NEW ENGLAND BioLabs Inc. be INSPIRED drive DISCOVERY stay GENUINE

NEBExpress® Cell-free *E. coli* Protein Synthesis System

NEB #E5360S/L 10/100 reactions

Version 2.0_1/21

Table of Contents

The NEBExpress Cell-free *E. coli* Protein Synthesis System Kit Includes

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E5360S) and 100 reactions (NEB #E5360L). T7 RNA Polymerase and RNase Inhibitor, Murine should be stored at –20°C and all other reagents should be stored at –80°C.

NEBExpress S30 Synthesis Extract

Thaw on ice just before use. Avoid multiple freeze-thaw cycles. Aliquot as necessary.

Protein Synthesis Buffer (2X)

Thaw on ice just before use. Avoid multiple freeze-thaw cycles. Aliquot as necessary.

T7 RNA Polymerase

Enables transcription of DNA templates with a T7 promoter. Supplied in 50 mM Tris-HCl, 100 mM NaCl, 20 mM β-ME, 1 mM EDTA, 50% Glycerol, 0.1% Triton™ X-100, pH 7.9.

RNase Inhibitor, Murine

Inhibits potential RNase contamination carried over from plasmid preparation. Supplied in 20 mM HEPES-KOH, 50 mM KCl, 8 mM DTT, 50% Glycerol, pH 7.6.

NEBExpress Control DHFR-His Plasmid

Encoding *E. coli* dihydrofolate reductase (DHFR, with N-terminal His-tag, ~20 kDa, 125 ng/µl) for use as a positive control.

Introduction

Cell-free protein synthesis (CFPS) has become a powerful and versatile alternative to the heterologous *in vivo* expression of proteins. CFPS offers many advantages, such as time savings, high throughput protein synthesis and production of proteins toxic to living cells. Coupled transcription/translation systems contain an RNA polymerase and the necessary cellular machinery needed to direct protein synthesis (e.g., ribosomes, translation factors and tRNAs). Supplements such as amino acids, an energy source and NTPs complete the system. The NEBExpress Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA template under the control of a T7 RNA Polymerase promoter.

The NEBExpress Cell-free *E. coli* Protein Synthesis System offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost-effective for high throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening. In addition, it can be used to generate proteins for biophysical and structure-function analyses.

Protein synthesis is achieved using a short incubation and the synthesized protein is compatible with downstream purification or analysis by SDS-PAGE, western blot or direct functional assay. The novel formulation of this system allows samples to be loaded directly onto SDS-PAGE, without the need for acetone or TCA precipitation. Additionally, the synthesized protein can be isolated from the reaction mixture by affinity purification techniques such as immobilized metal affinity chromatography (IMAC) for further structural and/or functional characterization.

Template Preparation

PCR products, linear or circular plasmid DNA, or mRNA can be used as a template. While higher yields are often obtained with circular plasmid DNA, PCR products can generate acceptable yields and can provide timesaving advantages. Furthermore, a DNA exonuclease inhibitor, such as NEBExpress GamS Nuclease Inhibitor (NEB #P0774), can be added to stabilize linear DNA and achieve close to plasmid-level yields.

Template purity is important for successful *in vitro* transcription/translation; use the Monarch® Plasmid Miniprep Kit (NEB #T1010) or Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) to isolate plasmid or linear DNA. Plasmid DNA prepared from some commercial kits may contain trace amounts of RNase A. The RNase A contamination can be inhibited by the supplied RNase Inhibitor, and therefore no additional clean-up steps are necessary.

The DNA template should be ≥ 100 ng/µl in a suitable buffer. For diluted DNA samples, nuclease-free water (NEB #B1500) should be used to prevent buffer carryover into the protein synthesis reaction. A starting point for template input is 250 ng of DNA (linear or circular) per 50 µl reaction. However, optimal DNA template can range from 25–1000 ng per reaction. Template input amount should be scaled up or down accordingly with the reaction volume.

