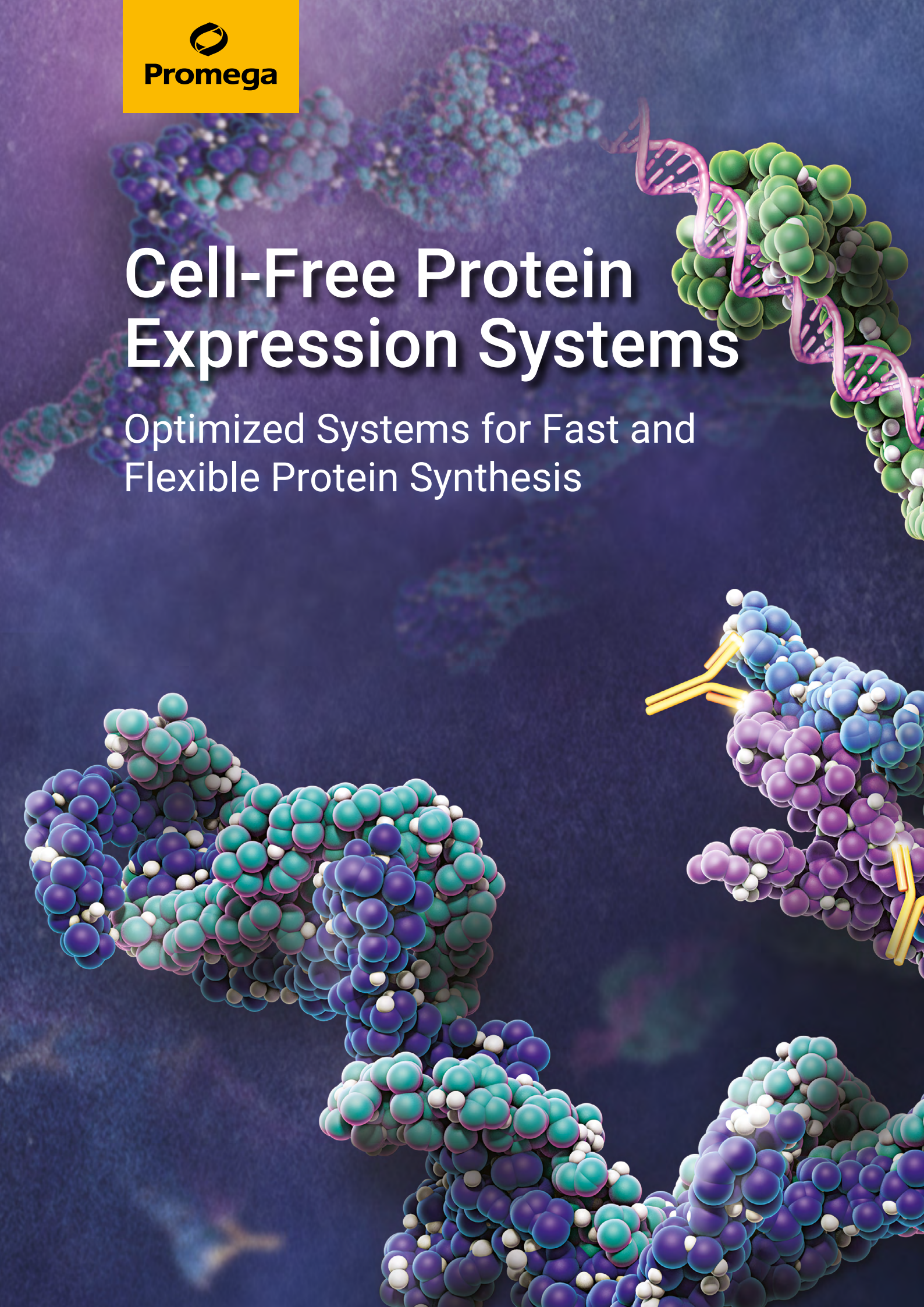
Promega

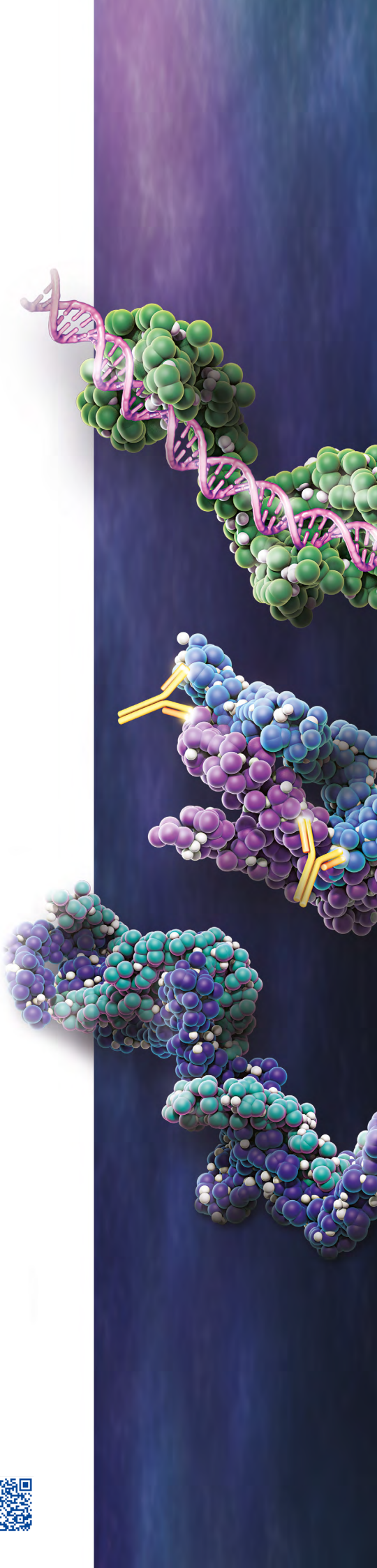
Cell-Free Protein Expression Systems

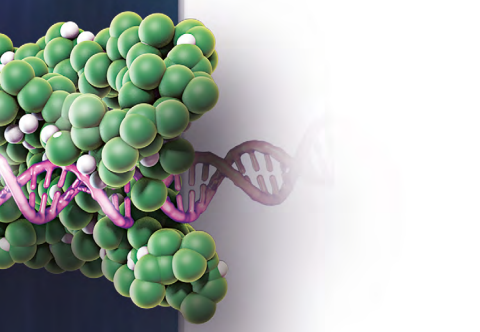
Optimized Systems for Fast and Flexible Protein Synthesis

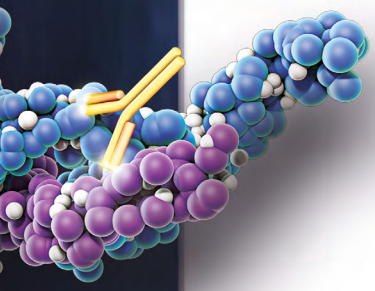


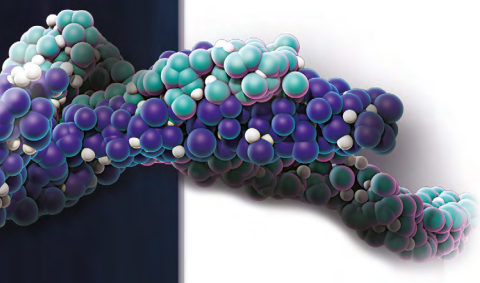
Introduction

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 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.









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1. Overview

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 1**). Both types 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.

Origins of Cell-Free Expression Systems

Many different cell-free expression systems derived from prokaryotic and eukaryotic source are available. The choice of the system is dependent on several factors, inluding the origin of the template (RNA or DNA) and the protein yield. We offer translation systems (mRNA-based) and coupled transcription/translation systems (DNA-based) from prokaryotic and eukaryotic sources. **Table 2** provides an overview of translational systems and **Table 3** provides an overview of coupled translation/transcription systems.

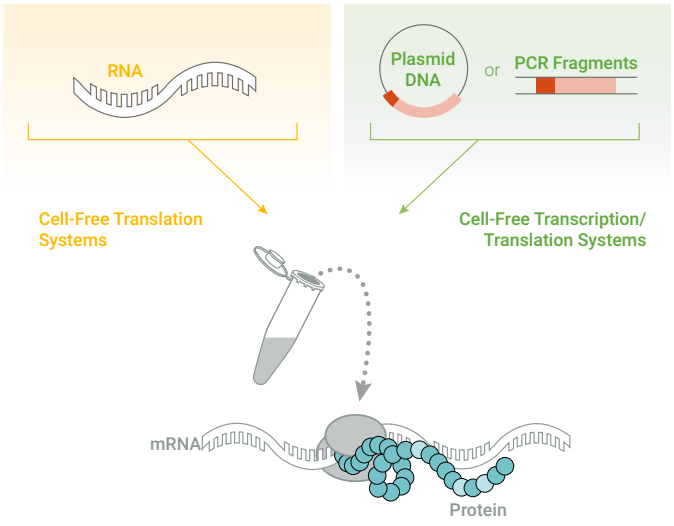


Figure 1. Cell-free protein expression systems are divided into mRNA-based translation systems and in DNA-based transcription/translation systems.

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
