SynthEasy
Cell-Free Protein Synthesis Kit

User Manual

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Introduction

SynthEasy Cell-Free Protein Synthesis Kit is a fast, practical, flexible and cost-effective tool to synthesize recombinant protein based on *E. coli* cell extracts. It was designed for *in vitro* transcription and translation of target DNA into protein, in a single reaction. The SynthEasy cell extract for *in vitro* translation contains functional ribosomes and all the essential components for the prokaryotic translation. Since results are available in a few hours, SynthEasy Cell-Free Protein Synthesis Kit saves time and is well suited for high-throughput synthesis and screening of gene products. This system may also be used to synthesize toxic proteins that are difficult to obtain using expression systems based on live cells, due to their negative effects on the host.

Contents

The kits are shipped with the following contents.

Component	Content and function
E.Coli Lysate	• Lysate from E.coli; • Contains components for transcription and translation
Energy Mix;	Energy mix to prepare reaction solution
Buffer CFPS Biolinker	Provides support for pH, energy and stability reaction.
Pool AA	Provides amino acids for protein synthesis and co-factor. In addition to prolonging reaction time

Warranty

Biolinker warrants the performance of all products in the manner described in our product literature. The customer shall determine whether our product is suited for its specific use. This guarantee shall take over from all other, stated or implied guarantees.

Ordering information

Kit	Cat. No.	mL	Suggested reactions
SynthEasy Protein Expression Kit	PEK1001	1 mL	Ten 200-µl, or one 1-ml reactions, depending on your needs
SynthEasy Membrane Protein Expression Kit	PEK2001		
SynthEasy Luminescent Protein Expression Kit	PEK3001		
SynthEasy Protein Expression Kit	PEK1002	5 mL	Fifty 200-µl, five 1-ml, or one 5-ml reactions, depending on your needs.
SynthEasy Membrane Protein Expression Kit	PEK2002		
SynthEasy Luminescent Protein Expression Kit	PEK3002		

You can place your order, as well as ask any questions, directly with us, via the following e-mail: **orders@biolinker.tech**

Materials and equipment provided by the user

The SynthEasy Cell-Free Protein Synthesis Kit does not include:

- Standard molecular biology equipment and reagents for PCR, cloning, DNA and protein handling
- Incubators
- Bench-top centrifuge
- Cooling and freezing apparatus, such as liquid nitrogen

Storage and shipping

The SynthEasy Cell-Free Protein Synthesis Kit is shipped on dry ice.

Once delivered, all components should be stored at appropriately:

- **SynthEasy E.coli Cell Extract**: to be stored at -80°C. The kits will be viable for 12 months.
- **Remaining components**: to be stored in the dark, at -20°C. The kits will be viable until the expiration date printed on the label. Avoid repeated freezing and thawing.

Product limitations

SynthEasy Cell-Free Protein Synthesis Kit was developed, manufactured and sold for the sole purpose of non-commercial research use. Commercial use of the SynthEasy Cell-Free Protein Synthesis Kit, however, requires specific licensing. Commercial use includes but is not limited to:

- The use of any protein or other substance produced by SynthEasy Cell-Free Protein Synthesis Kits as reagents in screening to discover and/or promote candidate compounds for sale to customers, distributors, retailers or other end-user in therapeutic, diagnostic, prophylactic, and/or veterinary areas;
- The manufacture, sale or offer to sell any product containing proteins or other substances produced with SynthEasy Cell-Free Protein Synthesis Kits;
- "Contract research" to any third party or "Contract manufacturing" for any third party that has not been granted a license to use SynthEasy Cell-Free Protein Synthesis Kit

For commercial use of the kit, please contact us at orders@biolinker.tech.

Safety information

When handling chemicals, wear protective clothing and eyewear, and disposable gloves.

We recommend all users to adhere to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines),* since they detail safety practices and containment procedures for basic and clinical research involving recombinant and/or synthetic nucleic acid molecules.

Quality Assurance

Quality and satisfaction are priorities here at Biolinker. We are committed to consistently delivering superior products, services, and quality support to our clients while encouraging an atmosphere of innovation and continuous improvement. We have implemented an extensive quality assurance policy that extends to raw materials, production stages, packaging and storage, that meets the highest national

requirements. In order to guarantee maximum process integrity, quality tests are a part of every batch. In order to keep the quality standards that satisfy our customers' expectations, we strive to constantly upgrade our equipment, internal policies and processes. Our in-house Quality Control laboratory is staffed by an experienced and qualified team. We have the latest analytical instruments and advanced microbiological testing equipment to guarantee the best possible quality, purity and formulation accuracy.