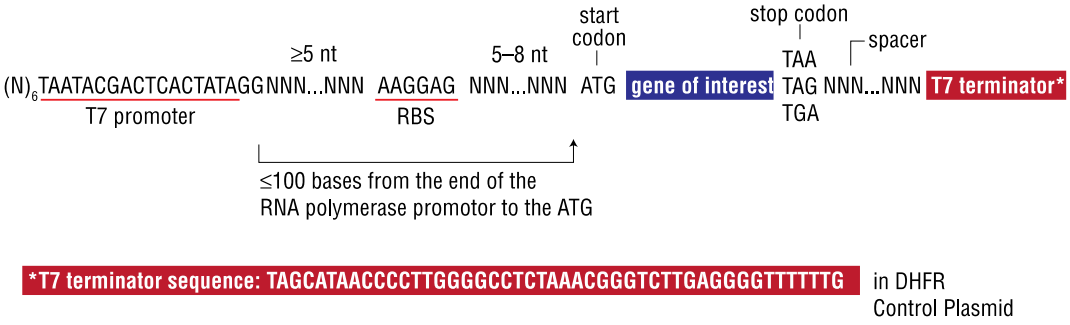
For convenience, the NEBExpress DHFR-His Control Plasmid can be used as a cloning vector (Figure 1A). This high copy vector contains the required T7 promoter, ribosome binding site, T7 terminator elements and ampicillin resistance. The DHFR gene can be replaced with other genes of interest for use with *in vitro* expression.

Figure 1A. Schematic of the NEBExpress DHFR-His Control Plasmid.

In addition to the desired protein coding sequence (in-frame), the template DNA must contain the following elements (Figure 1B):

- Start codon (ATG)
- Stop codon (TAA, TAG, or TGA)
- T7 promoter (20 to 100 nucleotides upstream of the coding sequence)
- Ribosome binding site (RBS, also known as a Shine-Dalgarno sequence) upstream, approximately 6-8 nucleotides, of the start of translation
- Spacer region 6 base pairs downstream from the stop codon (for PCR products)
- T7 terminator downstream from the stop codon (recommended for all templates)

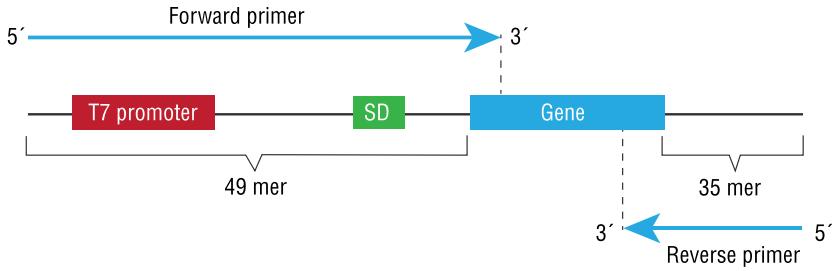
Figure 1B. Required Elements for Template DNA.



Generation of Template DNA by PCR

Gene specific primers are used to add adaptor sequences (homologous to part of the regulatory region DNA) to the 5′ and 3′ ends of the gene of interest (Figures 2 and 3).

Figure 2. Suggested Primer Design for PCR



5′ UTR sequence of Forward Primer (49 mer)

5′ GCG AAT TAA TAC GAC TCA CTA TAG GGC TTA AGT ATA
AGG AGG AAA AAA T . . . 27 bases matching gene of interest starting with ATG - 3′

3′ UTR sequence of Reverse Primer (35 mer)

5′ AAA CCC CTC CGT TTA GAG AGG GGT TAT
GCT AG TTA . . . 24 bases matching gene of interest - 3′

