Overview of the Expressway™ Cell-Free Expression Systems

The Expressway™ Cell-Free Expression Systems use an efficient coupled transcription and translation reaction to produce up to milligram quantities of active recombinant protein in a tube reaction format. The Expressway™ systems eliminate the time-consuming steps of traditional cell-based protein production such as transformation, cell culture maintenance, and expression optimization. In just a few hours, high yields of high-quality protein suitable for an array of downstream functional and structural studies can be produced with this *E. coli*-based *in vitro* system. The recent improvements to the Expressway™ Cell-Free Expression System with the *sly*D mutant *E. coli* extract and patented feed buffer produce the highest level of active proteins.

The improved Expressway™ Cell-Free Expression Systems are available in four versions for different applications (Table 1). Whether you're making protein from plasmid or linear DNA, for rapid expression screening or large-scale protein production, or for downstream structural or functional analyses, Expressway™ Cell-Free Expression Systems can greatly simplify your protein expression.

Table 1—Expressway™ Cell-Free Expression Systems and applications

Application	Product
Rapid, small-scale protein expression starting from plasmid or linear DNA template	Expressway™ Mini Cell-Free Expression System
Rapid, small-scale protein expression with direct N-terminal or C-terminal Lumio™ tag detection, starting from plasmid or linear DNA template	Expressway™ Lumio™ Expression and Detection System
Rapid, milligram-scale protein expression, or high-throughput protein expression screening, starting from plasmid or linear DNA template	Expressway™ Maxi Cell-Free Expression System
Rapid, milligram-scale protein expression for highly efficient labeling for protein NMR analysis, starting from plasmid or linear DNA template	Expressway™ NMR Cell-Free Expression System

Expressway™ Mini Cell-Free Expression System

Fast, easy, and versatile cell-free protein expression

Description:

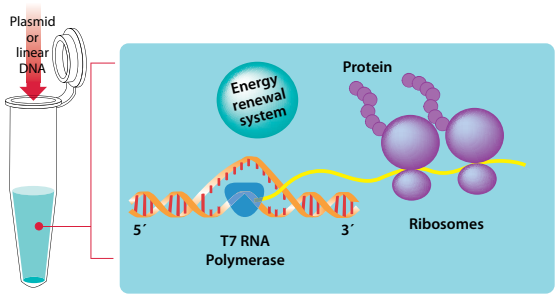
The Expressway™ Mini Cell-Free Expression System uses an efficient coupled transcription and translation reaction to produce active recombinant protein. This system eliminates the time-consuming steps of cell-based protein production, including transformation, cell culture, and expression optimization. In just two hours, you can produce protein suitable for an array of downstream applications and functional studies. The Expressway™ Mini Cell-Free Expression System offers flexibility in experimental design, allowing you to:

- Synthesize proteins from both circular plasmid and linear templates (e.g., PCR products)
- Produce proteins at high throughput
- Express genes toxic to *in vivo* systems

How it works

The Expressway™ Mini Cell-Free Expression System provides all the components you need for optimal cell-free protein production. The kit includes an *E. coli* extract containing the cellular machinery required to drive transcription and translation. An IVPS (*in vitro* protein synthesis) Reaction Buffer and an amino acid module are also included in the kit to provide the required amino acids and an ATP-regenerating system. Simply mix the Reaction Buffer, Amino Acid Mix, Methionine (labeled or unlabeled), T7 Enzyme Mix, and your DNA template (containing a T7 promoter) with the *E. coli* extract. As the DNA template is transcribed, the 5′ end of the mRNA is bound by ribosomes and undergoes translation as the 3′ end of the template is still being transcribed (Figure 1).