- Characterization of synthetic or *in vitro* transcribed mRNAs for RNA therapeutics

Expression of Difficult-to-Express Proteins

- Cell-toxic proteins, membrane proteins, 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

Table 1. Applications of Cell-Free Protein Synthesis

2. 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 micrococcal nuclease to destroy endogenous mRNA and thus reduce background translation to a minimum (**Table 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, yeasts or other fungi also may find WGE preferable to RRL.

Translation System	Nuclease-Treated	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™	+	3–6 µg/ml
Wheat Germ Extract (Cat.# L4380)	+	Met, Cys, Leu, FluoroTect™; Transcend™	+	0.6–3 µg/ml

* The lysates are provided with three Amino Acid Mixtures for the incorporation of labeled amino acids like methionine, cysteine & leucine. Transcend™ Non-Radioactive Translation Detection System (Cat.# L5070) and FluoroTect™ GreenLys in vitro Translation Labeling System (Cat.# L5001) can be used to incorporate biotinylated or fluorescently labeled lysine residues, respectively

** 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.

Table 2. Overview of Cell-FreeTranslation Systems that use mRNA as a Template

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.

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.

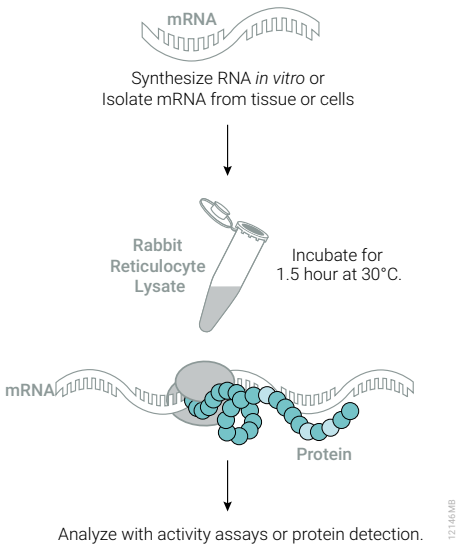


Figure 2. Flow chart of *in vitro* translation procedure using Rabbit Reticulocyte Lysate.