Figure 3. 3′ UTR Stem Loop of PCR Templates

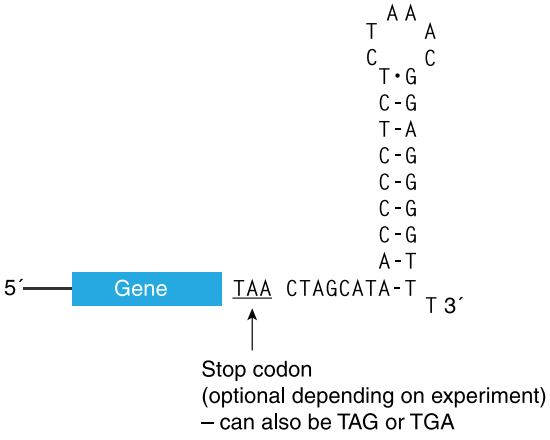


Illustration of the stem loop structure formed by the 3′ UTR Reverse Primer. This structure will be present in mRNA from templates generated by PCR using the suggested primer design in Figure 2. The 3′ UTR is based on the T7 Terminator sequence and will function as such.

Using RNA Templates

Although the NEBExpress Cell-free *E. coli* Protein Synthesis System is designed for coupled transcription and translation, direct translation from an mRNA template is possible. The amount of RNA (transcribed *in vitro*) will need to be determined empirically. As a starting point, use 1–5 µg mRNA template per reaction. The mRNA should contain a proper Ribosome Binding Site for efficient translation. The quality of the mRNA is very important; therefore, *in vitro* transcription reactions should be cleaned up to remove residual NTPs, salts and buffers.

NEBExpress Cell-free *E. coli* Protein Synthesis Reaction Protocol

Using a positive control template to verify protein synthesis can be useful when unfamiliar with *in vitro* transcription-translation protocols. To prevent nuclease contamination, wear gloves and use nuclease-free tubes and tips. Keep all reagents on ice before and during the assembly of reactions and avoid repeated freeze-thaw cycles of the tubes. Reactions are typically 50 µl but can be scaled down or up, as needed. Reactions are typically assembled in nuclease-free 1.5 ml microcentrifuge tubes. Components can be pre-assembled to create a master mix for a desired number of reactions. Use the master mix immediately, discard any unused master mix.

Standard Protocol

1. Thaw all components on ice.
2. Gently vortex the NEBExpress S30 Synthesis Extract and Protein Synthesis Buffer to mix.
3. Combine reagents in a 1.5 ml microcentrifuge tube on ice as follows:

COMPONENTS	NEGATIVE CONTROL (no DNA)	POSITIVE CONTROL	SAMPLE
NEBExpress S30 Synthesis Extract	12 µl	12 µl	12 µl
Protein Synthesis Buffer (2X)	25 µl	25 µl	25 µl
T7 RNA Polymerase	1 µl	1 µl	1 µl
RNase Inhibitor, Murine	1 µl	1 µl	1 µl
NEBExpress Control DHFR-His Plasmid (125 ng/ µl)	–	2 µl	–
Plasmid template (> 100 ng/µl)	–	–	250 ng
Water	11 µl	9 µl	to 50 µl

4. Incubate reactions at 37°C, with vigorous shaking, for 2–4 hours.
5. Analyze by method of choice or freeze at -20°C for later use.