Figure 1—How the Expressway™ Mini Cell-Free Expression System works

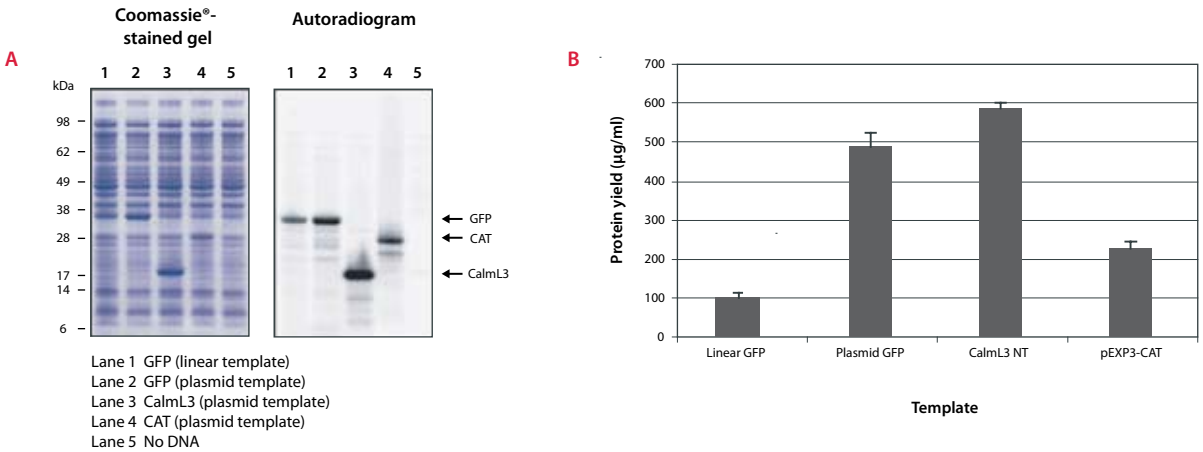


As a DNA template containing a T7 promoter is transcribed, ribosomes bind to the nascent 5′ end of the mRNA and begin translation. A specially engineered ATP energy renewal system coupled with early ribosome binding for transcript stability results in high protein yields.

Functional protein made easy

The Expressway™ Mini Cell-Free Expression System has been optimized to produce high yields of full-length, functional protein from *E. coli* cell lysates (Figure 2). A proprietary Feed Buffer is provided with the system for increased protein yields.

Figure 2—Performance of the Expressway™ Mini Cell-Free Expression System



Lane 1 GFP (linear template)
Lane 2 GFP (plasmid template)
Lane 3 CalmL3 (plasmid template)
Lane 4 CAT (plasmid template)
Lane 5 No DNA

Three proteins (GFP, CalmL3, and CAT) were synthesized *in vitro* using the Expressway™ Mini Cell-Free Expression System. For trace-labeling purposes, [^{35}S]-methionine was added to the reactions. A. Products were loaded onto a NuPAGE® gel, and proteins were visualized by both Coomassie® staining and autoradiography. B. Yields from the reactions shown in (A) were determined by TCA precipitation.

Contents and Storage:

The Expressway™ Mini Cell-Free Expression System is designed to perform twenty 50-µl reactions or one 1-ml reaction. This system includes the IVPS *E.coli* Extract, IVPS Reaction Buffer, Feed Buffer, 19 amino acid mix, methionine, DNase/RNase-free water, T7 Enzyme Mix, and a positive expression control vector. Suggested vectors for use with the Expressway™ Mini Cell-Free Expression System are listed below. Store vectors and T7 Enzyme Mix at –20°C. Store other kit components at –80°C.

Product	Quantity	Cat. no.
Expressway™ Mini Cell-Free Expression System	1 kit	K9901-00
pEXP1-DEST™ Vector Kit	6 µg	V960-01
pEXP2-DEST™ Vector Kit	6 µg	V960-02
pEXP5-NT/TOPO® Vector Kit	20 rxns	V960-05
pEXP5-CT/TOPO® Vector Kit	20 rxns	V960-06

Expressway™ Lumio™ Expression and Detection System

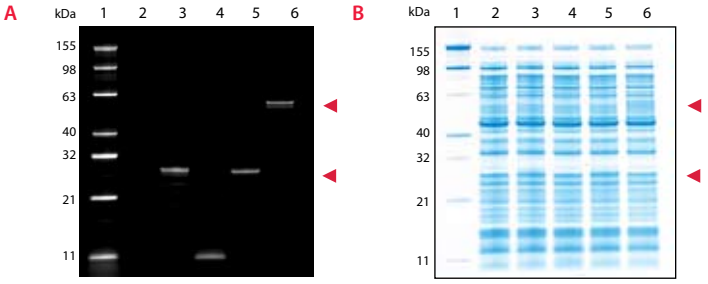
Direct in-gel detection and real-time expression analysis

Description:

The Expressway™ Lumio™ Expression and Detection System takes advantage of the small, six-amino acid Lumio™ recognition sequence (Cys-Cys-Pro-Gly-Cys-Cys). The Lumio™ detection reagent binds the recognition sequence with high specificity and affinity, resulting in a bright fluorescent signal for real-time protein production analysis and immediate in-gel protein detection. In addition, the Expressway™ specialized *E. coli* lysate, derived from a *slyD* mutant, eliminates nonspecific binding of the Lumio™ Green Detection Reagent to the endogenous SlyD protein and provides optimal background for detection of recombinant proteins (Figure 2). The Lumio™ Green Detection Kit is included in the system for real-time detection of protein synthesis, as well as easy in-gel protein detection. The Lumio™ vectors (Figures 3 and 4) also feature:

- *attR* sites for efficient recombination with any *attL*-flanked Gateway® entry vector
- N-terminal Lumio™ tag (pEXP3-DEST vector) with TEV cleavage site for efficient removal of the Lumio™ sequence following purification
- C-terminal Lumio™ tag (pEXP4-DEST vector) for easy and immediate in-gel detection
- T7 promoter, ribosome binding site, and T7 terminator optimally spaced for cell-free protein expression

Figure 2—In-gel detection of proteins synthesized using the Expressway™ Lumio™ Expression and Detection System



Several Lumio™ fusion proteins were expressed using the Expressway™ Lumio™ Expression System and Detection. Protein samples were labeled using the Lumio™ Green Detection Kit and electrophoresed on a NuPAGE® Novex® 4-12% Bis-Tris Gel. A. The Lumio™ Green signal. B. Total protein was detected with SimplyBlue™ SafeStain. Arrows indicate Lumio™ fusion proteins. Lane 1: BenchMark™ Fluorescent Protein Standard. Lane 2: Negative expression control. Lanes 3-6: Lumio™ fusion proteins human Krev, human c-Jun leucine zipper domain, CAT, and mouse E2F1, respectively.

Figure 3—pEXP3-DEST vector

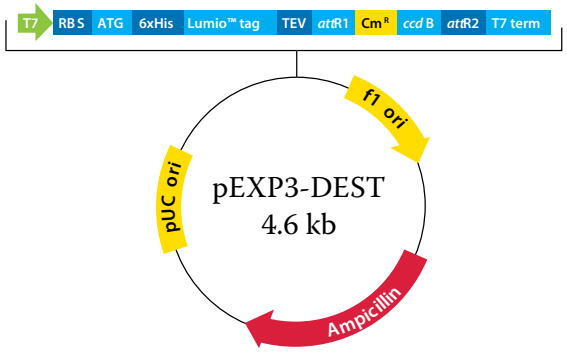
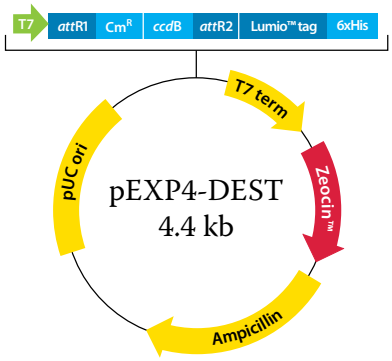


Figure 4—pEXP4-DEST vector



Expressway™ Lumio™ Expression and Detection System, continued

Contents and Storage:

The Expressway™ Lumio™ Expression and Detection System includes an IVPS *E. coli* Extract, IVPS Reaction Buffer, T7 Enzyme Mix, Feed Buffer, 19 amino acid mix, Methionine, a control plasmid, and a Lumio™ Green Detection Kit for 20 real-time reactions or 100 in-gel applications. In addition to these components, the Expressway™ N-term Lumio™ kit also includes 6 µg of the pEXP3-DEST vector and the Expressway™ C-term Lumio™ kit includes 6 µg of the pEXP4-DEST vector. Store the vectors, T7 Enzyme Mix, and the detection reagent at –20°C. Store all other components at –80°C. Guaranteed stable for 6 months when properly stored.

Product	Quantity	Cat. no.
Expressway™ N-term Lumio™ Expression and Detection System	1 kit	K9900-70
Expressway™ C-term Lumio™ Expression and Detection System	1 kit	K9900-90
Expressway™Lumio™ Expression and Detection System (without vector)	1 kit	K9900-60
pEXP3-DEST	6 µg	V960-03
pEXP4-DEST	6 µg	V960-04
Lumio™ Green Detection Kit	1 kit	LC6090

For more information on Lumio™ technology, visit **www.invitrogen.com**.