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 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. 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 (ribosomes, tRNAs, aminoacyl-tRNA synthetases, 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 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.

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.

Ordering Information

Wheat Germ Extract
(Cat.# L4380)

3. 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 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 as well as from prokaryotic *E. coli* extracts (**Table 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 4**.

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 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.

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

NR: Not Recommended

1 DNA templates for TnT® E.coli Systems requires the Shine Dalgarno sequence.

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

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

4 Translation reactions can be further optimized by adding Mg^{2+} and K^{+}.

5 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.

6 Not recommended for SP6 containing template.

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

8 Only linearized templates.

Table 3. Overview of Transcription and Translation Systems

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**). 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 10–150 kDa. 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 50 µl reaction for 60–90 minutes at 30°C. See **Table 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.

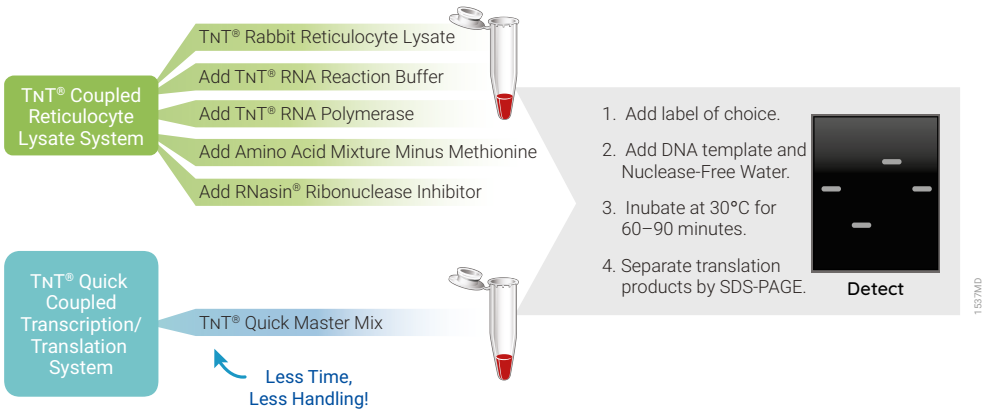


Figure 3. Comparison of the TNT® Coupled Reticulocyte Lysate System and the TNT® Quick Coupled Transcription/Translation System protocols.

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

12147MB

Figure 4. Forward primers used to generate PCR fragments for protein expression in TNT® Systems.

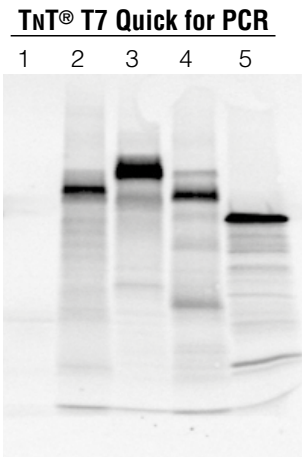


Figure 5. TNT® T7 Quick for PCR was used to express variants of the APC gene and BRCA1 gene. PCR fragments were used as starting material for the TNT® reaction. Transcend™ tRNA was included in the reaction for the incorporation of biotinylated lysine residues. **Lane 1** contains the no DNA controls; 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 100 µg/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 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 50 µl reaction for 2 hours at 25°C. Expressed proteins can be used directly or purified for related applications.

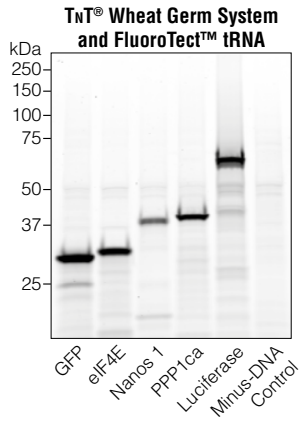


Figure 6. Proteins of different size and origin were expressed using TNT® SP6 High-Yield Wheat Germ Protein Expression System in the presence of FluoroTect™ tRNA for lysine residue labeling. Samples were separated by SDS-PAGE and imaged using a fluorescence scanner.

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

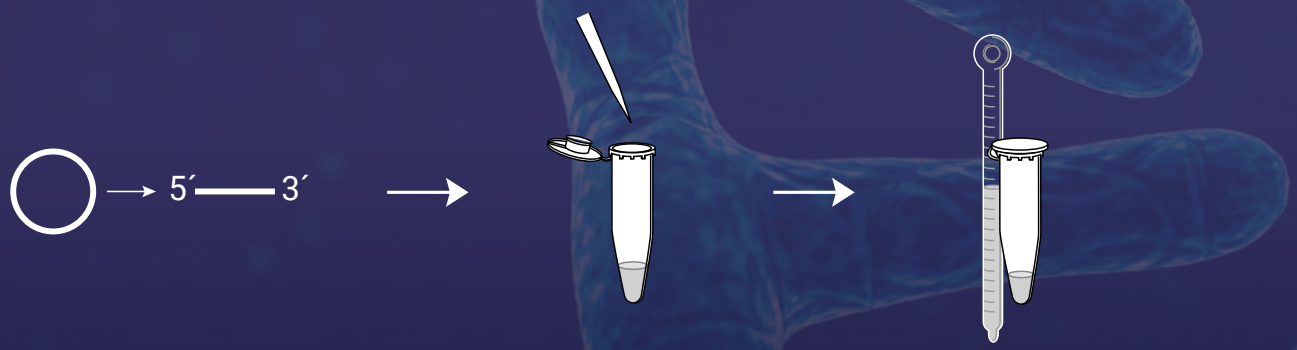
TNT® SP6 High-Yield Wheat Germ Protein Expression System (Cat.# L3260, L3261)

Maximize Your RNA Output with RiboMAX™

Enhance your research with the RiboMAX™ Large Scale RNA Production System. Capable of producing 2–5 mg/ml of both capped and uncapped RNA using SP6 or T7 RNA polymerase for high-efficiency synthesis.