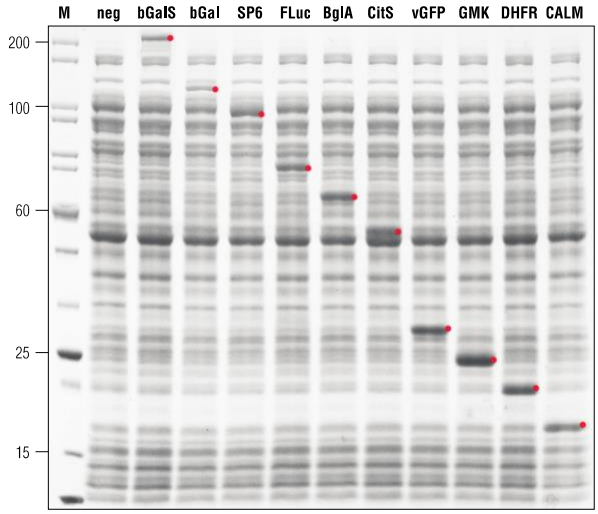
Optimizing Protein Synthesis

- Typical reaction conditions use a 50 µl reaction volume in 1.5 ml microcentrifuge tube. When using other vessels, leave enough head space for aeration. Cover 96 well plates with a breathable seal.
- For aeration, use a plate shaker or thermal mixer. Reactions can be performed without agitation; however, yields may be slightly lower.
- The typical incubation temperature is 37°C. Lowering the incubation temperature to 25°C can help the synthesis of some target proteins.
- If required, the reaction can be incubated at higher or lower temperatures, from 16°C to 42°C; however, protein yield may not be optimal.
- The target protein can be detected in as little as 30 minutes depending on the target and sensitivity of the assay method. Protein synthesis will continue steadily for 10 hours at 37°C or up to 24 hours at lower temperatures, depending on DNA input and aeration.
- Reactions can be scaled up or down linearly. When working with large reaction volumes it is necessary to include sufficient headspace and/or aeration. When working with small reaction volumes do not allow evaporation to occur.
- For target proteins requiring proper disulfide bond formation, supplement the reaction with PURExpress® Disulfide Bond Enhancer (NEB #E6820). Add 2 µl of Enhancer 1 and 2 µl of Enhancer 2 per 50 µl reaction.
- If using linear templates, add NEBExpress GamS Nuclease Inhibitor (NEB #P0774). As a starting point, use 1 µl GamS per 50 µl reaction.
- The protein yield is dependent on the nature of the target protein. The system routinely produces between 0.05 to 0.5 mg/ml (up to 25 µg per 50 µl reaction). The conditions for a given target will require optimization of the incubation temperature and time, as well as the concentration of the template and supplements.

Analysis of Synthesized Protein

Following synthesis, samples can be analyzed by SDS-PAGE (Figure 4) or western blot. Always analyze synthesis reactions side by side with a negative control sample, to compare the banding pattern. Alternatively, the synthesis reaction can be analyzed directly in a functional assay (provided the reaction mixture components do not cause interference). Due to the complexity of the reaction, it is strongly recommended to always compare the synthesis reaction with a negative control sample, or with a sample synthesizing an unrelated protein.

Figure 4. Protein Expression using the NEBExpress Cell-free *E. coli* Protein Synthesis System



50 µl reactions containing 250 ng template DNA were incubated at 37°C for 3 hours. 2 µl of each reaction were analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad range (NEB #P7717), "Neg" = negative control, no DNA.

SDS-PAGE Protocol

In vitro protein synthesis reactions produced by the NEBExpress Cell-free *E. coli* Protein Synthesis System can be directly loaded onto an SDS-PAGE gel without the need for acetone or TCA precipitation.

1. Combine 2 µl of a NEBExpress Cell-free *E. coli* Protein Synthesis System reaction with 6 µl of SDS-PAGE Blue Loading Buffer (NEB #B7703), and 10 µl H_2O. Also prepare a negative control sample.
2. Incubate at 100°C for 3–5 minutes.
3. Load 3 µl of the Unstained Protein Standard (NEB #P7717) into the first lane.
4. After a quick microcentrifuge spin, load samples directly on to the gel. To ensure uniform mobility, load an equal volume of SDS-PAGE Blue Loading Buffer into any unused wells.
5. Run the gel according to the manufacturer's recommendations.
6. Stain with Coomassie Blue or another stain as directed or proceed to western blot.

After staining, the target protein is typically observed as a unique band, absent in the negative control reaction. However, sometimes, the target has the same apparent molecular weight as an endogenous protein. In this case, the target protein will enhance or "darken" the co-migrating band.