Expressway™ Maxi Cell-Free *E. coli* Expression System

Fastest tool for producing milligram levels of active protein or for performing efficient HTP expression screening

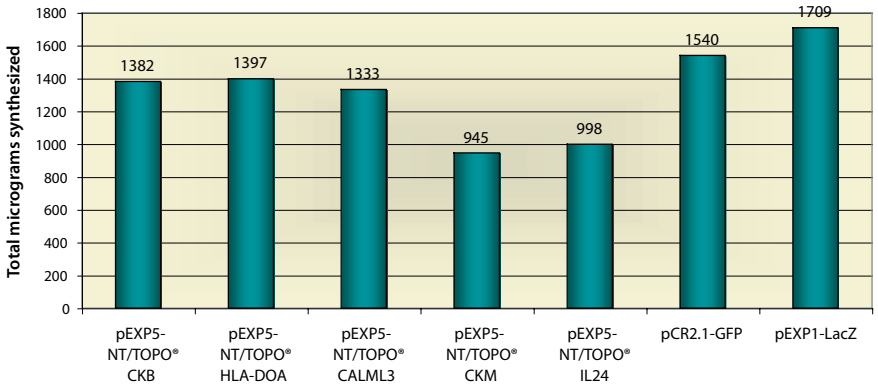
Description:

The Expressway™ Maxi Cell-Free *E. coli* Expression System uses an efficient coupled transcription and translation reaction to produce milligram quantities of soluble, functionally active protein in 4–6 hours. The procedure can be performed in a single reaction tube and is easily scalable without specialized equipment. The TOPO® TA Cloning® expression vectors (5-minute cloning with 95% efficiency) are provided for optimal expression results. In addition to all the advantages of an open expression system, Expressway™ Maxi technology provides a means to produce high levels of recombinant protein that may be easily detected and purified for various downstream applications.

Milligram level protein production within hours

The key to the Expressway™ Maxi technology is the unique formulation of the Feed Buffer that boosts the reaction productivity so that more than one milligram of protein is produced in a 2-ml reaction (final volume) within 4–6 hours (Figure 5). The formulation of the reaction buffer enables protein synthesis using either circular or linear templates.

Figure 5—High yields of various proteins using the Expressway™ Maxi Cell-Free *E. coli* Expression System



Two-milliliter (1 ml initial reaction plus 1 ml feeding) Expressway™ Maxi reactions were incubated for 6 hours at 37°C generating GFP, *LacZ*, and five additional ORFs in different expression vectors. Products were visualized on NuPAGE® Novex® 4-12% Bis-Tris Gels with Coomassie® staining. Expression levels were determined by [^{35}S] Methionine trace labeling.

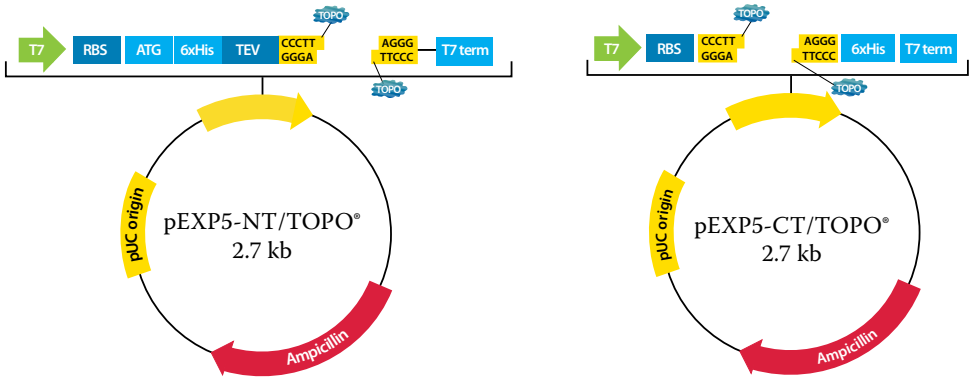
High throughput (HTP) compatible

This system is also designed for HTP expression. One kit is good for 200 25-µl reactions, which can be accommodated by two 96-well plates. Without Feed Buffer, such reactions need only 2 hours. Once positive expression is detected, scale-up protein production can be performed with the large Expressway™ cell-free format or in *E. coli* cell-based systems.

Optimized vectors

pEXP5-TOPO® TA vectors are optimized for use in the Expressway™ Maxi System (Figure 6). They are designed to minimizethe addition of extra amino acid sequences that may interfere with native protein folding and functionality. The pEXP5-NT/TOPO® expression vector provides an N-terminal histidine tag and TEV protease recognition site. The pEXP5-CT/TOPO® expression vector can be used for expressing proteins with a C-terminal histidine tag or for native protein expression by introducing a stop codon at the end of your gene of interest.