Features & Benefits:

- **High Yield:** Generate 10–20 times more RNA than standard systems with up to 5 mg/ml in a single reaction
- **Flexible:** Ideal for a variety of RNA studies, from CRISPR guide RNA production to development of mRNA therapeutics
- **Scalable:** Tailor the system to meet varying RNA production requirements



Linearize DNA template by restriction digestion. Clean up linearized template.

Assemble the reaction components appropriate for SP6 or T7 RNA Polymerase.

Incubate at 37°C for 2–4 hours.

Explore the potential of RiboMAX™ in your lab today!
www.promega.com/RiboMAX

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 lacUV5, 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 OmpT endoproteinase, lon protease and exonuclease V (recBCD). The absence of protease activity results in greater stability of expressed proteins. The recD mutation of the SL119 strain produces a more active S30 Extract for Linear DNA than the previously described nuclease-deficient, recBC-derived S30 extracts. An easy-to-perform, non-radioactive 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 500 µg/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).

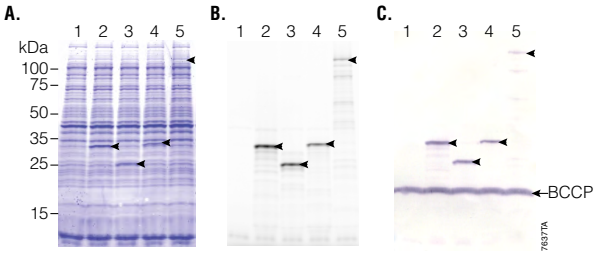


Figure 8. Coupled *in vitro* transcription/translation of circular DNA templates using the S30 T7 High-Yield Protein Expression System. The protein-coding sequences cloned into pFN6A (HQ) Flexi® Vector were expressed as described in the S30 T7 High-Yield Protein Expression System Technical Manual #TM306, 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, β-galactosidase (BCCP = *E. coli* biotin carboxyl carrier protein).

Features and Benefits

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

Ordering Information

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

4. 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).

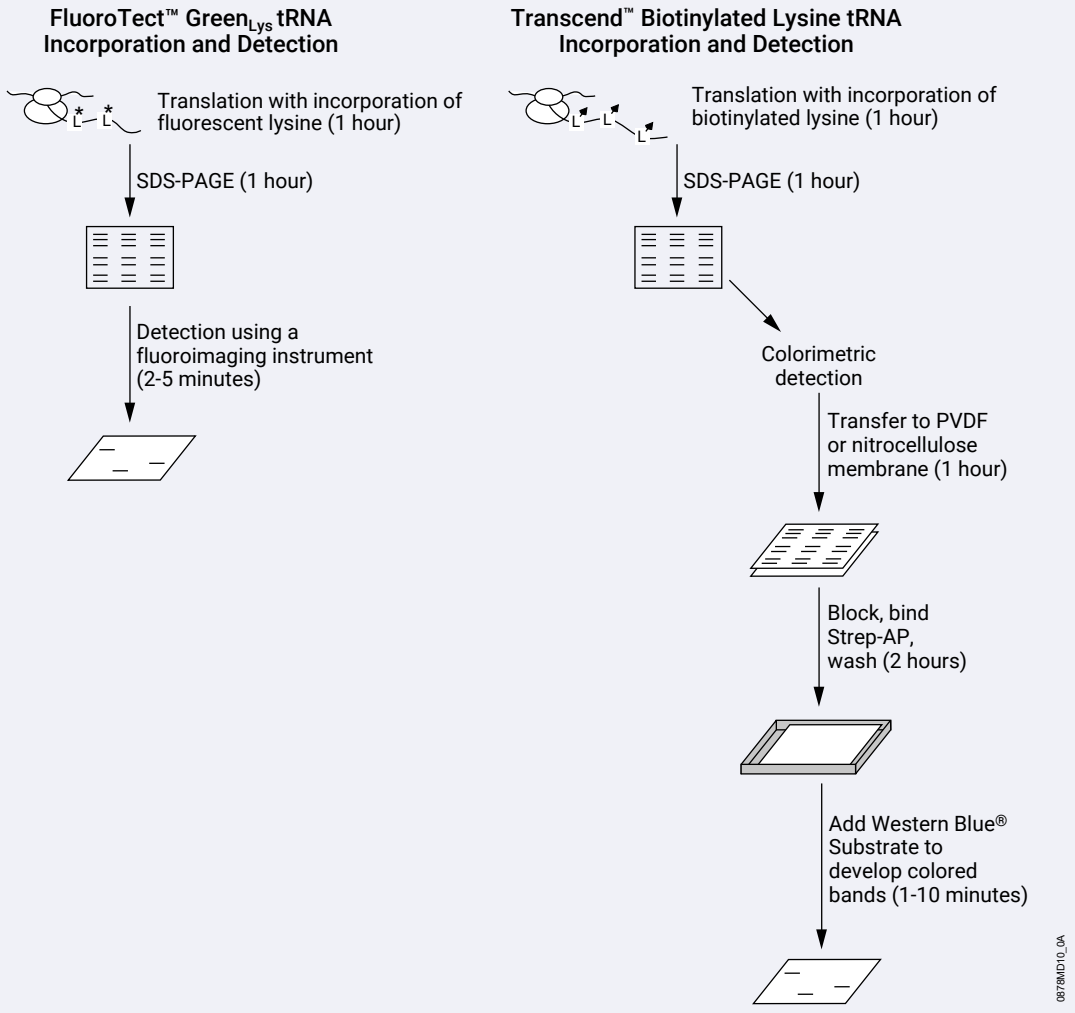


Figure 9. Detection protocols using FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System and Transcend™ Non-Radioactive Translation Detection System.