Disclaimer

Biolinker provides no other warranties of any type, expressed or implied, including those of profitability and readiness for a particular application. Biolinker is not responsible for any damage or loss resulting from the use, misuse, or inability to use its products.

2 Protocols |

How does it work?

Cell-free protein synthesis (CFPS) is based on a coupled transcription/translation reaction in solution (or in vitro), leading to the production of recombinant protein using cell extracts containing translation machinery that can come from various sources: while the T7-RNA- polymerase transcribes the template gene, the machinery in the lysate starts to translate the 5'-end of the nascent mRNA. Regulatory elements, such as T7 promoter, ribosomal binding site and T7 terminator are added to the gene of interest by PCR or cloning. The resulting DNA template is then added to the mixture. In a coupled in vitro reaction, the template DNA is first transcribed into mRNA by T7 RNA polymerase, followed by translation into protein by the ribosomal machinery present in the lysate. The expressed protein accumulates during the reaction and is harvested after 1-24 hours. The reaction will reach 90% completion after 16 hours.

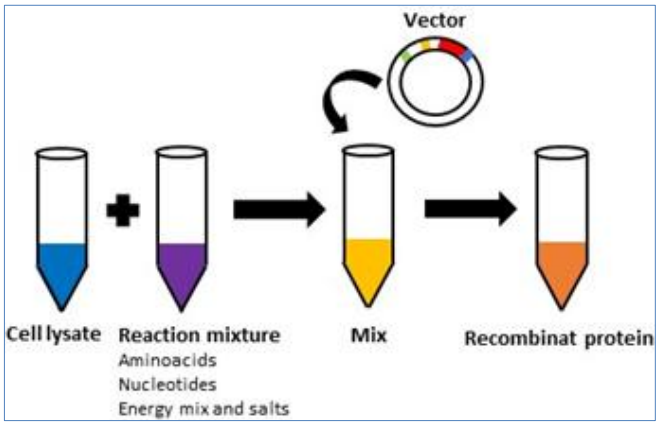

Figure 1: Simplified procedure

This approach is extremely advantageous:

- **It allows for faster and hassle-free expression of recombinant protein.** The desired protein can be obtained in a matter of hours and the lack of living cells in the process means that there is no need for safety regulations regarding recombinant organisms
- **It promotes efficiency**. Scientists can work on multiple synthesis in parallel.
- **It's flexible**. Scientists can now synthesize proteins that would, otherwise, be harder to express due to their inherent toxicity, as well as synthesize proteins with known folding or solubility issues.

Protocols

1. **RECOMMENDATIONS**

Template optimization. When dealing with low expression yields, we recommend that the sequence be optimized, since gene expression yields depend on the mRNA structure.

PCR and generation of expression templates. DNA template expression via PCR protocols is much faster than via subcloning. When optimizing your CPFS protocols, it is advised to have a significant amount of DNA template ready until a satisfactory performance is attained.

Notes:

- Do not combine reagents from different lots.
- Reconstitute only what is needed for a given experiment.
- When specified, use only the provided reaction reagents and/or salts (even if you have the equivalent from another manufacturer).
- Store reconstituted solutions at –15 to –25°C. Lysate should be frozen in liquid nitrogen and subsequently stored at –15 to –25°C. Reagents can withstand two freeze–thaw cycles without significant decrease in activity.
- Reconstituted plasmid should be stored at –15 to –25°C.
- Lyophilized reagents or thaw solutions must be reconstituted immediately before use.
- Always work on ice.

2. CFPS REACTION

Materials required:

- DNA template.
- RNase-free plastics and/or glassware
- Water bath or incubator. Temperature must be adjusted to 30°C.
- Pipettes (0–10 µl, 10–200 µl, 200–1,000 µl) and autoclaved tips.
- UV lamp or UV lamp-equipped transilluminator for detection of GFP (360nm).

Protocol

- Reconstitute the reaction components. Make sure all solutions are clear, except for the lysate, which is always cloudy.
- Prepare the working solution, depending on the number of reactions to be performed.
- Close the reaction tubes thoroughly, using adhesive film and place them into thermocycler/incubator/water bath or similar device, at 30°C.

