Protein Synthesis Reaction using PURExpress (E6800)

New England Biolabs[1]

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May 29, 2020

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ABSTRACT

PURExpress® is a reconstituted protein synthesis system based on the PUREsystem™ (Shimizu et al., 2001) where all necessary components needed for in vitro transcription and translation are purified from *E. coli*.

- Defined system with all his-tagged proteins for coupled transcription/translation; Ribosome is not his-tagged
- T7 RNA Polymerase drives *in vitro* transcription
- Minimal nuclease and protease activity for stability of synthesized protein and encoding target
- Templates can be either plasmid DNA, linear DNA or mRNA
- Protein of interest can be synthesized and visualized in a few hours
- Synthesizes various target peptides and proteins
- Synthesized protein can be co-translationally radiolabeled or fluorescently labeled
- Protein can be reverse-purified or subject to direct functional analysis
- Applications include high throughput screening/directed evolution, synthetic biology, toxic or difficult to express protein synthesis, studies on protein folding, activity and protein-protein interactions
- Due to reconstituted nature, several kits are offered where translation factors or macromolecules have been omitted to facilitate specific studies (see companion products)
- Compatible with the PURExpress Disulfide Bond Enhancer (NEB #E6820)

EXTERNAL LINK

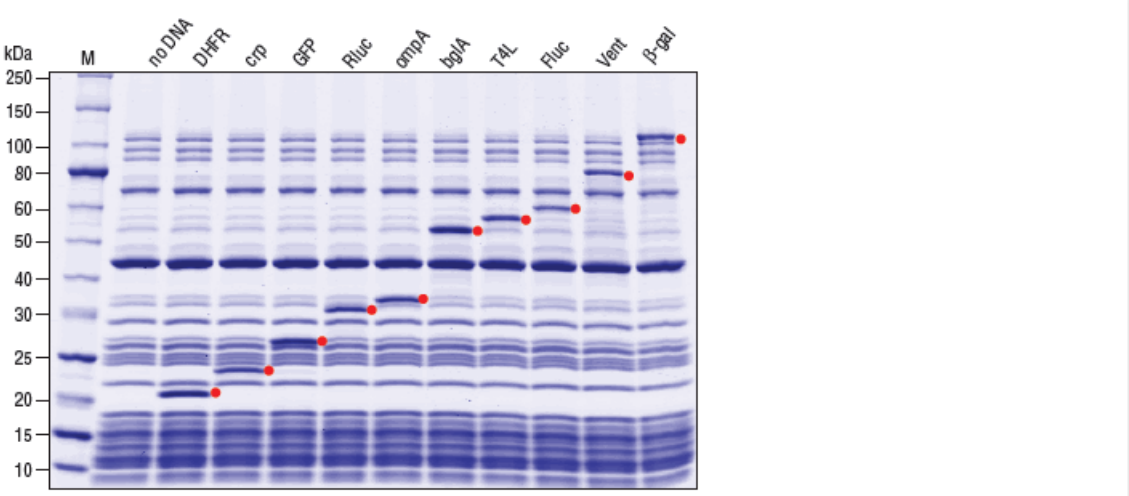
https://www.neb.com/protocols/0001/01/01/protein-synthesis-reaction-using-purexpress-e6800