Figure 6—pEXP5 NT/TOPO® and pEXP5 CT/TOPO® vectors



Simple and fast procedure

The Expressway™ Maxi Cell-Free *E. coli* Expression System provides all the components needed for high-yield cell-free protein production. To start a 2-ml reaction, mix the reaction buffer, 19 amino acid mix, your choice of labeled or unlabeled Methionine, T7 enzyme mix, and your DNA template (with T7 promoter) with the *E. coli* extract. After a 30-minute incubation, add the Feed Buffer. Milligram levels of active protein will be synthesized within 4–6 hours. To perform HTP expression, simply premix all the components and aliquot into a HTP format.

Contents and Storage:

The Expressway™ Maxi Cell-Free *E. coli* Expression System is designed to perform 200 25-µl reactions, five 1-ml, or one 5-ml reaction (initial volume). This system includes the IVPS *E. coli* Extract, IVPS Reaction Buffer, Feed Buffer, 19 Amino Acid Mix, Methionine, DNase/RNase-free water, T7 Enzyme Mix, and a positive expression control vector. The Expressway™ Maxi Cell-Free *E. coli* Expression System with pEXP5-NT/TOPO® and pEXP5-CT/TOPO® vectors also includes the pEXP5-NT/TOPO® and pEXP5-CT/TOPO® TA Expression Kits. Each kit contains the pEXP5-NT/TOPO® or pEXP5-CT/TOPO® vector, other reagents to facilitate TOPO® Cloning, and One Shot® TOP10 Chemically Competent *E. coli*. Store the vectors and T7 Enzyme Mix at –20°C. Store all other components at –80°C. Guaranteed stable for 6 months when properly stored.

Product	Quantity	Cat. no.
Expressway™ Maxi Cell-Free *E. coli* Expression System with pEXP5-NT/TOPO® and pEXP5-CT/TOPO® vectors	1 kit	K9900-96
Expressway™ Maxi Cell-Free *E. coli* Expression System	1 kit	K9900-97
pEXP5-NT/TOPO® TA Expression Kit	10 rxns	V960-05
pEXP5-CT/TOPO® TA Expression Kit	10 rxns	V960-06

Expressway™ NMR Cell-Free *E. coli* Expression System

Fast and cost-effective production of soluble proteins for NMR spectroscopy

Description:

The Expressway™ NMR Cell-Free *E. coli* Expression System produces milligram quantities of recombinant proteins efficiently labeled with a stable isotope for NMR spectroscopy and mass spectrometry. This system uses the Expressway™ Maxi technology to produce milligram levels of soluble, functionally active proteins, while allowing highly efficient labeling in a very small volume, greatly reducing the need for expensive stable isotope-labeled amino acids. The reaction is scalable and can easily be adapted for high-throughput expression. This system also includes convenient TOPO® TA Cloning® expression vectors for optimal expression and easy purification of the recombinant proteins.

Most cost-effective protein production for NMR analysis

Because the reaction is performed in a single-tube format without any specialized instrumentation, the amount of stable isotope-labeled amino acid needed for efficient protein labeling is minimized, making this method more cost-effective than any other method available for producing labeled protein. The individual packaging of the amino acids provides maximal convenience and flexibility, allowing production of labeled recombinant protein using any labeled amino acid.

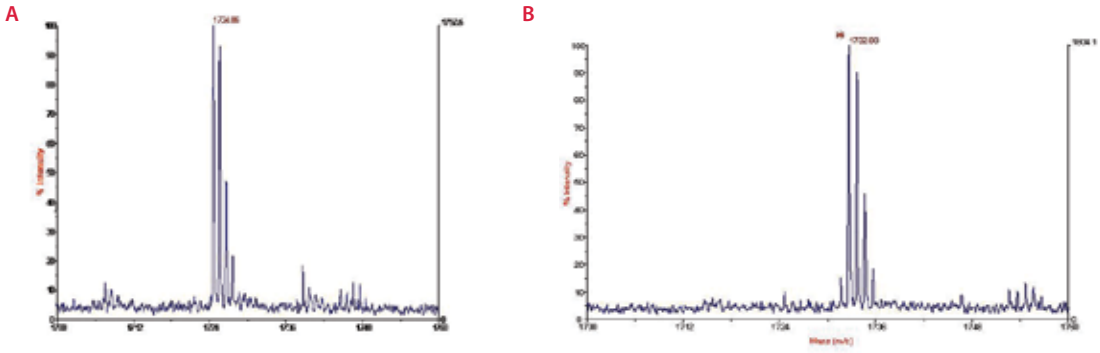
Simple and fast procedure

The Expressway™ NMR Cell-Free *E.coli* Expression System provides all the components needed for optimal cell-free protein production. The kit includes the *slyD* mutant *E. coli* extract, IVPS reaction buffer, 2X IVPS Feed Buffer, T7 enzyme mix, and 20 amino acids in individual tubes. To start a reaction, simply mix reaction buffer, unlabeled amino acids, your choice of labeled amino acids, T7 enzyme mix, and your DNA template (with T7 promoter) with the *E. coli* extract. After 30 minutes of incubation, add the Feed Buffer. Milligram levels of soluble protein are synthesized and efficiently labeled in 4–6 hours.