Protocol

Initially the components of the Cell-free Biolinker Kit should be previously thawed on ice for 1 hour. After thawing the components should be added in the order they are presented for maximizing transcription and translation. In a 1.5 mL microtube, add the following components in the volumes described (**Table 1)**:

Table 1 The composition of the CFPS reaction mixture.

Components	**Control and user**	**Negative**
Buffer Cell-free Biolinker	108	73
Pool AA	10	10
Energy mix	12	12
S30-T7 lysate	60	60
DNA template (controle or user)	10µl (concentration 10ng/ul)	0
Water	x	10
Total volume (µL)	200	200

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- We recommend that the user of the Cell-free Biolinker Kit perform a positive and negative control consisting of all components of the reactive mix except the DNA template. The reaction mixture should be incubated at 30°C from 2 hours to 24 hours.
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Notes:

- If using modules, refrain from using a water bath, for it is unsuitable. After 6 hours remove the reactions from the Eppendorf thermocycler/incubator or water bath and store the reaction solution at –25°C (or at 0–4°C until purification or further processing)

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- The optimal temperature for most proteins is 30°C; however, lower temperatures may be used for proteins that tend to aggregate.
- Even though the reaction is 80-90% complete after 4 hours, protein synthesis takes up to 6 hours.

Control reactions

1. GFP

- Reconstitute bottles according to Table 1.
- Prepare reaction solution in one of the reaction tubes
- Add 1µg (approx 1µl) of reconstituted control vector GFP and 9µl of DEPC water.
- Heat at 30°C for 6 hours in the thermocycler.
- Store the reaction solution 2-8°C for 24h.
- Measure the fluorescence of GFP using a blue lamp (488nm).
- Apply 2–5 µl of the reaction onto SDS-polyacrylamide gels.
- Run the gel, and stain with Coomassie ® Blue.

Notes:

- The GFP protein can also be detected on Western blots by using an anti-His6 antibody.

- The yield of properly-folded fluorescent GFP is further increased by storing the reaction solution at 2–8°C for 24h right after expression.

- GFP expression and detection via UV. are for qualitative purposes only and should not be used to quantify the expression yield.

Troubleshooting

Please always follow the instructions and recommendations from this User Manual. The current Troubleshooting is designed to explain the most common undesired outcomes.

1. No control protein synthesis

Possible explanation: *Kit has expired*
Possible measure: *Get a new kit.*

Possible explanation: *Kit was not stored at -20°C*
Possible measure: *Get a new kit.*

Possible explanation: *Kit component(s) are not active anymore*
Possible measure: *Kit components are required to be stored at –80°C (SynthEasy E.Coli Cell Extract) or -20°C (rest). The number of freeze-thaw rounds should be minimized.*

Possible explanation: *Nuclease contamination*
Possible measure: *Always wear gloves and use nuclease-free Tubes to avoid nuclease contamination. You may want to apply an RNase Inhibitor for your reactions.*

2. Control protein synthesis manifests low color development

Possible explanation: *Kit was not stored at -20°C*
Possible measure: *Get a new kit.*

Possible explanation: *Reaction mixtures were mixed unequally*
Possible measure: *Make sure to gently pipette reaction mixtures during preparation to get thorough mixing.*

3. Target protein not present or present in low yield, but normal synthesis of control protein

Possible explanation: *Protein concentration is too small*
Possible measure: *Use the maximum amount of sample on the gel.*

Possible explanation: *Protein is not soluble*
Possible measure: *Chech both soluble (supernatant) and insoluble (pellet) parts.*

Possible explanation: *Hidden tag*
Possible measure: *Try a different protein-specific antibody or tag position. Also: in addition to Western Blotting, use a Coomassie-stained gel*

or a gel stained by other means.

Possible explanation: *Error in cloning*
Possible measure: *Verify the sequence of your clones before using them for experiments.*

Possible explanation: *Low purity of plasmid DNA*
Possible measure: *The usual purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Adequate DNA has an A260/A280 ratio of 1.7–2.0. A reading of 1.6 does not make the DNA inadequate for applications, but lower ratios tell that there are more contaminants.*

Further measures include to (a) prepare a new plasmid preparation, or (b) carry out a phenol extraction.