GUIDELINES

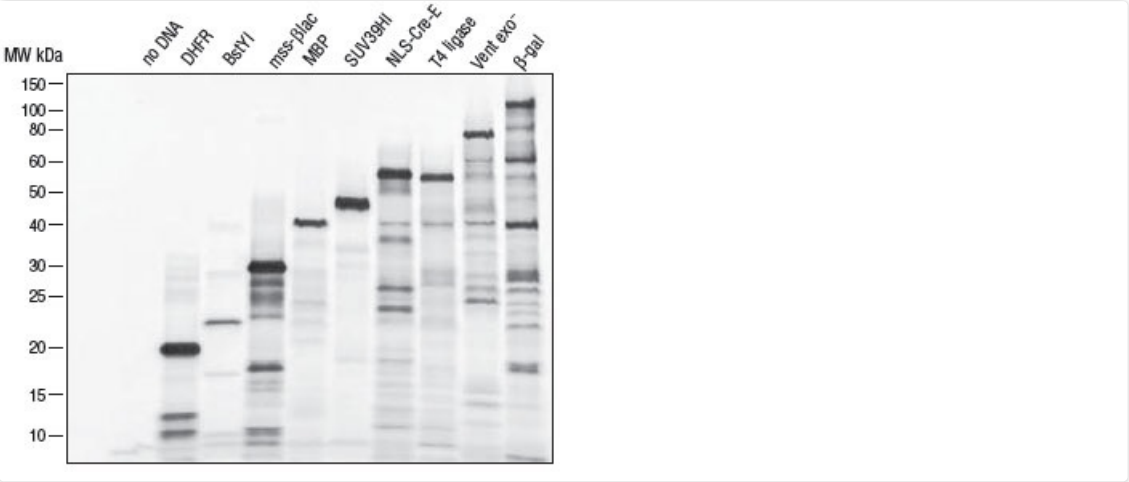
A rapid method for gene expression analysis, PURExpress®is a novel cell-free transcription/translation system reconstituted from the purified components necessary for*E. coli*translation. With minimal nuclease and protease activity, the PURExpress system preserves the integrity of DNA and RNA templates/complexes and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

PURExpress Citations

Figure 1: Protein expression using the PURExpress® In Vitro Protein Synthesis Kit



25 µl reactions containing 250 ng template DNA and 20 units RNase Inhibitor were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. Marker M is the Protein Ladder (NEB #P7703).**Figure 2: Incorporation of 35S-methionine enables visualizationof protein by autoradiography**



25 µl reactions containing 250 ng template DNA, 20 units RNase Inhibitor and 2 µl 35S-met were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE, the gel was fixed for 10 minutes, dried for 2 hours at 80°C and exposed to x-ray film for 5 hours at -80°C.**Figure 3: Schematic diagram of protein synthesis and purification by PURExpress**