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.

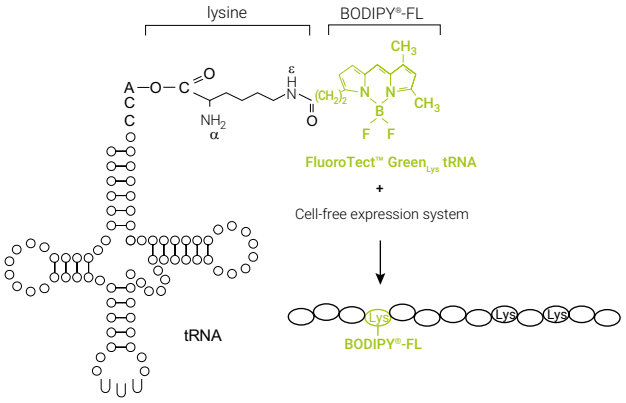


Figure 10. Schematic diagram of the incorporation of FluoroTect™ Green$_{Lys}$-labeled lysine into nascent protein.

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™ Non-radioactive Translation Detection Systems

Labeling and detection of *in vitro* synthesized proteins.

Description

The Transcend™ Non-radioactive 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

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 by colorimetric detection. Typically, these methods can detect 0.5–5 ng 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.

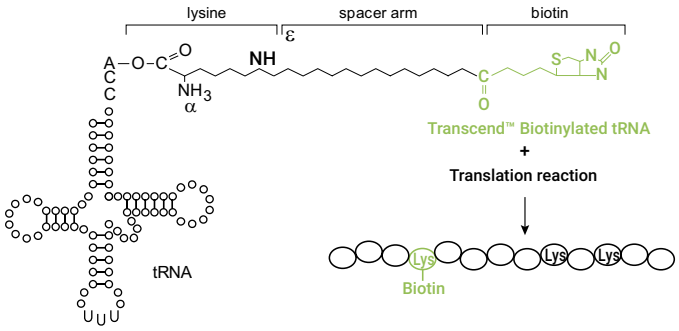


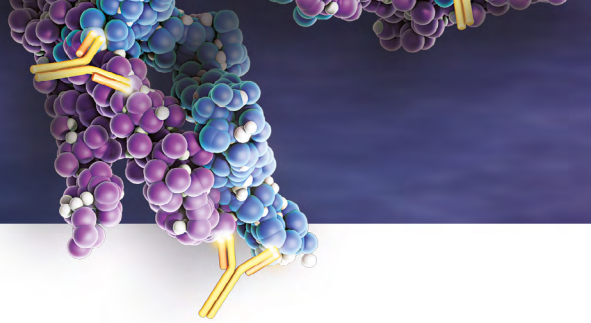
Figure 10. Schematic diagram of the incorporation of Transcend™ Biotinylated tRNA-labeled lysine into nascent protein.

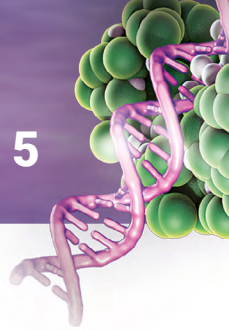
Features and Benefits

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

Ordering Information

Transcend™ Non-Radioactive Translation Detection System (Cat.# L5070)





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Functional Genome/Proteome Analysis

Gene Mutation/Deletion Analysis (e.g., Enzyme Kinetics)

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Protein Domain Mapping

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Characterization of Protein Interactions

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Generation of Protein Arrays

Wright, C. *et al.* (2012) Detection of Multiple Autoantibodies in Patients with Ankylosing Spondylitis Using Nucleic Acid Programmable Protein Arrays. *Mol. Cell. Proteomics*. **11**(2), M9.00384.

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Protein Evolution/Enzyme Engineering

Display Technologies (e.g., Ribosome, mRNA Display, *in vitro* Compartmentalization)

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Eukaryotic Ribosome Display Selection Using Rabbit Reticulocyte Lysate. J.A. Douthwaite, ed. In: *Ribosome Display and Related Technologies*. 2012, Springer.

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Evolution of Antibodies in vitro by Ribosome Display

Evolution of Antibodies in vitro by Ribosome Display; B.M. Edwards, M. He, eds. In: *Antibody Engineering*, 2012, Springer.

Expression of Difficult-to-Express Proteins

Cell-toxic Proteins, Membrane Protein, Viral Proteins, Kinases

Schwarz, D., Dotsch, V. and Bernhard, F. (2008) Production of membrane proteins using cell-free expression systems. *Proteomics* **8**(19) 3933–46.

Nozawa, A., Nanamiya, H. and Tozawa, Y. (2010) Production of membrane proteins through the wheat germ cell-free technology. *Methods Mol. Biol.* **607**, 213–8.

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Screenings

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Galam, L. *et al.* (2007) High-throughput assay for the identification of Hsp90 inhibitors based on Hsp90-dependent refolding of firefly luciferase. *Bioorg. Med. Chem*. **15**(5), 1939–46.