Highly efficient protein labeling

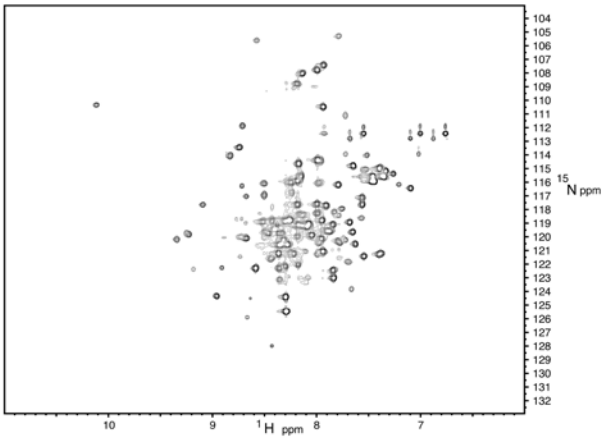
Two-dimensional NMR spectra were successfully obtained from multiple proteins produced by the Expressway™ NMR Cell-Free *E. coli* Expression System. The labeling efficiency of the proteins was close to 100% for the majority of labeled amino acids as tested by MALDI-TOF MS analysis (Figures 7 and 8).*

Figure 7—MALDI-TOF MS spectra of an internal peptide (113-118) derived from human calmodulin-like 3 protein synthesized using the Expressway™ NMR Cell-Free *E. coli* Expression System



A. Unlabeled peptide from calmodulin-like 3 protein (MW=1724.65 Da). B. $^{15}N_4/^{13}C_4$ lysine-labeled calmodulin-like 3 peptide (MW=1732.88 Da).

Figure 8—^{15}N-^{1}H HSQC spectrum (800 MHz, Varian Inova spectrometer) of the uniformly labeled CALML3 protein produced in the Expressway™ NMR Cell-Free *E. coli* Expression System



Ten milligrams of protein were produced from one kit of the Expressway™ NMR Cell-Free *E. coli* Expression System, purified using Ni-NTA resin, and dialyzed before analysis. The ^{15}N-^{1}H HSQC spectrum was obtained at 25°C and pH 6.0 in 10% D_2O, 90% H_2O. Six hundred microliters of 0.5 mM labeled protein were used.

Contents and Storage:

The Expressway™ NMR Cell-Free *E. coli* Expression System includes the Expressway™ Maxi Expression Module and the Expressway™ NMR Amino Acids Module. The Expressway™ Maxi Expression Module contains Expressway™ *slyD* mutant *E. coli* extract, 2.5X IVPS Reaction Buffer (lacking amino acids), 2X IVPS Feed Buffer, T7 enzyme mix, and a positive control plasmid. The Expressway™ NMR Amino Acids Module contains 20 amino acids in individual tubes. The Expressway™ NMR Cell-Free *E. coli* Expression System with pEXP5-NT/TOPO® and pEXP5-CT/TOPO® vectors also includes the pEXP5-NT/TOPO® and pEXP5-CT/TOPO® TA Expression Kits. Each kit contains the pEXP5-NT/TOPO® or pEXP5-CT/TOPO® vector and One Shot® TOP10 Chemically Competent *E. coli*. Store vectors and T7 enzyme mix at –20°C. Store all other components at –80°C. Guaranteed stable for 6 months when properly stored.

Product	Size	Cat. No.
Expressway™ NMR Cell-Free *E. coli* Expression System with pEXP5-NT/TOPO® and pEXP5-CT/TOPO®	1 kit	K9900-98
Expressway™ NMR Cell-Free *E. coli* Expression System	1 kit	K9900-99
pEXP5-NT/TOPO® TA Expression Kit	10 rxns	V960-05
pEXP5-CT/TOPO® TA Expression Kit	10 rxns	V960-06

* For protein labeling with asparagine, glutamic acid, or glutamine, please contact Custom Services or Technical Services for custom formulation of the buffers.