Protein Purification

Any affinity purification method can be applied, as none of the endogenous proteins in the NEBExpress Cell-free *E. coli* Protein Synthesis System are tagged. If the synthesized protein contains a Maltose-Binding Protein (MBP) tag, then use Amylose Resin (NEB #E8021), Amylose Resin High Flow (NEB #E8022) or Amylose Magnetic Beads (NEB #E8035). If the synthesized protein has a Chitin Binding Domain (CBD) tag, then use Chitin Resin (NEB #S6651) or Chitin Magnetic Beads (NEB #E8036). If the synthesized protein contains a His-tag, then use NEBExpress Ni-NTA Magnetic Beads (NEB #S1423), NEBExpress Ni Spin Columns (NEB #S1427) or NEBExpress Ni Resin (NEB #S1428), as described below.

NEBExpress Ni-NTA Magnetic Beads Quick Protocol (NEB #S1423)

Visit www.neb.com/S1423 for full-length protocol

1. Gently vortex and thoroughly suspend magnetic beads.
2. Aliquot 50 µl of bead suspension to a sterile microcentrifuge tube, apply magnet, to pull beads to the side of the tube and remove supernatant.
3. Equilibrate beads by mixing with 200 µl of Lysis/Binding buffer (20 mM sodium phosphate, 300 mM NaCl, 10 mM Imidazole, pH 7.4), apply magnet to pull the beads aside and remove the supernatant.
4. Add 50 µl of Lysis/Binding buffer and 50 µl of the protein synthesis sample. Mix for 30 minutes. Apply magnet and remove supernatant.
5. Wash beads 3X with 500 µl of Wash buffer (20 mM sodium phosphate, 300 mM NaCl, 20 mM Imidazole, pH 7.4) as per Step 3.
6. Elute fusion protein by incubating with 100 µl Elution buffer (20 mM sodium phosphate, 300 mM NaCl, 500 mM Imidazole, pH 7.4) for ≥ 2 minutes with mixing. Apply magnet, remove and keep supernatant. Elution can be repeated, and eluates combined.

NEBExpress Ni Spin Columns Quick Protocol (NEB #S1427)

Visit www.neb.com/S1427 for full-length protocol

1. Remove the bottom tab of the column and place the column in a collection tube, loosen the cap.
2. Centrifuge column at 800 x *g* for 1 minute, discard the buffer.
3. Add 250 µl of Lysis/Binding buffer to the column. Centrifuge column at 800 x *g* for 1 minute, discard the Lysis/Binding buffer.
4. Add 150 µl of Lysis/Binding buffer and 50 µl of the protein synthesis sample to the column, tap to mix and allow binding for 2–60 minutes.
5. Centrifuge column at 800 x *g* for 1 minute, retain flow through.
6. Place the column in a new 2 ml microcentrifuge tube.
7. Add 250 µl of Wash Buffer to the column and centrifuge at 800 x *g* for 1 minute, repeat twice. Retain the washes.
8. Add 200 µl of Elution Buffer to the column. Tap the column repeatedly to mix. Centrifuge at 800 x *g* for 1 minute, retain the eluate.
9. Repeat elution step once, collecting fraction in a new microcentrifuge tube.

Troubleshooting

1. Control Protein (DHFR, ~20 kDa) is not synthesized

1.1 Kit component(s) were inactivated: Store NEBExpress S30 Synthesis Extract and the Protein Synthesis Buffer at –80°C. Minimize number of freeze-thaw cycles (if necessary, aliquot components).

1.2 Nuclease contamination: Wear gloves and use nuclease-free water, pipette tips and microcentrifuge tubes.

1.3 T7 RNA Polymerase was absent in the reaction make sure to add T7 RNA Polymerase.

2. Control Protein is synthesized, but the target protein is not present or present in low yield

2.1 RNase contamination: Commercial mini-prep kits are useful for preparing template DNA but can also introduce RNase A into the reaction. Make sure RNase Inhibitor has been added to the reaction.