Drug Screening

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Protein Labeling

Labeling of Proteins in Cell-Free Expression Systems Using FluoroTect™ System

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Labeling of Proteins in Cell-Free Expression Systems using Transcend™ System

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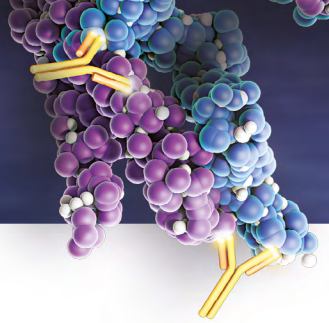
6. Overview of Protein Expression Vectors

Vector Name[1]	Cat. #	Drug Selection[2]	Expression Application	Promoter for Expression			Peptide Fusion Tag	Features
				E. coli	Mammalian Cells	Cell-Free Transcription/ Translation		
pTnT™ Vector	L5610	Ampicillin	Inducible expression of native protein			T7, SP6		5´β-Globin, poly-A
pCMVTnT™	L5620	Ampicillin	Constitutive high expression of native protein		CMV	T7, SP6	N-terminus GST	
pF1A T7 Flexi® Vector pF1K T7 Flexi® Vector	C8441 C8451	Ampicillin Kanamycin	Inducible expression of native protein	T7		T7		
pFN2A (GST) Flexi® Vector pFN2K (GST) Flexi® Vector	C8461 C8471	Ampicillin Kanamycin	Soluble expression and purification via an N-terminal GST tag	T7		T7	N-terminus GST	
pF3A WG (BYDV) Flexi® Vector pF3K WG (BYDV) Flexi® Vector	L5671 L5681	Ampicillin Kanamycin	*In vitro* wheat germ expression of native protein			T7, SP6		
pF25A ICE T7 Flexi® Vector pF25K ICE T7 Flexi® Vector	L1061 L1081	Ampicillin Kanamycin	*In vitro* insect cell free expression of native protein			T7		
pF4A CMV Flexi® Vector pF4K CMV Flexi® Vector	C8481 C8491	Ampicillin Kanamycin	Constitutive high expression of native protein		CMV	T7		
pF5A CMV-neo Flexi® Vector pF5K CMV-neo Flexi® Vector	C9401 C9411	Ampicillin/ Neomycin Kanamycin/ NeomycinM	Constitutive high expression of native protein with selection for stable transfectants		CMV	T7		
pF9A CMV hRluc-neo Flexi® Vector	C9361	Ampcillin NeomycinM	Constitutive high expression of native protein with selection and reporter screening for stable transfectants		CMV	T7		

1 The "pF" indicates the vector is a Flexi® Vector suitable for Flex® cloning.The letter after "pF" indicates the position of any expression tags ("N" for an N-terminal expression tag and "C" for a C-terminal expression tag). The letters "A" and "K" designate the bacterial drug selection for the vector ("A"= ampicillin and "K"= kanamycin).

2 The "M" indicates the vector provides resistance to the indicated drug in mammalian cells.

Table 4.



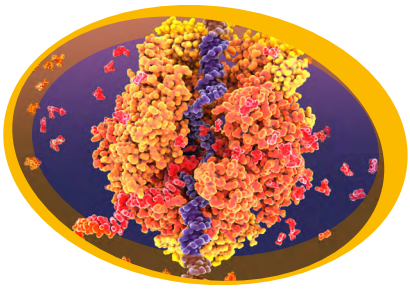
7. Ordering Information

Cell-Free Protein Expression Systems	Size	Cat.#
mRNA-based Translation Systems		
Rabbit Reticulocyte Lysate System, Nuclease Treated	30 reactions	L4960
Flexi® Rabbit Reticulocyte Lysate System	30 reactions	L4540
Wheat Germ Extract	5 x 200 µl	L4380
DNA-based Transcription and Translation Systems: Rabbit Reticulocyte Lysate Systems		
TnT® SP6 Coupled Reticulocyte Lysate System	8 reactions (Trial Size)	L4601
	40 reactions	L4600
TnT® T7 Coupled Reticulocyte Lysate System	8 reactions (Trial Size)	L4611
	40 reactions	L4610
TnT® T3 Coupled Reticulocyte Lysate System	40 reactions	L4950
TnT® T7/T3 Coupled Reticulocyte Lysate System	40 reactions	L5010
TnT® T7/SP6 Coupled Reticulocyte Lysate System	40 reactions	L5020
TnT® T7 Quick Coupled Transcription/Translation System	5 reactions (Trial Size)	L1171
	40 reactions	L1170
TnT® SP6 Quick Coupled Transcription/Translation System	5 reactions (Trial Size)	L2081
	40 reactions	L2080
TnT® T7 Quick for PCR DNA	40 reactions	L5540
DNA-based Transcription and Translation Systems: Wheat Germ Extracts		
TnT® SP6 Coupled Wheat Germ Extract System	40 reactions	L4130
TnT® T7 Coupled Wheat Germ Extract System	40 reactions	L4140
TnT® T7/SP6 Coupled Wheat Germ Extract System	40 reactions	L5030
TnT® SP6 High-Yield Wheat Germ Protein Expression System	10 reactions	L3261
	40 reactions	L3260
DNA-based Transcription and Translation Systems: *E. coli* S30 Extract Systems		
E. coli S30 Extract System for Linear Templates	30 reactions	L1030
E. coli S30 Extract System for Circular DNA	30 reactions	L1020
E. coli T7 S30 Extract System for Circular DNA	30 reactions	L1130
S30 T7 High-Yield Protein Expression System	8 reactions	L1115
	24 reactions	L1110
Luciferase Control RNA	20 µg (1 mg/ml)	L4561
Magnesium Acetate	100 µl (25 mM)	L4581
Potassium Chloride	200 µl (2.5 M)	L4591
Amino Acid Mixture, Complete	175 µl (1 mM)	L4461
Amino Acid Mixture Minus Cysteine	175 µl (1 mM)	L4471
Amino Acid Mixture Minus Methionine and Cysteine	175 µl (1 mM)	L5511
Amino Acid Mixture Minus Leucine	175 µl (1 mM)	L9951
Amino Acid Mixture Minus Methionine	175 µl (1 mM)	L9961
Cell-Free Protein Labeling Reagents		
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System	40 reactions	L5001
Transcend™ Non-Radioactive Translation Detection System	30 reactions	L5070
Cell-Free Protein Labeling Reagents		
Transcend™ tRNA	30 µl	L5061

Table 5.