Possible explanation: *Nuclease contamination*
Possible measure: *Always wear gloves and use nuclease-free Tubes to avoid nuclease contamination. You may want to apply an RNase Inhibitor for your reactions.*

Possible explanation: *Translation did not start due to strong secondary structures of the mRNA*
Possible measure: *Use N- or C- terminal tagging.*

Possible explanation: *Reaction mixtures were mixed unequally*
Possible measure: *Make sure to gently pipette reaction mixtures during preparation to get thorough mixing.*

Possible explanation: *Expressed protein interfered with the translation or transcription process*
Possible measure: *Possibly your active protein cannot be expressed with SynthEasy. Try to express your protein of interest jointly with the control protein. If the expression of control protein is hindered, then this is the case.*

Possible explanation: *High nuclease concentration present in the prepared plasmid*
Possible measure: *Try to extract with phenol/chloroform. Then follow by alcohol precipitation. As last step, remove all remaining phenol by proper washing with alcohol.*

Possible explanation: *Low plasmid concentration*
Possible measure: *Try to concentrate the plasmid by alcohol precipitation.*

Possible explanation: *Kit was not stored at -20°C*
Possible measure: *Get a new kit.*

Possible explanation: *Inefficient Template DNA concentration*
Possible measure: *The concentration of template DNA is crucial since cell-free protein synthesis is a balance between transcription and translation. Insufficuent template diminishes the amount of actively translated mRNA, while excesive template manifests in the surplus production of mRNA and burdening of the translational apparatus.*

If your case has signifcant amounts of RNA or chromosomal DNA, the effective amount of template DNA may be lower than the calculated amount. The usual calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Adequate DNA has an A260/A280 ratio of 1.7–2.0.

Possible explanation: *Contaminated Template DNA*
Possible measure: *There may be inhibitors of transcription or translation present in the DNA. Try to mix control DNA and target DNA – and compare it to control DNA alone. This experiment will show whether there were inhibitors present. Those inhibitors in the target DNA will lower the yield of your control protein.*

Remaining SDS from plasmid preparation protocols can also be a cause for contamination. You can remove it by phenol/chloroform, then followed by alcohol precipitation. Pay attention to remove alchohol as well.

Ensure that templates made by PCR need to be free of non-specifc amplifcatiors. They may act as contaminants that contain transcription signals.

Possible explanation: *Compromised Template DNA design*
Possible measure: *Verify that you have a correct template DNA sequence.*

A successful translation initiation is crucial for successful protein synthesis. This initiation process can be comprimised by secondary structure or rare codons at the beginning of the mRNA. A possible solution is to add an optimal initiation region.

4. Target protein synthesized but product smaller than expected

Possible explanation: *Proteolytic degradation*
Possible measure: *Use protease inhibitor cocktail tablets to protect your proteins from a range of proteases.*

Possible explanation: *Translation initiation and/or termination not correct*
Possible measure: *Try to supplement the lacking tRNA.*

5. Sufficient protein expression, but low yield of active protein

Possible explanation: *Flawed protein folding*
Possible measures: *The E. coli lysate cannot introduce the formation of post-translational modifications, such as glycosylation, phosphorylation, or signal sequence cleavage.*

The formation of up to 3 disulfide bonds in proteins requires a sufficiently oxidizing environment.

You can add or adjust co-factors and/or chaperones.

6. Product shows up in the pellet after centrifugation

Possible explanation: *Aggregation*
Possible measure: *Protein aggregation leads to loss of biological activity. You may add a low concentration of non-denaturing detergent, e.g. 0.1 % CHAPS, 0.05 % Tween20, or add non-detergent sulphobetaines.*

You can also add or adjust co-factors and/or chaperones.

You can adapt the experimental environment (e.g., temperature, time etc.)

About BIOLINKER |

Headquartered in São Paulo, BIOLINKER is the leading Brazilian biotechnology company focused on cell-free protein synthesis (CFPS) and protein purification based on aptamers.

Our interdisciplinary and multinational team includes specialists in areas such as biochemistry (PhD), nanosciences and nanotechnologies (PhD), medical biochemistry (MSc), chemistry (MSc), veterinary medicine (2 doctors), chemical engineering (BSc), astrophysics/astrobiology (Honours Degree) and business administration (BSc).
Biolinker was chosen as one of the 500 best deep-tech startups in the world (HelloTomorrow 2019) among 4,500 applications from 119 countries.

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