2.2 Template DNA design is compromised:

2.2.1. The coding region and regulatory sequences of the DNA template must be correct and in-frame to ensure translation is initiated properly and that full-length product is produced. Make sure the DNA template contains the T7 terminator or a UTR stem loop to stabilize the mRNA in order to increase the yield.

2.2.2. Non-optimal regulatory sequences and/or spacing may adversely affect translational efficiency. Translation initiation is critical, secondary structure or rare codons at the beginning of the mRNA may compromise the initiation process.

2.2.3. If tolerable to the target, the addition of an adequate initiation region (e.g., first 10 codons of maltose binding protein) may help. Alternatively, using PCR to modify the 5´ end of the target gene may eliminate secondary structural elements or rare codons.

2.2.4. Certain eukaryotic proteins might require modifying the gene sequence for expression in a bacterial system (codon optimization, addition of tags to enhance solubility, removal of transmembrane domains, etc.)

2.3. Template DNA is contaminated

2.3.1. Inhibitors of transcription or translation may be present in the DNA and will reduce protein yield. A simple mixing experiment (control DNA + target DNA, compared to control DNA alone) will reveal whether inhibitors are present.

2.3.2. Do not use DNA purified from agarose gels as they often contain inhibitors of translation (e.g., ethidium bromide). Residual SDS from plasmid preparation protocols is another common contaminant. Re-purify the DNA using the Monarch® Plasmid Miniprep Kit (NEB #T1010) or Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030).

2.3.3. Templates produced by PCR need to be free of non-specific amplification products. These contaminants may contain transcription signals and thus compete for and titrate out transcription and/or translation components. As a result, yields may decrease, and truncated products may be produced.

2.3.4. Make sure to add RNase Inhibitor (NEB #M1018) to the reaction. The supplied RNase Inhibitor will reduce the effects of RNase contamination that may be present after using commercial plasmid prep kits.

2.4. Template DNA concentration is not optimal

2.4.1. The concentration of template DNA is important to maintain the balance between transcription and translation. Too little template reduces the amount of actively translated mRNA while too much template results in the overproduction of mRNA and overwhelming of the translational apparatus. 250 ng of template DNA is recommended for a 50 µl reaction. Optimization with different amounts of template DNA (e.g., 25–1000 ng) may improve yield of a particular target protein.

2.4.2 If UV absorbance was used to calculate the concentration of the template DNA, be aware that RNA or chromosomal DNA will also absorb UV light. If the sample has significant amounts of RNA or chromosomal DNA, the actual amount of template DNA may be lower than the calculated amount. The 260 nm/280 nm ratio should be 1.8. Running a sample of template DNA on an agarose gel can determine if it is the correct size, has been degraded, and/or if it contains other nucleic acids.

3. Target protein synthesized but the full-length product is not the major species

3.1 Translation initiation and/or termination is not correct. The system can express large molecular weight proteins; however, obtaining full-length product requires proper initiation and termination. Internal ribosome entry sites and/or premature termination can produce truncated proteins.

3.2 Bands smaller than the expected full-length protein can be proteolytic fragments produced during the reaction. Adding protease inhibitors may help to solve the issue.

4. Target protein synthesized but it is inactive or insoluble

4.1 Incubating at lower temperatures (down to 16°C), for longer periods of time (up to 24 hours), can help solubilize proteins that otherwise are synthesized in an insoluble form.

4.2 Alone or in combination with changes in incubation temperature, supplement the reaction with PURExpress Disulfide Bond Enhancer (NEB #E6820). Add 2 µl of Enhancer 1 and 2 µl of Enhancer 2 per 50 µl of reaction.