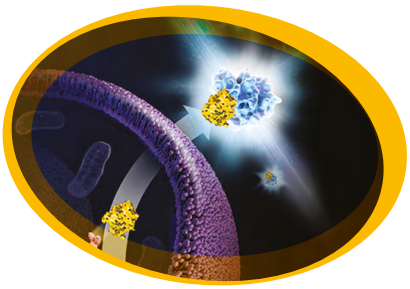
Product Portfolio

The Foundation for Scientific Progress & Innovation



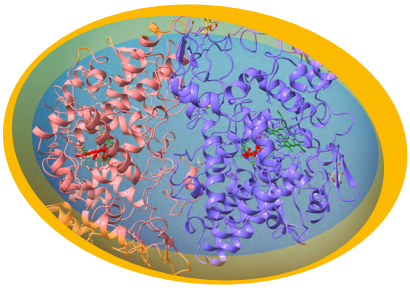
DNA & RNA Analysis

- Cloning, Enzymes, and DNA Markers
- Transfection and Epigenetics
- DNA and RNA Purification
- DNA and RNA Quantification
- DNA Amplification, PCR, and qPCR
- Reverse Transcription
- RNase Inhibitors
- NGS Sample Preparation
- Sanger Sequencing
- STR Analysis



Cell Biology

- Gene and Protein Reporters
- Viability, Cytotoxicity, and Apoptosis
- Cell Metabolism
- Oxidative Stress
- 3D Culture Analysis
- Real-time Analysis
- Signal Transduction
- Cellular Imaging
- Immunoassays

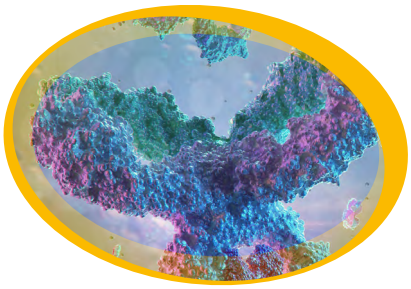


Protein Analysis

- Protein Expression
- Protein Quantification
- Protein Degradation
- Pull-down Assays
- Protein Interaction
- Protein Purification
- Mass Spectrometry

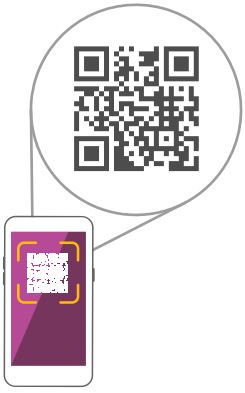
Instruments

- Luminometer, Fluorometer, and Multimode-Reader
- Automated DNA and RNA Extraction
- DNA and RNA Concentration Measurement
- Capillary Electrophoresis for Sanger Sequencing and Fragment Analysis



Reporter Potency Assays

- Fc Effector Function
- Cytokines & Growth Factors
- Immune Checkpoints
- T Cell Activation
- Drug-Target Interaction and Activity



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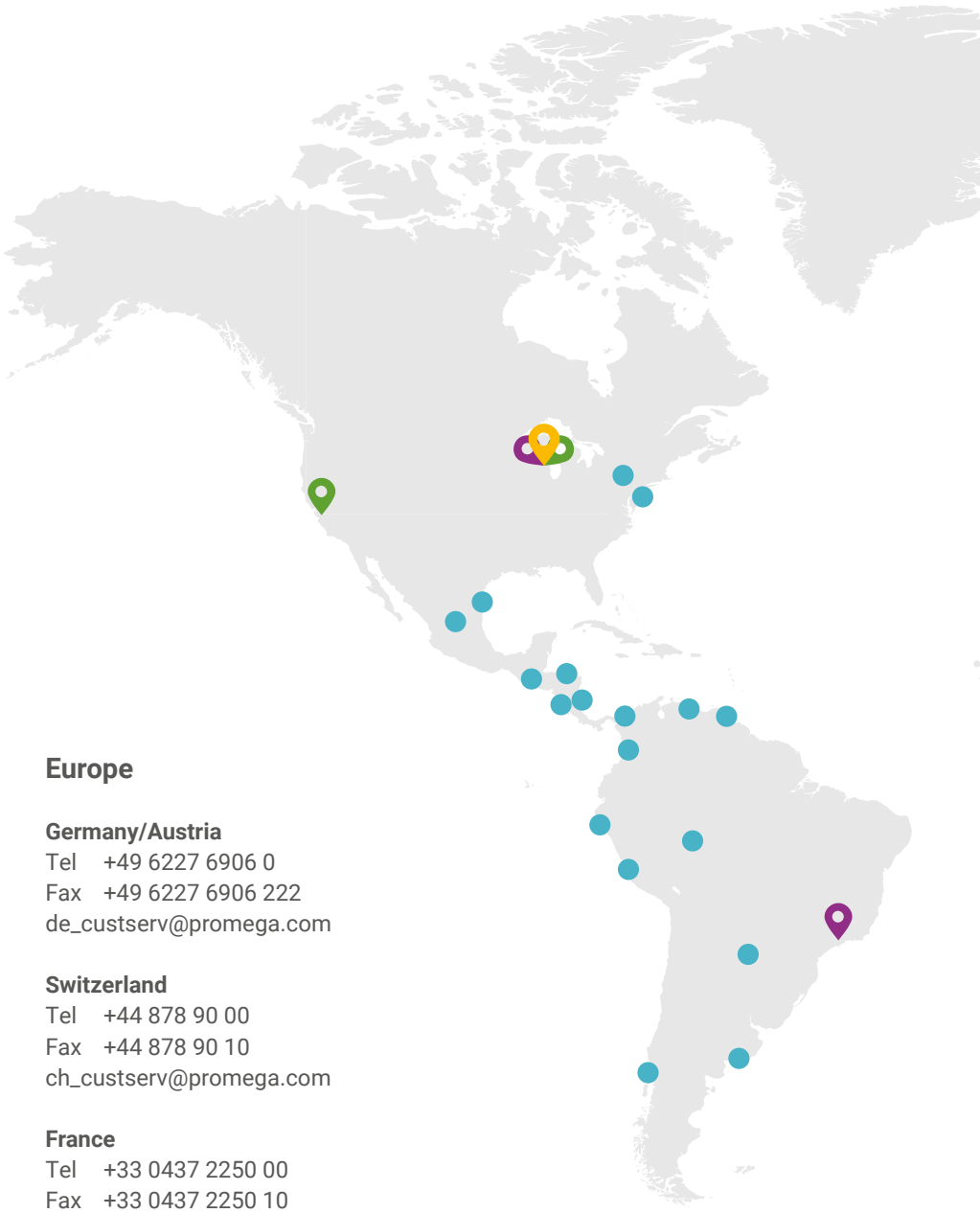