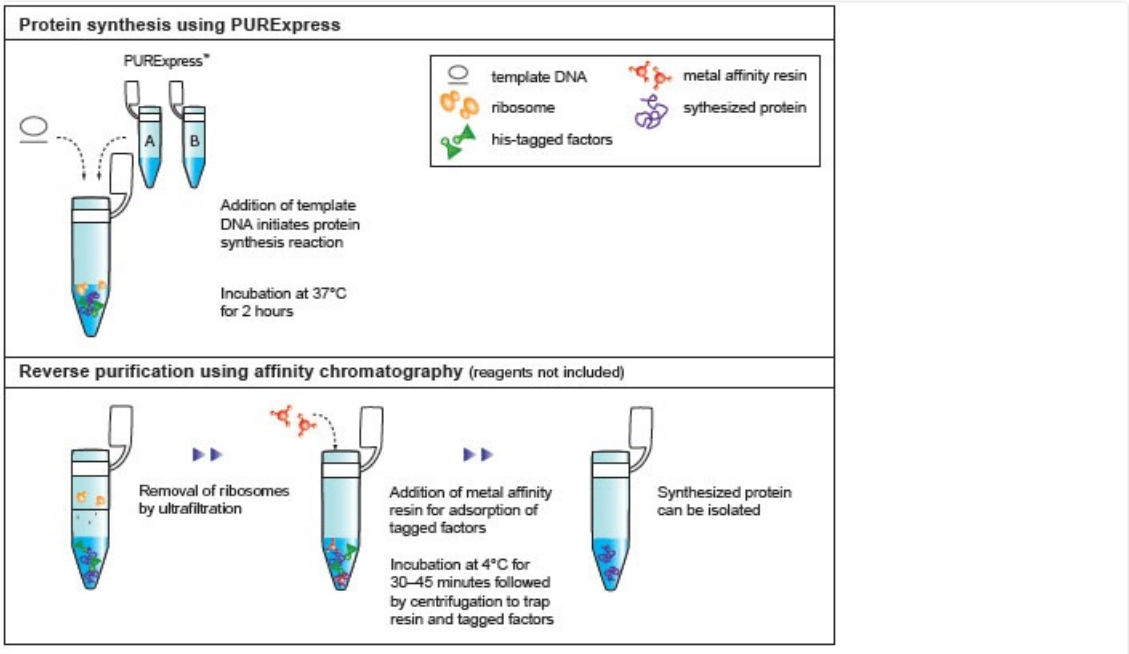
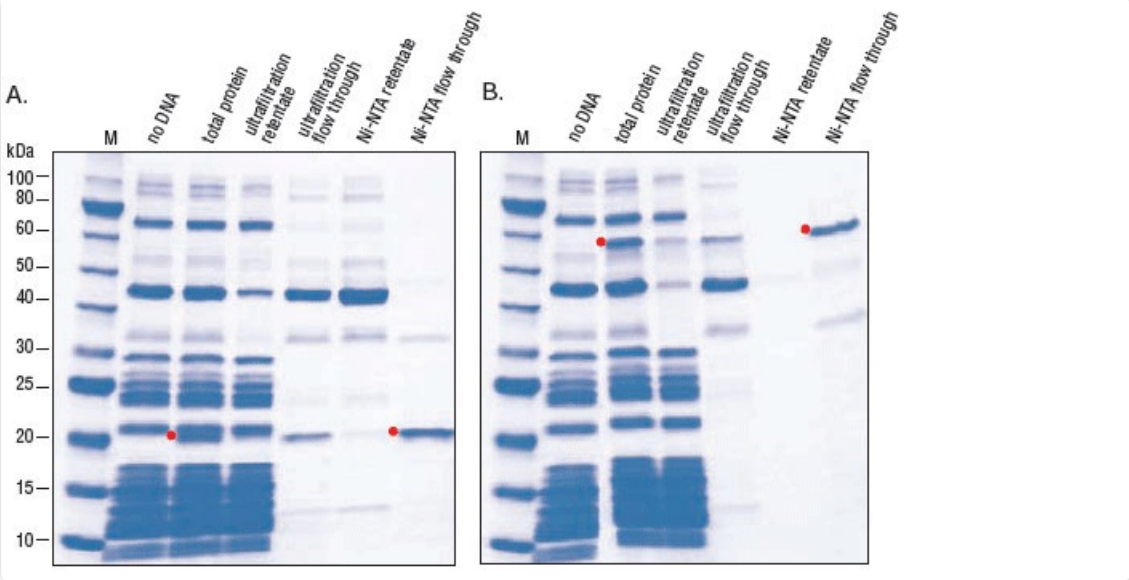


Figure 4: Expression and reverse purification of DHFR (A) and T4 DNA Ligase (B) using PURExpress



125 µl reactions were carried out according to recommendations in the accompanying manual. Samples were analyzed on a 10–20% Tris-glycine gel and stained with Coomassie Blue. Note that in both cases, the desired protein can be visualized in the total protein fraction. The red dot indicates the protein of interest. Marker M is the Protein Ladder (NEB #P7703).

MATERIALS

NAME	CATALOG #	VENDOR
PURExpress In Vitro Protein Synthesis Kit - 100 rxns	E6800L	New England Biolabs
PURExpress In Vitro Protein Synthesis Kit - 10 rxns (25 microliter)	E6800S	New England Biolabs

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Using a positive control template to verify protein synthesis can be useful when unfamiliar with *in vitro* transcription-translation protocols. We recommend wearing gloves and using nuclease-free tubes and tips to avoid introducing nucleases to your samples. Please keep all reagents on ice before and during the assembly of reactions and avoid multiple freeze-thaw cycles of the tubes. Reactions are typically 25 µl but can be scaled down or up, as needed. Reactions are usually assembled in nuclease-free 0.5 ml microfuge tubes.