Frequently Asked Questions

For a complete list of FAQs, please visit the product page at www.neb.com

Q1. ***Based on my application, should I use the NEBExpress Cell-free E. coli Protein Synthesis System or PURExpress®?***

A1: The NEBExpress Cell-free *E. coli* Protein Synthesis System and PURExpress are suited for high yield protein synthesis of a variety of protein targets, under the control of T7 RNA Polymerase. Both products offer convenient reaction analysis by SDS-PAGE or by direct assay. Both systems are compatible with plasmid and linear DNA templates; mRNA can also be used as a template.

PURExpress is a reconstituted system and therefore provides a more defined reaction environment with minimal nuclease and protease activity. Downstream applications such as directed protein evolution, translation studies and protein/protein interactions are particularly suited to PURExpress. PURExpress is also ideal for applications where components of the translational machinery are selectively left out (PURExpress Δ (aa, tRNA) Kit, PURExpress Δ RF123 Kit, PURExpress Δ Ribosome Kit), including non-natural amino acid incorporation, radiolabeling and ribosome studies. In PURExpress, all protein components (except ribosomes) are recombinant and His-tagged allowing for reverse purification.

The NEBExpress Cell-free *E. coli* Protein Synthesis System is preferable to isolate proteins that are His-tagged. In addition, it has a lower cost per reaction, making it more easily scalable to large volume applications.

To compare PURExpress and the NEBExpress Cell-free *E. coli* Protein Synthesis System by application, reference the selection chart on the product page at www.neb.com.

Q2: ***What is the difference between NEBExpress Cell-free E. coli Protein Synthesis System and PURExpress?***

A2: PURExpress is a reconstituted protein synthesis system where all necessary components needed for *in vitro* transcription and translation (including T7 RNA polymerase) are purified from *E. coli* and mixed in a multicomponent solution. All protein components are His-tagged (except the ribosome). PURExpress contains minimal nuclease and protease activities. The protein mix is combined with an optimized reaction buffer for coupled transcription and translation.

NEBExpress Cell-free *E. coli* Expression System is a high activity lysate from *E. coli*, which in combination with an optimized reaction buffer, T7 RNA polymerase, and RNase inhibitor, allow coupled transcription and translation.

Both systems are compatible with plasmid and linear DNA templates; mRNA can also be used as a template.

To compare PURExpress and the NEBExpress Cell-free *E. coli* Protein Synthesis System by application, reference the selection chart on the product page at www.neb.com.

Q3: ***Are the yields with NEBExpress Cell-free E. coli Protein Synthesis System comparable with PURExpress?***

A3: Protein yield, using either PURExpress or the NEBExpress Cell-free *E. coli* Protein Synthesis System, is dependent on the nature of the target protein and the reaction conditions. The NEBExpress Cell-free *E. coli* Protein Synthesis System routinely produces up to 0.5 mg/ml (up to 25 µg per 50 µl reaction). The PURExpress system routinely produces up to 0.25 mg/ml (up to 12.5 µg per 50 µl reaction). The conditions for a given target will require optimization of the incubation temperature and time, as well as the concentration of the template and supplements.

Q4: ***Can I express a large protein with NEBExpress Cell-free E. coli Protein Synthesis System?***

A4: NEBExpress Cell-free *E. coli* Protein Synthesis System can produce high molecular weight targets due to stabilization of the mRNA template in a low RNase environment. Proteins from 17 to 230 kDa have been successfully synthesized in an active form (unpublished results). For example, a functionally active 230 kDa protein, β-galactosidase from *Streptococcus*, was expressed using the NEBExpress Cell-free *E. coli* Protein Synthesis System (see Figure 4).

Q5: ***Can I use the NEBExpress Cell-free E. coli Protein Synthesis System to incorporate unnatural amino acids or radiolabeled amino acids?***

A5: For these applications, the PURExpress *in vitro* Protein Synthesis Kit (NEB #E6800) is the best option.