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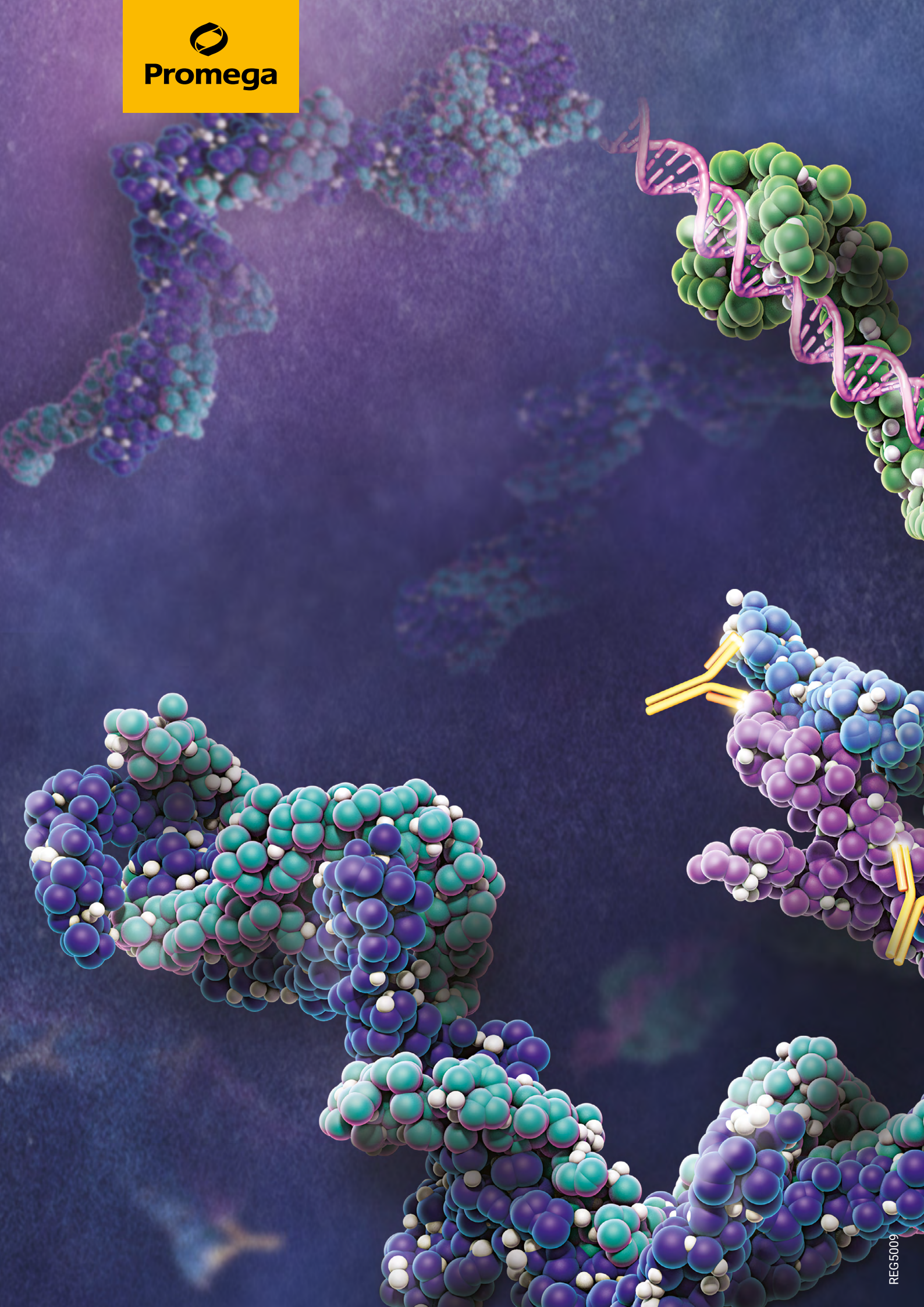
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