1 Thaw the necessary number of aliquots of solution A and B **On ice** . Pulse-spin in microfuge to collect solutions to bottom of tube.

Certain components in Solution A may precipitate during storage. Be sure to mix it well prior to assembling reactions. The performance of the kit will not be compromised.

2 Assemble the reaction **On ice** in a new tube in the following order:

COMPONENT	AMOUNT
Solution A	10 µl
Solution B	7.5 µl
Supplements (RNase Inhibitor, 35S-met, etc.)	X µl
Nuclease-free H2O	X µl
Template DNA	X µl

Total Volume	**25 µl**

These formulations allow an increase in the "user added" volume (for template, supplements, etc.); tolerating up to 20% over volume (30 µl reaction total) without an appreciable drop in productivity.

The DHFR control template is supplied at 125 ng/µl. Use 2 µl for the positive control reaction. Template DNA, particularly plasmid DNA prepared by mini-prep (e.g. Qiagen) is often the major source of RNase contamination. We strongly recommend adding 20 units Murine RNase Inhibitor (NEB #M0314) in each reaction.

For target proteins requiring disulfide bonding, we suggest supplementing the reactions with the PURExpress Disulfide Bond Enhancer (PDBE,NEB #E6820).

Add Solution B to Solution A, do not dilute Solution B unbuffered. We recommend a starting concentration of 250 ng template DNA per 25 µl reaction. The optimal amount of input DNA can be determined by setting up multiple reactions and titrating the amount of template DNA added to the reaction. Typically, the optimal amount will fall in a range of 25-1000 ng template. (NEB #E3313)The standard reaction contains 60 pmoles of ribosomes in a 25 µl reaction. The supplied control ribosomes are enough for two reactions. Using a smaller amount of ribosomes is possible but the protein yield may be lower.

3 Mix gently and pulse-spin in microfuge to collect mixture at the bottom of the tube.

4 Incubate at **37 °C** for **02:00:00** .

We recommend using an incubator rather than a water bath, to prevent evaporation. Some reactions can benefit from an additional hour of incubation to achieve maximum yield. Some proteins are also more soluble at reduced temperatures; however, incubating reactions below 37°C will likely reduce yield.

5 Stop the reaction by placing the tube(s) **On ice** .

6 Use samples for analysis or purification or freeze at **-20 °C** for use at a later time.

Some material may precipitate during storage at -20°C. Please ensure everything is resuspended by flicking the reaction tube after thawing.

The PURExpress components are highly purified and present in known quantities. The reconstituted nature of this product makes it amenable to modifications. As such, it is easy to perform *in vitro* labeling reactions with ^{35}S-methionine to allow visualization of the product. It is also straightforward to supplement the reactions with a component under investigation that is believed to have an effect on transcription or translation. *In vitro* labeling with ^{35}S-methionine can be performed by setting up a standard reaction with the addition of 2 µl of ^{35}S-methionine.

All amino acids, including methionine, are present at approximately 0.3 mM in PURExpress (with the exception of NEB #E6840). Labeled amino acids will compete with existing normal amino acids and the observed signal from the label depends on the efficiency of incorporation into the protein of interest. When

supplemented with 1.2 µM ^{35}S- L-methionine, we observe levels of incorporation compatible with autoradiographic detection of the synthesized protein. Reactions (1–5 µl) can then be directly resolved by SDS-PAGE (no need for acetone precipitation), the gels are then briefly fixed in a methanol /acetic acid solution (45%/10%) for 5 minutes at 25°C and dried down onto filter paper (2 hrs at 80°C). The dried gel is then exposed to autoradiographic film (overnight at -20°C) or detected with a phosphorimager.

We encourage safe handling of radioisotopes and suggest consulting with your institution's radiation safety officer for guidelines and advice on the practical aspects of performing labeling reactions in your workplace.