Q6: ***Can the reaction be scaled to smaller or larger volumes?***

A6: Yes, the typical reaction volume is 50 µl, but can be scaled up or down linearly. All reaction components, including the DNA template, must be scaled proportionally. The system has been tested with reaction volumes as small as 1 µl and as large as 2 ml. Protein synthesis yields are comparable within this range. For small volumes, such as 1 µl, an acoustic droplet ejection liquid handler (or similar) should be used. For large volumes use a vessel with adequate headspace to allow for aeration and incubate with agitation.

Q7: ***What methods can be used to purify the synthesized protein?***

A7: Any affinity purification method can be applied, as none of the endogenous proteins in the NEBExpress Cell-free *E. coli* Protein Synthesis System are tagged. If the synthesized protein contains a His-tag, then use NEBExpress Ni-NTA Magnetic Beads (NEB #S1423), NEBExpress Ni Spin Columns (NEB #S1427) or NEBExpress Ni Resin (NEB #S1428). If the synthesized protein contains a Maltose-Binding Protein (MBP) tag, then use Amylose Resin (NEB #E8021), Amylose Resin High Flow (NEB #E8022) or Amylose Magnetic Beads (NEB #E8035). If the synthesized protein has a Chitin Binding Domain (CBD) tag, then use Chitin Resin (NEB #S6651) or Chitin Magnetic Beads (NEB #E8036).

Kit Components

NEB #E5360S Table of Components

NEB #	PRODUCT	SIZE
P0864S	NEBExpress S30 Synthesis Extract	125 µl
B0864S	Protein Synthesis Buffer (2X)	250 µl
M1019A	T7 RNA Polymerase	15 µl
M1018A	RNase Inhibitor, Murine	15 µl
N3273A	NEBExpress Control DHFR-His Plasmid	10 µl

NEB #E5360L Table of Components

NEB #	PRODUCT	SIZE
P0864S	NEBExpress S30 Synthesis Extract	10 x 125 µl
B0864S	Protein Synthesis Buffer (2X)	10 x 250 µl
M1019AA	T7 RNA Polymerase	150 µl
M1018AA	RNAse Inhibitor, Murine	150 µl
N3273A	NEBExpress Control DHFR-His Plasmid	10 µl

NEB #E5360 Companion Products

NEB #	PRODUCT	SIZE
B1500S/L	Nuclease-free Water	25/100 ml
P0774S	NEBExpress GamS Nuclease Inhibitor	75 µg
E6820S	PURExpress Disulfide Bond Enhancer	50 reactions
T1010S/L	Monarch Plasmid Miniprep Kit	50/250 preps
T1030S/L	Monarch PCR & DNA Cleanup Kit (5 µg)	50/250 preps
P7717S/L	Unstained Protein Standard, Broad Range (10–200 kDa)	150/750 gel lanes
B7703S	Blue Loading Buffer Pack	8 ml
S1423S/L	NEBExpress Ni-NTA Magnetic Beads	1/5 ml
S1427S/L	NEBExpress Ni Spin Columns	10/25 columns
S1428S	NEBExpress Ni Resin	25 ml bed volume
E8021S/L	Amylose Resin	15/100 ml
E8022S/L	Amylose Resin High Flow	15/100 ml
E8035S	Amylose Magnetic Beads	25 mg
S6651S/L	Chitin Resin	20/100 ml
E8036S	Chitin Magnetic Beads	5 ml
S1506S	6-Tube Magnetic Separation Rack	6 tubes
S1511S	96-Well Microtiter Plate Magnetic Separation Rack	96 well

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
2.0	Name change: “Control DHFR-His Template to NEBExpress Control DHFR-His Plasmid”	1/21

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

This product is covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. For more information about commercial rights, please email us at busdev@neb.com While NEB develops and validates its products for various applications, the use of this product may require the buyer to obtain additional third party intellectual property rights for certain applications.

Information presented herein is accurate and reliable to the best of our knowledge and belief but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary.

B CORPORATION™ is a trademark of B Lab, Inc.
TRITON™ is a trademark of MilliporeSigma.

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com